PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF *CARICA PAPAYA*

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By

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CERTIFICATE

This is to certify that this dissertation entitled **"PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITY OF *CARICA PAPAYA*"** submitted by Y. ABINAYA Reg. No. 20APBO01 to **ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI** in partial fulfilment for the award of the degree of **"Master of Science in Botany" is done by her under my supervision.** It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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DECLARATION

I do hereby declare that this work has been originally carried out by me under the guidance and supervision of **Dr. P. Hermalin, M.Sc., M. Phil., Ph.D**, Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi and this work has not been submitted elsewhere for the award of any other degree.

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INTRODUCTION

Plants, the greatest gift of God, also they are the 'lungs' of our planet. It is just like the 'treasure box' hidden with a lot of active components that are essential for the process of developing new medicine. Medicinal plants not only considered as a readily available and affordable source, but they are also able to synthesize diverse active compounds which are effective in controlling and treating many diseases. These active compounds are known as secondary metabolites, such as phenols, tannins, alkaloids, flavonoids, glycosides, saponins and carbohydrates. Medical uses of plants range from extraction and decoction of leaf, bark, root, flower, seeds and stem portion of the plant. Described and accepted number of plant species as 3,74,000 of which approximately 308,312 are vascular plants, with 295,383 flowering. Among these flowering plant, *Carica papaya* is one of the medicinal plants that contributed as a remedy against a variety of diseases.

Papayas are the fourth most traded tropical fruit following bananas, mangoes, and pineapples. Approximately 75 percent of the world's papayas are produced in only ten countries. India leads the world in papaya production followed by Brazil, Indonesia, Nigeria, and Mexico. Papaya trees are fast growing, woody, tree-like plants that produce best in temperatures between 70º to 90ºF. They prefer full sun and well-drained porous soils that are moist in hot weather and dry in cold weather. This plant generally grow to between 10 and 12 feet tall, but can reach up to 30 feet in height. Plants used in commercial production are kept short because it is easier for crews to harvest the fruit from shorter trees. It is easily flourished well in many soil types as long as there is adequate drainage.

Papaya fruits are smooth skinned and vary widely in size and shape,

depending on variety and type of plant. The fruits usually contain many seeds surrounded by a sweet smooth yellow to orange-red flesh. It has three basic plant types: Male, Female, and Hermaphrodites. The males produce only pollen, never fruit. Only female and hermaphrodite plants typically produce fruit. Wind and insects are needed for pollination of female plants; however, hermaphroditic plants can self-pollinate. Almost all commercial orchards contain only hermaphrodites. Plants mature in six to nine months in warmer growing regions, and in 9 to 11 months in cooler regions. A mature papaya plant can produce as many as 100 fruits per growing season. Mature papaya plants naturally flower in the early spring and produce mature fruit in the summer.

The fruit contain rich source of vitamins, minerals and reduces inflammation. In fact, a cup of raw papaya contains 150 percent of the recommended daily intake of Vitamin C as well as 31 percent of Vitamin A and 10 percent of potassium (G. H. de Oviedo, 1535). It possesses high amount of beta carotene, which has been known to cause carotenemia, a harmless and temporary yellowing of the skin if you eat a ton of it. However, the latex may cause an allergic reaction. The plant contains many biologically active compound, two important compounds chymopapain and papain which are widely useful for digestive disorder and disturbance of the gastrointestinal tract, papaya derived papain, caricain, chymopapain and glycine endopeptids can survive acidic pH conditions and pepsin degradation. Thus, they may need to be protected against both acid denaturation and proteolysis for them to be effective in the gut after oral administration for the control of gastrointestinal nematodes. Carica papain lipase which is tightly bound to water insoluble fraction of crude papain and is thus considered as a “naturally immobilized” biocatalyst (Jose V. Sinistera, *et al*., 2006). The dried leaf infusion is taken for stomach trouble. Inner barks are used for

sore teeth and latex is used for syphilis in psoriasis ringworm and prescribed for the removal of cancerous growth, the flowers have been used for a hypoglycemic drug

The papaya oil seed contains saturated fatty acid (plasmatic satiric and arachnidan) and unsaturated fatty acid oleic, linoleum, and the seed yields 660-760 carpasemine. The 106 volatile components were identified in papaya (Robert A. Flath and Ralph R. Forrey, 1997). Fermentation with brew's yeast and distillation yielded alcohol (ethanol), which is externally applied to burns and scalds. The extract from fruit showed effective anti-microbial activity against *Staphylococcus aurous*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas shigella* (Bijoor Shivananda Nayak *et al*., 2012). Moreover, the chemical constituent of papaya in prevention of anthelmintic, anti- amoebic activities have been reported.

In view of these facts, the present investigation is under taken with following objectives

- To identify the phytochemicals present in the different parts of *Carica papaya.*
- The values of medicinal plants in pathogenic bacteria such as *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus*
- The phytochemical screening in different plant parts (leaf, stem, root, fruit and seeds) of *Carica papaya* using various solvents (ethanol, acetone, chloroform, benzene, aqueous).
- It can be concluded that presence of various pharmacological compounds in plant samples using FT - IR analysis.
- The result of the study also emphasizes the cost effective, bio friendly resources which would be tapped for development of effective drug in future.

REVIEW OF LITERATURE

Papaya originated from lowland of eastern Central America (Nakasone & Paull, 1998). The genus name Carica is derived from the Latin word which means a fig because of similarity in shape of the leaves and the fruits of papaya to those of figs. The plant can now be found and grown in all tropical countries and many sub-tropical regions of the world. Among a wide array of tropical fruits, papaya is deemed to be one of the most economical fruits, which not only is cultivated roughly in 60 countries but also is marketed worldwide (Hardur Venkatappa Annegowda & Bhat, 2016). It is otherwise known as 'fruit of the angels' or 'the most magnificent fruit in the world' (Singh, 2012). Almost all parts of the plant are used for either food, beauty or health products. Papaya has been cultivated for its edible fruits for hundreds of years but it is now also used for the production of jams, preserves, soft drinks, ice-cream, cocktail, crystallized fruit and canned in syrup (Ezike, Akah, Okoli, Ezeuchenne, & Ezeugwu, 2009; Nafiu *et al.*, 2019; Villegas, 1997; Workneh, Azene, & Tesfay, 2012). The fruits are widely used as a treatment of paediatric burns in Africa (Starley, Mohammed, Schneider, & Bickler, 1999). The green mature fruit, or the unripe fruit, is eaten as a vegetable in some Asian countries, usually after cooking or boiling. It is used as a substitute for marrow and applesauce (Villegas, 1997).

Apart from that, it is traditionally used to treat skin problems and is forbidden for consumption during pregnancy (Amenta, Camarda, Di Stefano, Lentini, & Venza, 2000; Anuar, Zahari, Taib, & Rahman, 2008; Fasihuddin & Ghazally, 2003). In South-East Nigeria, extracts of the unripe fruit including the seed were taken daily for management of ulcers for which it is claimed to be highly effective (Ezike *et al.*, 2009). In Philippines, the fruit is regarded as a medicinal fruit and fermented for use as food (Priscilla, 2008; Quisumbing, 1978). The fermented fruit has been

commercialized as a health product in Japan since the 19th century (Imao, Wang, Komatsu, & Hiramatsu, 1998).

The leaf of the plant was reported as being consumed by people living on the Gold Coast of Australia for its purported anti-cancer activity (Harald, 2003). In addition, the leaf extracts have also been used for a long time by indigenous people as a remedy for various disorders, including cancer and infectious diseases. In Malaysia, young papaya leaf are consumed for treating diabetes and high blood pressure (Ong & Norzalina, 1999) whereas in Nigeria, the fruits and roots are used in the management of diabetes mellitus (Abo, Fred-Jaiyesimi, & Jaiyesimi, 2008). The seeds of the plant are normally discarded and not eaten due to their spicy and pungent flavour and are believed to have antifertility and contraceptive effects.

The market value of papaya fruit is very much dependent on its taste, structure and appearance. Ripe fruit can be fragile, with a short shelf life and messy to eat. However, ripe fruit have a high antioxidant activity due to mainly to the content of ascorbic acid and carotenoids (Hernández *et al.*, 2006; Wall, 2006). Unripe fruit on the other hand is not marketable in countries like Brazil and Australia, but in Asian countries, it is used in salads and in cooking. Unripe fruit contains high levels of carbohydrate in the form of starch (Oloyede, 2005).

There are numerous commercially available papaya products such as beauty products, skin treatment cream and health supplements. Many studies have been published on the antioxidant and health properties of the plant. Krishna, Paridhavi, and Patel (2008) briefly discussed the nutrient, uses and medicinal value of the papaya plant.

Papaya fruits have a good nutritional health profile being an excellent source of provitamin A and ascorbic acid. They rank as one of the top fruit for ascorbic acid

content (Chandrika, Jansz, Wickramasinghe, & Warnasuriya, 2003; Wall, 2006) however, the ascorbic acid variation depends upon ripening stages of the fruit (Gayosso-García Sancho *et al*., 2011). The ripening process begins when the chlorophyll is degraded, which coincides with carotenoid synthesis and results in significant color changes from green to yellow-orange color (Andersson, Olsson, Johansson, & Rumpunen, 2008; Yahia & Ornelas-Paz, 2010). Many tropical fruits such as mango have similar behaviour as papaya in that the colour is conferred by the carotenoid level. Carotenoids like β-cryptoxanthin, and β-carotene are found in all types of papaya. However, contradictory results are found on lycopene availability in yellow fleshed fruits. Lycopene in yellow-fleshed fruits was not detected or found in a low amount compared with the red-fleshed fruits. (Gayosso-García Sancho *et al*., 2011; Marelli de Souza *et al*., 2008; Wall, 2006). Colour intensity resulting from carotenoid content plays a vital role in fruit acceptability by consumers (Yahia & Ornelas-Paz, 2010). Ascorbic acid (AA) is widely distributed in plant cells where it plays many crucial roles in growth and metabolism. As a potent antioxidant, ascorbic acid has the capacity to eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant a-tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes by keeping metal ions in the reduced state, appears to be the substrate for oxalate and tartrate biosynthesis and has a role in stress resistance (Arrigoni & De Tullio, 2002).

Due to its equivalence in biological activity to ascarbic acid, recent studies often consider the sum of DHAA and AA as vitamin C activity (Martí, Mena, Cánovas, Micol, & Saura, 2009). Papaya is a rich food source of AA with an average range of between 45-60 mg/100g, with the highest reported 9 value of 154 mg/100g. Most studies have shown no significant difference in the AA content of papaya

between cultivars (Tripathi *et al*., 2010; Wall, 2006). However, the AA content measured depends on the availability of light to the crop and to individual fruits and also the methods used to determine the AA content. The preferred choice for AA determination are separation techniques: capillary electrophoresis (Y. Tang & Wu, 2005; Versari, Mattioli, Parpinello, & Galassi, 2004), gas chromatography (F. O. Silva, 2005) and liquid chromatography (Nováková, Solich, & Solichová, 2008). Determination of actual dehydroascorbic acid (DHAA), an oxidized form of AA has not always been done due to the analytical challenge (Nováková *et al*., 2008). Usually, DHAA is determined as the difference between the total AA after DHAA reduction and the AA content of the original sample prior DHAA reduction.

There are very few available reports on vitamin E content of papaya. Food composition databases from countries like US, Japan and Malaysia indicate 0.3 mg/100g value of vitamin E content for papaya (Hagiwara, 2001; Tee, Noor, Azudin, & Idris, 1997; USDA, 2015

Benzylglucosinolate was found to be detected in all of the tissues of papaya (Aruna & Subrata, 2008; Jiao, Deng, Li, Zhang, & Cai, 2010; Tripathi *et al.*, 2010). Nonetheless, in papaya fruits, the amount detected was very low compared to other parts of the plant such as leaves and seeds (Bennett *et al.*, 1997; MacLeod & Pieris, 1983). In addition, the leaves also contain alkaloids 2d(including carpain and pseudocarpain), enzymes (papain, chymopapain, cystatin), tocopherol, ascorbic acid, flavonoids, tannins, nicotinic acid, saponins and phenolics (Bennett *et al.*, 1997; Duke, 2011; Seigler, Pauli, Nahrstedt, & Leen, 2002; C. S. Tang, 1979; Tee *et al*., 1997).

Papaya fruit pulp contains nonvolatile organic acids such as citric, fumaric, malic, malonic, succinic, and tartaric acids (pH 4.5–5.9). Furthermore, the level of

organic acids fresh weight (FW) in ripe papaya (per 100mg) contains the following: citric acid, 335mg; l-malic acid, 209mg, succinic acid, 52mg, quinic acid, 52mg, tartaric acid, 13mg, oxalic acid 10mg, and fumaric acid, 1.1mg (Hernández, Lobo, & González, 2009; Spínola, Pinto, & Castilho, 2015).

The seeds represent a rich source of biologically active isothiocyanate (Nakamura *et al*., 2000; Nakamura *et al*., 2007). Studies have confirmed that benzyl isothiocyanate (BITC) is the predominant compound in papaya seed extracts (Duke, 2011; Kermanshai *et al.*, 2001; Wilson, Kwan, Kwan, & Sorger, 2002). Several epidemiological studies have indicated that the dietary consumption of isothiocyanates or isothiocyanate containing foods inversely correlates with the risk of developing cancers and potential evidence of cancer prevention in humans (Cavell, Syed Alwi, Donlevy, & Packham, 2011; Hwang & Lee, 2006; Seow *et al*., 2002). In contrast, there are also many studies published on the toxicological effects of the seeds and its 11 contraceptives properties, especially to men. More research is required on the health benefits and toxicology of the seeds for human consumption. The seed was also high in lipid content, with oleic acid (77.7%) as the predominant fatty acid (Dakare *et al*., 2011).

Fermented papaya is used as a health product. A commercial Fermented Papaya Preparation (FPP) is produced as a white granular food supplement product by yeast fermentation of non-genetically modified papaya. It has been sold as a natural functional healthy food in Japan and other countries. Nutritional analysis reveals that FPP (Immun' Age®) has amino acids and carbohydrates (Osato Research Institute, 2003). Fermented papaya seed was also shown to have essential amino acids, protein (24%) and lipids (54%). Moreover, fermentation reduced the level of antinutritional factors of the seeds. Oxalate reduced from 210.1 to 40.2 mg/100g, phytic acid from

102.0 to 68mg/100g, tannin from 15.5 to 8.3 mg/100g and typsin inhibitor from 2431.2 to 63.0 mg/100g (Dakare *et al.*, 2011).

Plants contain a large variety of substances called phytochemicals that possess antioxidant activity. Some of the compounds that exhibit high antioxidant capacity (AOC) include vitamins and polyphenol compounds. Most studies have shown that papaya has a high AOC in comparison with other fruits (Faller & Fialho, 2010; Isabelle *et al.*, 2010; Leong & Shui, 2002; Lim, Lim, & Tee, 2007; Mehdipour *et al*., 2006; Osato, Santiago, Remo, Cuadra, & Mori, 1993). However, there are some studies that detected a low AOC in comparison to other fruits (Patthamakanokporn, Puwastien, Nitithamyong, & Sirichakwal, 2008; Stangeland, Remberg, & Lye, 2009).

Due to the lack of a standard method for AOC, it is difficult to compare the results reported from different research groups. Several reviews have been published, and the opinions vary considerably (D. Huang, Ou, & Prior, 2005; Prior, Wu, & Schaich, 2005; Sánchez-Moreno, 2002). However, for botanical samples or juices, the most commonly used methods used for AOC are 2,2-di(4- tertoctylphenyl)-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) and total radical-trapping antioxidant parameter (TRAP). In the case of AOC of papaya plant, DPPH seems to be the most prominent method used for majority researchers. However, the reason for this maybe because this method is easy, relatively quick and suitable for fruit and vegetables juices or extracts (Sánchez-Moreno, 2002). In contrast, Huang and colleagues (2002) in their review indicated that DPPH assay was much less chemically sound as a valid assay for antiradical activity measurement due to its reaction with other compounds. However, there are pros and cons of all these AOC assays and different methods are needed to fully evaluate and compare any one product.

Lim and co-workers (2007) observed that the papaya fruit variety ‘Solo’ was a very potent radical scavenger with a low IC50 (the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration) of 3.5 mg/ml. This result was supported by other researchers, who found similar results of high radical scavenging activity. Scavenging activity of papaya fruits were found to be higher compared to other fruits like mango, tangerine, apple as well as standard vitamin E using the same method (Faller & Fialho, 2010; Mehdipour *et al.*, 2006). On the contrary, using a ferrous ion-chelating capacity method the peel showed relatively low radical scavenging activity but much higher ability to chelate pro-oxidant metal ion (Matsusaka & Kawabata, 2010). Reports on AOC of non-edible parts of the plant such as the peel, seeds and leaves are very rare in the literature, thus the AOC of these parts could not be confirmed from reported studies. .

Leong and Shui (2002) examined the AOC of the papaya varieties ‘Solo’ and ‘Foot long’ papaya using two methods: DPPH and ABTS free radical decolorization assay. They found that AA contributes the most to the antioxidant properties of the fruits with a contribution percentage of 48% for ‘Solo’ and 62.3% for ‘Foot long’ papaya. The result was later confirmed by Isabelle and coworkers (2010) who found that AA contributes to 97% of AOC of the fruits. However, the result was only based on the hydrophilic ORAC (H-ORAC) method which found a relatively low AOC level compared to other tropical fruits. This result was supported by Patthamakanokporn, *et al*. (2008) who found a relatively low AOC using the same method. On the other hand, the lipophilic antioxidant content of the fruit was found to be relatively high, probably due to the high amounts of ß-cryptoxanthin, lycopene and ß-carotene (Isabelle *et al.*, 2010).

A low AOC was found in papaya fruits using the FRAP method

(Patthamakanokporn *et al*., 2008; Stangeland *et al*., 2009). The FRAP method is an electron transfer-based antioxidant capacity assay and the mechanism is quite similar to the Folin Ciocalteu method (Singleton, Orthofer, & LamuelaRaventós, 1999) used to determine the 'total phenolic content'. Numerous publications have applied the total phenolic content assay using the Folin Ciocalteu reagent and an electron transfer-based antioxidant capacity assay (such as the FRAP). They have often found excellent positive correlations between the total phenolic content and AOC (D. Huang *et al*., 2005; Netzel, Netzel, Tian, Schwartz, & Konczak, 2006; Prior *et al*., 2005). This is expected because the chemistry of these assays is quite similar.

The AOC level in FPP (PS-501®) was found to be high with an IC50 value of 12.5mg/ml (Imao *et al*., 1998). In this study, FPP had the ability to scavenge 80% of hydroxyl radicals generated by Fenton reagents and 50% lipid soluble radicals and was dose-dependent in the range of 5-50mg/ml by DPPH method. Moreover, oral administration of the FPP for 4 weeks was found to decrease the elevated lipid peroxide levels in iron-injected cortex of rats (Imao *et al*., 1998). The result of this study was further confirmed by Noda and co-workers (2008), using the same product (PS-501). FPP was found to have an IC50 value of 8mg/ml which inhibits hydroxy radical generation from methylguanidine, a type of neurotoxin/nephrotoxin which generates reactive oxygen species (ROS) (Noda *et al*., 2008). Thus, FPP may have a beneficial effect in reducing ROS as well as preventing methylguanidine related diseases (Noda *et al*., 2008). In contrast, Calzuola and colleagues (2006) showed that FPP (Immun'Age®) had lower AOC levels compared to wheat sprouts, white tea and Morinda citrifolia extracts. The result indicated that 1g of FPP could only reduce potassium ferricyanide reagent by 1.05±0.09 µmol compared with standard compound of vitamin C (4.8 ± 0.7 µmol of reduced ferricyanide/mg compound), rutin (3.8 ± 1.2

µmol of reduced ferricyanide/mg 17 compound) and quercetin (4.8 ± 1.7 µmol of reduced ferricyanide/mg compound), respectively (I. Calzuola *et al*., 2006; Isabella Calzuola, Marsili, & Gianfranceschi, 2004). However, the discrepancy with the results of the AOC observed in these studies could be restricted to the method used, and the type and concentration of the sample. In addition, a study by Bolling *et al*. (2012) found that dilution factors may also affects the AOC of the sample. In general, for routine determination of AOC in extracts or beverages is normally diluted in a buffer or solvent in order to be within the linear standard graph. However, in their study, it is found that increased dilution factor resulted in higher AOC in pomegranate and grape juice studied. Therefore, dilution factors should be considered in AOC assays for more accurate AOC interpretation (Bolling *et al*., 2012).

Fruits and vegetables contain many types of phytochemicals, of which many such as vitamin C, vitamin E and carotenoids are antioxidant compounds, (Prior *et al*., 1998). However, polyphenol compounds, such as flavonoids, procyanidins and phenolic acids also contribute to the beneficial effects of this group of foods (Del Rio, Costa, Lean, & Crozier, 2010; Fraga, Galleano, Verstraeten, & Oteiza, 2010; Petti & Scully, 2009). Polyphenols are the most abundant bioactive compounds in our diet, and their content is much higher than that of all other classes of phytochemicals and known dietary antioxidants. Their total dietary intake could reach as high as 1 g/day, which is approximately 10 times higher than the vitamin C intake (Scalbert, Johnson, & Saltmarsh, 2005). However, the polyphenolic composition in papaya pulp had not been unambiguously determined until Gayosso- García Sancho *et al*., (2011), recently listed and quantified some of the major polyphenols compounds found in papaya pulp of ‘Maradol’ cultivars. They were ferulic acid, pcoumaric acid, and caffeic acid. The result coincides with the first report on ‘Maradol’ cultivars which found a similar

profile pattern of phenolics compounds (Rivera-Pastrana, Yahia, & Gonzalez-Aguilar, 2010) but the concentration was very different.

Other researchers suggested only low amounts or traces of phenolic compounds in papaya pulp or only identified compounds without quantifying (Franke, Custer, Arakaki, & Murphy, 2004; Jindal & Singh, 1975; Lako *et al*., 2007; Simirgiotis, Caligari, & Schmeda-Hirschmann, 2009). The low content and number of polyphenols in papaya pulp found in all these studies could explain the limited data for polyphenols in papaya pulp.

Total phenolic content in papaya pulp, determined using the standard Folin Ciocalteu's method (Singleton *et al*., 1999) was found to be quite low, ranging from 0.02-2.08 (mg GAE/100g fresh weight (FW)) (Faller & Fialho, 2010; Isabelle *et al.*, 2010). The low value of total phenolics perhaps explained the low AOC using the FRAP method as discussed earlier. Nevertheless, some 18 researchers had found moderate levels of total phenolics in this fruit, with the highest value of 54 mg GAE/100g FW (Lako *et al.*, 2007; Patthamakanokporn *et al*., 2008). The values of total phenolics using the Folin Ciocalteu's reagent may possibly result from the formation of bluemolybdenum-tungsten complex from AA in the fruits rather than from the phenolics compounds. It is necessary to correct the absorbance originating from AA especially in fruits that have high AA like Papaya. The literature reports do not indicate whether correction had been made for ascorbic acid.

The content of polyphenols was found to be high in the leaves and in the peels of papaya. The quantitative analysis on phenolic compounds of papaya leaves was done by Canini *et al*., (2007) and Husin *et al*. (2019) which revealed the presence of phenolic acids (i.e. caffeic acid, p-coumaric acid and protocatechuic acid) as the main compounds. Chlorogenic acid was found in trace amounts, compared to the

flavonoids and coumarin compounds. On the contrary, Miean & Mohamed (2001) detected a high level of total flavonoids (126.4 mg/100 g) in young leaves of papaya, with quercetin and kaempferol as the main compounds. Quercetin and kaempferol are compounds found abundantly in most edible plants including leafy vegetables, fruits and beverages. Phenolic acids on the other hand are present in appreciable amounts in a large number of vegetables. Both compounds demonstrate antioxidant and antioxidative properties (Lu *et al*., 2006; Moon, Tsushida, Nakahara, & Terao, 2001; Rusak *et al*., 2010; Sasaki, Toda, Kaneko, Baba, & Matsuo, 2003; Sternberg *et al.*, 2008). In papaya peel, the polyphenols content was found to be higher than the pulp (Faller & Fialho, 2010; Matsusaka & Kawabata, 2010). Recent quantitative analysis indicated the presence of ferulic and caffeic acids as the most abundant compounds (Rivera-Pastrana *et al.*, 2010) in the peel of the fruits.

The quantity of polyphenols in foods can be affected by agricultural practices such as the use of synthetic fertilizers that offer more bioavailable sources of nitrogen which can accelerate plant and the production of secondary metabolites. Polyphenol content could also be affected by higher exposure of the plant to stressful environment such as the absence of synthetic pesticides. Attack by insects or fungi can induce the production of natural defense substances such as phenolic compounds (Winter & Davis, 2006; Woese, Lange, Boess, & Bogl, 1997). Both hypotheses would result in foods with higher antioxidant capacity as a consequence of the higher polyphenol content. Faller and Fialho (2010) had compared the polyphenol content of organic and conventional plant foods. They found that among fruits studied, papaya seems to be more affected by alteration in agricultural management with approximately 70% higher hydrolysable polyphenols in organic fruit compared to the conventional counterpart. This could be one of the reasons for different polyphenol 19 value found

in the same type of papaya cultivars discussed earlier. Nonetheless, polyphenol composition in food can also be altered by storage, location, handling, and processing conditions (Rinaldo, Mbéguié-A-Mbéguié, & Fils-Lycaon, 2010).

Wound healing effects

Animal studies addressed the ability of papaya to treat skin disorders specifically wound healing properties. The extract of peels of mature green fruit (unripe) have proven to gives faster epidermal wound healing on induced wounds on mice (Anuar *et al.*, 2008) and rats (Nayak, Pinto Pereira, & Maharaj, 2007) compared to peels of ripe fruit. This was further supported by wound healing evaluation using latex from the skin on mice burn models (Gurung & Skalko-Basnet, 2009). The latter suggested that the latex was responsible for its wound healing properties. The latex from unripe papaya fruits contains a mixture of cysteine endopeptidases such as papain, chymopapain A and B, caricain, papaya endopeptidase II, papaya endopeptidase IV, omega endopeptidase chitinase II, protease inhibitors, glutaminyl cyclase and unknown-function proteins (Azarkan, El Moussaoui, van Wuytswinkel, Dehon, & Looze, 2003; Azarkan, Wintjens, Looze, & Baeyens-Volant, 2004). Proteolytic enzymes such as papain, chymopapain and leukopapain were reported to be effective for debridement of necrotic tissues, preventing infection, promoting growth and improving the quality of the scar (Anuar *et al*., 2008; Starley *et al.*, 1999). Thus, in agreement with traditional beliefs, papaya has a high potential as a treatment for wound healing.

Anti-ulcer effects

Papaya leaves and unripe fruits may potentially serve as a good therapeutic agent for protection against gastric ulcers. A gastric ulcer index was significantly reduced in rats pretreated with papaya extracts as compared with alcohol treated

controls (Ezike *et al.*, 2009; Indran, Mahmood, & Kuppusamy, 2008), and the standard drugs cimetidine (Ezike *et al.*, 2009) and indomethacin (Owoyele *et al*., 2008), respectively. The unripe fruit was also shown to have the ability to inhibit gastrointestinal propulsion (Ezike *et al.*, 2009). This has proven beneficial in ulcer therapy as delaying of gastrointestinal motility will increase the absorption of oral anti-ulcer drugs. Moreover, the unripe fruit was also shown to possess antimicrobial properties (Osato *et al.*, 1993), which are probably beneficial in treating/preventing peptic ulcers by acting against Helicobacter pylori. The extracts of unripe C. papaya contain terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins, and steroids. Saponins are known to possess anti-ulcer activity mediated by the formation of protective mucus on the gastric mucosa and protection of the mucosa from acid. The cytoprotective and antimotility properties of the extracts may account for the anti-ulcer property of the leaves and unripe fruit.

Anti-inflammatory and immune modulatory effects

Researchers have suggested the possibility of anti-inflammatory and immune modulatory effects of papaya pulp, leaves and seeds. A recent published study with 15 healthy subjects suggested that ingestion of papaya fruit is inversely associated with the reduction of IFN-λ+CD4+ T cells which play a vital role in mediating inflammatory responses (Abdullah *et al*., 2011). The anti-inflammatory effects of papaya leaf extract has also been found to significantly reduce carrageenan-induced paw oedema, cotton pellet granuloma and formaldehyde-induced arthritis in rat models (Owoyele *et al*., 2008). The leaf extract was also shown to have immunomodulatory activities by enhancing Th 1 types cytokines from human lymphocytes, that relate to antitumor immunity (i.e. IL-12p40, IL-12p70, IFN-λ or TNF-α) in the human immune system (Otsuki *et al*., 2010). Similarly, the seed extract

has also shown the ability to enhance phytohemagglutinin reactivity of lymphocytes isolated from human blood (Mojica-Henshaw, Francisco, De Guzman, & Tigno, 2003). This implies that the seed extract contains substances or components which have or display growth-promoting actions and thus act as immunomodulators. In the same manner, some bioactive fractions of the seed extract are also reported to have the ability to inhibit cell lysis as shown using an *in vitro* classical complement-mediated hemolytic pathway assay. This indicates a possible effect towards inhibiting inflammation (Mojica-Henshaw *et al*., 2003). As mentioned earlier, papaya especially the leaves contain flavonoids, saponins, tannins and glycosides that have all been associated with various degrees of anti-inflammatory activities (González Mosquera *et al*., 2011; Pelzer, Guardia, Juarez, & Guerreiro, 1998; Sparg, Light, & van Staden, 2004; Thomas & Filho, 1985). Therefore, the anti-inflammatory effects may be due to the activity of one or a combination of some of the identified constituents.

Anti-cancer effects:

Papaya is rich in glucosinolates, isothiocyanates and BITC and thus is presumably a promising plant source for use in chemoprevention of cancer. Many scientific studies validate that BITC induces apoptosis specifically in cancer cells and protects against tumorigenesis. It has been suggested that anticarcinogenic effects of isothiocyanates are related to their capacity to induce phase II enzymes such as glutathione *S*-transferase, nicotinamide adenine dinucleotide phosphate and quinine reductase (Cavell *et al*., 2011; Nakamura *et al*., 2000). Moreover, plant sources of glucosinolates, have been of great interest for potential use in the chemoprevention of cancer (Aruna & Subrata, 2008; Cavell *et al*., 2011).

Enzymatic action of plant-specific myrosinase or intestinal flora in the human body (Aruna & Subrata, 2008). Interestingly, only recently was the effect of

inhibitory activity of *C.* papaya leaf extract on tumor cell lines documented. The leaf extracts were shown to significantly inhibit the proliferative responses of solid tumor cell lines derived from cervical carcinoma, breast adenocarcinoma, prostate cancer, hepatocellular carcinoma, lung adenocarcinoma, pancreatic epithelioid carcinoma, and mesothelioma (Otsuki *et al*., 2010; Pandey *et al*., 2017). This studies reporting on the antitumor properties of the leaf extracts and suggested that the extract may potentially provide the means for the treatment and prevention of selected human diseases such as cancer, and may also serve as immunoadjuvants for vaccine therapy (Otsuki *et al*., 2010). There is no current scientific data available on the exact compound that demonstrated the anti-cancer and immunomodulatory effects, however isothiocyanates extracted from papaya fruits have been proven to act as a glutathione *S*-transferase inducers (Nakamura *et al*., 2000). Otsuki *et al* (2010) have done fractionation of papaya leaf and suggested that a fraction of lower than molecular weight 1000 could increase the Th 1 cytokines and exhibited inhibitory effects of proliferative response of tumor cell lines and may also have the possibility to prevent various allergic disorders. In addition, papaya fruit was also rich in lycopene content. Several *in vitro* studies with human cancer cells, particularly prostate cancer cell lines, have indicated that lycopene can promote apoptosis in these cells and therefore might have potential as a chemotherapeutic agent (van Breemen & Pajkovic, 2008).

Other health benefits

Antimalarial activity of papaya is mostly anecdotal but in the literature there is a report on the efficacy of a crude aqueous extract of papaya leaf on mice infested with malaria parasite (Pietretti *et al.*, 2010). It was found that the extracts exhibited plasmodicidal activity and reduction of parasitemia (Pietretti *et al*., 2010; Sannella *et al*., 2009). A study by Eliagita *et al*. (2017) reported that consuming papaya (mature

ripe pulp) has a significant effect on changes in hemoglobin and hematocrit levels in pregnant women. It is suggested that consuming papaya should be one of alternative treatments for midwives to prevent anemia in pregnant women.

Diseases such as dengue result in a low thrombocyte count in the blood and need a rapid response of thrombocyte levels and plasma transfusions (World Health Organization, 1997). Papaya leaf crude formulations have been successfully employed in folk medicine in Malaysia for the treatment of dengue infections with haemorrhagic manifestations (Ching *et al*., 2015; Subenthiran *et al*., 27 2013). The formulations use suspensions of powdered leaves in palm oil as a vehicle.

A recent published study indicated that papaya leaves contain high α-glucosidase and lower α-amylase inhibitory activities that could be considered potential dietary supplement approaches to manage early stages of hyperglycemia (Loh & Hadira, 2011). Inhibition of these enzymes delays and prolongs carbohydrate digestion time, causing a reduction in the rate of glucose absorption and therefore blunting the postprandial plasma glucose rise (Rhabasa-Lhoret & Chiasson, 2004). In addition, fruits and roots are also claimed to be effective in the management of diabetes mellitus (Abo *et al.,* 2008).

MATERIALS AND METHODS

Classification:

Class	:	Dicotyledons
Order	:	Brassicales
Family	:	Caricaceae
Genus	:	*Carica*
Species	:	*papaya*

Morphology of *Carica papaya*:

Plants are medium trees, with latex, dioecious, roots are tap root and branched. The stems are soft-wooded, trunks thin barked. Leaves are Large, long, petioled, alternate, simple-with lobed or entire lamina and exstipulate. Inflorescence are pendant raceme or corymb, multiflowered. Flowers are bisexual, actinomorphic and hypogynous. Calyx are small, rotate, cupular or shortly tubular, teeth or segments

long, in pistillate flower calyx segments are comparatively longer than that of staminate ones. Petals in female flower or pistillate flower free or comate at the base. Carpels are 5, united or syncarpous, ovary superior, unilocular, parietal placentation, ovules many, anatropous; style short, terminating into five partite linear. Fruits are Berry, large, many seeded, one celled with large cavity inside. Seeds are oval, ellipsoid, more or less compressed, sometimes, smooth or tubuled or spiny.

Carica papaya Linn. Collected in February 2022 from Sorispuram, Tuticorin, Tamil Nadu, India. The plant material was washed, shade dried for few days and then dried completely in an oven at 38ºC. The plants were coarsely powdered and stored in air tight containers for further analysis.

Extractive values

Soluble extractives

10 gram of *carica papaya* plant sample (leaf, stem, root, fruit and seed) was sequentially extracted with 200 ml of aqueous, acetone, benzene, chloroform and petroleum ether solution using in soxhlet apparatus. The prepared extract was used for further analysis.

Phytochemical screening of different extracts

The shade dried and coarsely powdered leaves were extracted successively with different solvents by using Soxhlet apparatus and analyzed using simple chemical tests for preliminary screening of various groups of phytochemicals such as alkaloids, flavonoids, phenolic acids, sterols, cardiac glycosides, tannins, saponin. The chemical tests for various phytoconstituents in the extract are carried out as described below.

Determination of Alkaloids

The amount 0.50 g of plant material was refluxed with 2 mL of dilute hydrochloric acid for 10 min on water bath, cooled and filtered. The filtrate was

tested carefully with various alkaloidal reagents as follows.

A. Dragendorff's test

For the preparation of Dragendorff's reagent, Solution A: 0.17 g of bismuth subnitratein 2 ml glacial acetic acid diluted upto 10 mL with distilled water. Solution B: 4 g of potassium iodide was taken in 10 ml of glacial acetic acid and diluted upto 20 mL with water. Solution A and B were mixed together and diluted upto 100mL.

Dragendorff's test was performed by adding 2 ml of Dragendorff's reagent to 2 mL of filtrate. A prominent yellow precipitate indicated the test to be positive.

Determination of Carbohydrates

Decoction of 2 g of plant material were prepared by 20 mins refluxing in 10 mL distilled water and filtered through Whatman no. 1 filter paper. The filtrate was subjected to the following tests.

A. Molish's test

Two drops of alcoholic solution of α-naphthol were added to 2 mL of filtrate, the mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.

B. Fehling's test

Fehling's solution A was prepared by dissolving 0.35 g of Copper sulphate in 5.0 mL of distilled water. Fehling's solution B was prepared by dissolving potassium sodium tartrate (0.17g) and sodium hydroxide (0.50 g) in 5.0 mL of water.

Fehling's test was performed by boiling 1 mL of filtrate with 1 mL each of Fehling solutions A and B on a water bath. A red precipitate indicated the presence of sugar.

Determination of Proteins and Amino Acids

Decoction of 2 g of plant material were prepared by 20 mins refluxing in 10 mL distilled water and filtered through What man No.1 filter paper. The filtrate was subjected to tests for proteins and amino acids.

A. Biuret test

An aliquot of 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1 mL of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, pink color in the ethanolic layer indicated the presence of proteins.

B. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of the filtrate. A characteristic purple color indicated the presence of amino acid.

Determination of Glycosides

For detection of glycosides, 5 g of water extract of *Carica papaya* was hydrolyzed with 2 N concentrated hydrochloric acid for 2 h on water both, filtered through Whatman no. 1 filter paper and the hydrolysate was subjected to the following tests.

Borntrager's test

3 mL chloroform was added in 2 mL filtered hydrolysate, well shaken. The chloroform layer was separated and 10% ammonia solution was added to it, a pink color indicated the presence of glycosides.

Determination of Saponins by Foam Test

2g decoction of papaya plant material was shaken in a graduated cylinder for

15mins. 2 cm layer of foam indicated the presence of saponins.

Determination of Fixed Oils and Fats by Spot test

A small quantity of dried leaves was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oil.

Determination of phenolic compounds and Tannins

A. Ferric chloride test

Five drops of neutral 5% ferric chloride solution were added in the decoction of Papaya sample (5 mL). A dark green color indicated presence of phenolic compounds.

Lead acetate test

The decoction of papaya sample (3 ml) was diluted with distilled water and 3 mL of 10% lead acetate solution was added. A bulky white precipitate indicated presence ofphenolic compounds.

Antibacterial activity

The test organisms were obtained from the Department of Microbiology, St. Mary's College (Autonomous), Thoothukudi. The two gram positive bacteria viz. *Bacillus subtilis, Staphylococcus aureus* (G +ve) and two gram negative bacteria *Escherichia coli, Vibriyo cholerae* were used in the present study. *Bacillus* responsible for food borne gastroententis. *E. coli, Staphylococcus aureus* cause diseases like mastitis, abortion and upper respiratory complications, while *Vibrio cholera* cause disease like cholera. The test bacterial strains were inoculated into nutrient broth and incubated at 37°C for 24 hrs. After the incubation period, the culture tubes were compared with the turbidity standard.

Disc diffusion assay

Anti-bacterial activity was evaluated by agar disc diffusion method. Test solutions were prepared with known weight of different solvent extracts dissolved in 5% dimethyl sulphoxide (DMSO). What man No.1 sterile filter paper discs (5mm) were impregnated with 20 µl of these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculum. Each sample loaded disc was placed in the seeded agar plate. After 24-48 hours of 37°C incubation, the diameter of the inhibition zone was measured. For positive control, ampicillin disc (100µg/ml) was used, whereas for negative control, respective solvents were loaded on the sterile disc.

FT-IR Procedure

FT-IR analysis was performed using KBr (Potassium bromide) pellet method, which was used to detect the characteristic peaks and their functional groups. The peak values of FT-IR was recorded. Each and every analysis was repeated twice for the spectrum confirmation. A small amount of powdered sample was respectively placed directly on the germanium piece of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from $4000cm^{-1}$ to $400cm^{-1}$ and computerized for analyses. The reference spectra were acquired from the cleaned blank crystal blank prior to the presentation of each sample replicate.

RESULT AND DISCUSSION

Phytochemical constituents in plants samples are considered to be biologically active compounds with a variety of functions including antioxidant, anti-microbial, antifungal, hypoglycemic, anti-diabetic, anti-inflammatory, anti-carcinogenic, antimalarial, anticholinergic properties.

The phytochemical analysis of different leaf extracts (aqueous, chloroform, acetone, benzene and petroleum ether) of *Carica papaya* were shown in Table 1 - 5. Phytochemicals such as alkaloids, carbohydrates, Sugar, Protein, Phenolic compounds, Flavonoids, Tannins, saponins and amino acid were analysed. The most number of phytochemical tests were reported in chloroform and acetone extract. Some of the test were reported in water and benzene extract (Table no. 1). Alkaloids which make up one of the most diverse classes of phytochemicals found in plants, have remarkable effects on humans, leading to the production of effective pain relievers. Tannin react with proteins to produce the tanning effect, which is beneficial in the treatment of inflamed or ulcerated tissues. Herbs are those that contain tannins as one of their main components and are used to treat intestinal problem like diarrhea and dysentery. In *Carica papaya* saponin component was absent in chloroform, petroleum ether and aqueous leaf extract whereas, saponin is more responsible for anti-inflammatory, anti-hepatotonic, wound healing, veinotonic, expectorant, spasmolytic, hypoglycemic, antimicrobial, and antiviral properties.

The phytochemical analysis of different stem extracts (aqueous, Acetone, Benzene, Chloroform and petroleum ether) of *Carica papaya* were shown in Table 2. The results clearly showed that the chloroform stem extract of C. papaya contain alkaloids, carbohydrates, sugar, protein, phenolic compounds, flavonoids, tannins, saponins and mino acid. Next to these, the maximum number of phytochemicals were

reported in aqueous and acetone extract. The lowest number of tests resulted in benzene extract.

According to the fruit extracts (aqueous, acetone, benzene, and chloroform and petroleum ether) of *Carica papaya* (Table 3) phytochemicals such as alkaloid, carbohydrate, sugar, protein, phenol, flavonoid, amino acids are reported in acetone, benzene and petroleum ether. Similar result was observed in Gupta (2020) revealed the presence of flavonoids, alkaloids, tannin, coumarins, terpenoids, proteins and amino acids in aqueous, petroleum ether extracts. Similar results were obtained in seed extracts (Table 4). The most number of phytochemical tests were resulted in aqueous and chloroform extract. next to this the maximum number of tests provided the results in acetone extract. The papaya frit is rich source of antioxidant and various types of enzymes which is excellent aid to digestion. (Dewkin *et al.,* 2003). In root extracts the phytochemicals were reported in acetone, benzene, chloroform and petroleum ether (Table 5). The lowest number of tests resulted in acetone extract.

Infectious diseases are a major cause of morbidity and mortality in India. The number of multiple drug resistant strains and the appearance of the strains with reduced susceptibility to antibiotics are continuously increasing. The antimicrobial activities of *Carica papaya* plant material (leaf, stem, fruit, seeds and root) with different solvents were investigated in the present study to identify the presence of bioactive substances which have been reported to confer resistance to plants against bacteria, therefore explains the demonstration of antibacterial activity by the plant extracts used (Srinivasan *et al*., 2001).

In the present study, the aqueous extracts of *Carica papaya* leaf showed inhibitory activity against *Escherichia coli* (6mm), *Bacillus substilis* (5mm), *Vibrio cholerae* (3mm) and *Staphylococcus aureus* (3mm); Acetone extract of leaf exhibits

inhibitory activity against *E. coli* (3mm), *Bacillus substilis* (4mm), *Vibrio cholerae* (3mm) and *Staphylococcus aureus* (9mm); the chloroform extract of the leaf showed inhibitory action against *Escherichia coli* (8mm), *Bacillus substilis* (3mm), *Vibrio cholerae* (4mm) and *Staphylococcus aureus* (5mm); the benzene extract of the leaf showed activity inhibitory action against *Escherichia coli* (7mm), *Bacillus substilis* and *Vibrio cholerae* (3mm) and *Staphylococcus aureus* (7mm); the Acetone extract of the leaf showed activity inhibitory action against *Escherichia coli* (3mm), *Bacillus substilis* (4mm), *Vibrio cholerae* (3mm) and *Staphylococcus aureus* (9mm); the petroleum ether extract of the leaf showed inhibitory action against *E. coli* (4mm) *Bacillus substilis* (3mm), *Vibrio cholera* (1mm), *Staphylococcus aureus* (2mm). The most number of anti-bacterial activities were resulted in chloroform and Benzene extract. The maximum number of anti-bacterial activities provided the results in water and acetone extract. The lowest number of anti-bacterial activities resulted in petroleum ether extract.

The aqueous extracts of *Carica papaya* stem showed activity on *E. coli* (8mm) *Bacillus substilis* and *Vibrio cholera* (3mm), *Staphylococcus aureus* (4mm); the acetone extract of the stem exhibits inhibitory activity against *E. coli* (3mm) *Bacillus substilis* (4mm) *Vibrio cholera* and *Staphylococcus aureus* (3mm); the benzene extract of the stem showed inhibitory action against *E. coli* (7mm) *Bacillus substilis* (6mm), *Vibrio cholera* (4mm), *Staphylococcus aureus* (5mm); the chloroform extract of the stem showed inhibitory action against *E. coli* (7mm) *Bacillus substilis* (10mm), *Vibrio cholera* (5mm), *Staphylococcus aureus* (4mm); the petroleum ether extract of the stem showed inhibitory action against *E. coli* (4mm) *Bacillus substilis* (5mm), *Vibrio cholera* (2mm), *Staphylococcus aureus* (6mm). The most number of anti-bacterial activities were resulted in aqueous and chloroform extract. The maximum

number of anti-bacterial activities provided the results in benzene and petroleum ether extract. The lowest number of anti-bacterial activities resulted in acetone extract.

The aqueous extract of the fruit showed inhibitory action against *E. coli* (5mm) *Bacillus substilis* (2mm), *Vibrio cholera* (4mm), *Staphylococcus aureus* (5mm); the acetone extract of the fruit showed inhibitory action against *E. coli* (2mm) *Bacillus substilis* (4mm), *Vibrio cholera* (8mm), *Staphylococcus aureus* (6mm); the benzene extract of the fruit showed inhibitory action against *E. coli* (3mm) *Bacillus substilis* (2mm), *Vibrio cholera* (5mm), *Staphylococcus aureus* (1mm); the petroleum ether extract of the fruit showed inhibitory action against *E. coli* (6mm) *Bacillus substilis* (4mm), *Vibrio cholera* (2mm), *Staphylococcus aureus* (3mm); the chloroform extract of the fruit showed inhibitory action against *E. coli* (7mm) *Bacillus substilis* (6mm), *Vibrio cholera* (4mm), *Staphylococcus aureus* (5mm). The most number of anti-bacterial activities were resulted in chloroform and petroleum ether extract. The maximum number of anti-bacterial activities provided the results in aqueous and acetone extract. The lowest number of anti-bacterial activities resulted in benzene extract.

The aqueous extract of the seed showed inhibitory action against *E. coli* (2mm) *Bacillus substilis* (3mm), *Vibrio cholera* (8mm), *Staphylococcus aureus* (2mm); the acetone extract of the seed showed inhibitory action against *E. coli* (6mm) *Bacillus substilis* (4mm), *Vibrio cholera* (3mm), *Staphylococcus aureus* (5mm); the benzene extract of the seed showed inhibitory action against *E. coli* (3mm) *Bacillus substilis* (6mm), *Vibrio cholera* (4mm), *Staphylococcus aureus* (3mm); the chloroform extract of the seed showed inhibitory action against *E. coli* (4mm) *Bacillus substilis* (7mm), *Vibrio cholera* (7mm), *Staphylococcus aureus* (5mm); the petroleum ether extract of the seed showed inhibitory action against *E. coli* (5mm)

Bacillus substilis (5mm), *Vibrio cholera* (4mm), *Staphylococcus aureus* (4mm). The most number of anti-bacterial activities were resulted in acetone and chloroform extract. The maximum number of anti-bacterial activities provided the results in benzene and petroleum ether extract. The lowest number of anti-bacterial activities resulted in aqueous extract.

The aqueous extract of the root showed inhibitory action against *E. coli* (9mm) *Bacillus substilis* (2mm), *Vibrio cholera* (5mm), *Staphylococcus aureus* (1mm); the acetone extract of the root showed inhibitory action against *E. coli* (2mm) *Bacillus substilis* (4mm), *Vibrio cholera* (8mm), *Staphylococcus aureus* (6mm); the benzene extract of the root showed inhibitory action against *E. coli* (3mm) *Bacillus substilis* (2mm), *Vibrio cholera* (5mm), *Staphylococcus aureus* (1mm); the chloroform extract of the root showed inhibitory action against *E. coli* (7mm) *Bacillus substilis* (6mm), *Vibrio cholera* (4mm), *Staphylococcus aureus* (5mm); the petroleum ether extract of the root showed inhibitory action against *E. coli* (6mm) *Bacillus substilis* (4mm), *Vibrio cholera* (2mm), *Staphylococcus aureus* (3mm). The most number of anti-bacterial activities were resulted in aqueous and acetone extract. The maximum number of anti-bacterial activities provided the results in benzene and chloroform extract. The lowest number of anti-bacterial activities resulted in petroleum ether extract.

This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. It is important to investigate scientifically these plants, which have been used in traditional medicines as potential sources of novel antimicrobial compounds (Hema *et al*., 2013).

The results obtained from the above data represents the fact that the plant materials with organic solvents were exhibited significant anitimicrobial activities,

especially the solvent chroloform and aqueous showed inhibitory activities against all test pathogens. In those two solvents, chloroform extracts were showed good significant inhibitions. The difference in the composition of the crude extracts is likely to be due to the varying degrees of solubility of the active constituents in the four solvents used. Different solvents have been reported to have the capacity to extract different phyto constituents depending on their solubility or polarity in the solvent (Marjorie, 1999). Other studies reported that most active constituents are mainly aromatic or saturated organic compounds which have better solubility in organic solvents (Marjorie, 1999). Also the gram positive bacteria were more susceptible than the gram negative bacterium especially *E. coli* and *Vibrio cholera.*

FT-IR Result and discussion

Carica papaya Linn leaves are promising in providing wide variety of phyto-constituents for various herbal formulation using in the current era. Previously useful study was conducted for diagnostic setting for authentication and identification in making profile of *Carica papaya* plant (Zunjar, 2011).

Development of quality standards of leaves of *Carica papaya* Linn were also studied and established (Anjum *et al*., 2013) to confirm purity and authenticity of leaf of *Carica papaya* L. on the basis of guidelines of WHO. This study showed that leaves are more rich in various compounds as compared to male leaves.

Fourier Transform Infrared Spectrophotometric analysis was also carried out to evaluate various functional groups. Aromatic Carbonyl group is dominating in female leaves while O–H group is dominating in male leaves as indicated by FTIR. On the basis of results obtained in the current study, it can be concluded that the leaf of plant showed more O-H group indicating presence of more phenolic or acidic contents. FTIR Analysis also reported the characteristic functional groups like

carboxylic acids, amides, polysaccharides, aldehydes etc. responsible for various medicinal characteristics of the plants.

FT-IR was used to analyse the functional group present in *Carica papaya* Leaf and fruit. The FTIR spectroscopy analysis of *Carica papaya* leaf obtained peaks at516.89 cm $^{-1}$, 636.47 cm $^{-1}$,665.4 cm $^{-1}$, 780.5 cm $^{-1}$, 890.09 cm $^{-1}$, 1080.88cm $^{-1}$,1108.03cm $^{-1}$, 1242.07 cm $^{-1}$,1317.29cm $^{-1}$, 1379.01 cm $^{-1}$, 1455.19 cm $^{-1}$, 1512.09 cm $^{-1}$, 1545.84 cm $^{-1}$, 1648.24 cm $^{-1}$, 1844.79 cm $^{-1}$, 2360.71 cm $^{-1}$, 2848.67 cm $^{-1}$ 2920.03 cm $^{-1}$, 3443.66 cm $^{-1}$, 3738.75 cm $^{-1}$. These absorption peaks are known to be associated with the vibration for O-H in aromatic amines, H-C-=O in Aldehydes, C=O in Carbonyls, C=C in Alkylnes, C-N in Aliphatic amines, C-H in Alkenes, C-Br in Aromatic compound (Table. 11).

The FTIR spectroscopy analysis of *Carica papaya* fruit obtained peaks at 517.85 cm $^{-1}$, 634.54 cm $^{-1}$,704.93 cm $^{-1}$, 778.22 cm $^{-1}$, 818.73 cm $^{-1}$, 865.98cm $^{-1}$,919.02cm $^{-1}$, 1058.85 cm $^{-1}$,1243.04cm $^{-1}$, 1317.29 cm $^{-1}$, 1339.47 cm $^{-1}$, 1362.61 cm $^{-1}$, 1420.48 cm $^{-1}$, 1456.16 cm $^{-1}$, 1510.16 cm $^{-1}$, 1545.84 cm $^{-1}$, 1645.35 cm $^{-1}$ 1747.39 cm $^{-1}$, 1845.75 cm $^{-1}$, 2317.31 cm $^{-1}$ 2684.73 cm^{-1}, 2934.49 cm^{-1}, 3397.38 cm^{-1}, 3754.18 cm^{-1}. These absorption peaks are known to be associated with the vibration for O-H in aromatic amines, H-C-=O in Aldehydes, C=O in Carbonyls, C=C in Alkylnes, C-N in Aliphatic amines, C-H in Alkenes, C-Br in Aromatic compound (Table 14)

From the spectral data presence of C-H,C-Cl, C-Br, C-O, N-O C-H-O H-C-O, C=H, C=O, H-C=O were identified. These bonding are responsible for the presence of sulphinic acid, amine group, aldehyde group, aromatic group, nitro group, ether, carboxylic group, and aliphatic group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing fever (Dengue), head ache, treatment

of rheumatic joint pain. Amides, amino acid are the main groups which are involved in protein synthesis. The study revealed that the *Carica papaya* leaf and fruit contain a considerable amount of secondary metabolites and it may considered in future to be used human disease management.

SUMMARY AND CONCLUSION

India is encountered with vast variety of vegetation having medicinal value. These plants are commonly used by people from all walks of life, either as folk remedies or as medicinal preparations for modern medicine. Phytochemical with biological activity have a lot of applications in term of pharmaceuticals and pharmacological effects. The preliminary phytochemical studies exposed the presence of phytoconstituents such as flavonoids, alkaloids, phenols, saponins and tannin. In this study the leaf of *Carica papaya* has a various chemical group in their chemical composition. It exposed some differences in the constituent of the one part of the plant tested. Alkaloids and tannins were found in all five extracts of *Carica papaya*. Hence this plant should be evaluated further to assess its Phyto therapeutic properties.

The antibacterial activity of *carica* has reported in different solvents. The plant parts of *Carica papaya* leaves, root, stem, and seed, showed good activity against dermatophytic and pathogenic bacteria. Traditional medicines are always at the centre of attention to cure various ailments. *Carica papaya* is native plant of India and all the parts of this plant have been medicated. Various crude fractions and purified components have shown potential medicinal and pharmacological activities. The antioxidant and free radical scavenging activities of phyto-components isolated from this plant give us an impression that the plant might be the future drug for diversified panel of tumors and cancers.

In this study, chloroform and aqueous extracts showed maximum antimicrobial activity. Petroleum ether, acetone and benzene showed the least antibacterial activity, suggestive of the active compounds having antimicrobial potential be extracted using appropriate solvent. This research gives a scientific validation to the fact that bioactive components in the plant *Carica papaya* are

exhibited highly promising antibacterial and inhibitory activity. This study revealed that the herbal medicine can be as effective as modern medicine to combat pathogenic microbes. Using different purification, isolation and characterization methods, antimicrobial principals can be obtained and thus the activity of antimicrobial compounds can be improved for further pharmaceutical uses.

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PHYTOCHEMISTRY, FTIR, GC-MS ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF CITRUS PEELS

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By

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CERTIFICATE

This is to certified that this dissertation entitled, **'PHYTOCHEMISTRY, FTIR, GC-MS ANALYSIS, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF CITRUS PEELS'** submitted by **CHITRA S Reg.No. 20APBO02** to ST. MARY'S COLLEGE (Autonomous) Thoothukudi - in partial fulfillment for the award of the degree of **'Master of Science in Botany'** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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CONTENTS

INTRODUCTION

INTRODUCTION

Nature has been bestowed upon as a vast botanical wealth with a large number of diverse plant species growing in various part of the world. Fruits and vegetables had conferred to be capable of delivering health benefits besides fulfilling physiological needs (kaur and Kapoor, 2001). Among various fruits that are consumed, citrus fruits are widely used in almost all countries. *Citrus* fruits are rich sources of bioactive compounds having beneficial effect on human health such as vitamin C, carotenoids, flavonoids, limonoids, essential oils, alkaloids, minerals and vitamin B complex. The peel of citrus fruits is an important byproduct of citrus processing industries. A large amount of peel is produced and is considered as waste. The citrus peels contain high quantity of phenolic compounds including several flavonoid compounds. The citrus peel extracts and essential oils are known to exhibit various biological activities such as antimicrobial and antioxidant activities.

Citrus fruits make up the largest sector of the world's fruit production, with more than 100 million tons produced each season. About 34% of citrus fruits are made into juices; therefore, large amounts of residues are formed every year3. Citrus peels, which comprise the dominant residue, exhibit potent antioxidant, antimicrobial and anti-inflammatory activities, and are considered potential sources of functional components. Except for ascorbic acid, citrus peels contain more bioactive compounds, such as phenolic acids, flavonoids, limonoids, and fibre than do juices (Lin *et al*., 2008)

Among the well-known citrus bioactive compounds, flavonoids, especially the citrus unique polymethoxy flavones and flavanone glycosides, attract considerable attention for their significant biological activities. Due to their high flavonoid content, citrus peels could be exploited by both pharmaceutical and food industries. In spite of this, the compounds present in

citrus peel are usually processed as by-products or wasted, resulting in environmental pollution. One of the main reasons for this is the absence of effective extraction procedures to obtain the flavonoids from the citrus peels (Kumar *et al* ., 2011).

Citrus peel is used to treat a variety of microbial and non-microbial disease as a consequence of their beneficial impact in health care. Plants have produced a bio active molecule, making them a rich source of different types of Medicine. Natural products have an important role to play in drug development programme in the pharmaceutical industry. In comparison of synthetic drugs citrus peel contain numerous phytochemicals with enormous therapeutic value and are considered natural and healthy since many infectious microorganisms are immune to a synthetic drug microbial pathogenesis is critical for control. Long before prehistoric period plants have been used for medicinal purpose. Now a days, we move away from nature as our lifestyle become more techno savvy, but we cannot avoid nature because we part of it. Because nature herbs are free of side effects, they are relatively healthy, eco - friendly and locally accessible. They must be promoted in order to keep human life safe compared to synthetic drug, that are considered to be hazards to humans and the environment and herbal products have become a sign of production. While herbs have been valued for their medicinal flavoring and aromatic qualities for centuries, synthetic products from the modern era have temporarily eclipsed their signification. However, the era of blind dependence on synthetic materials is over and people are turning back to the natural world in the hope of protection and security. *Citrus* fruits are cultivated in more than 100 countries, making them as one of the most important commercial fruit crops of the world. Mandarins (*Citrus reticulata* L. Blanco), one of the ancestral species of *Citrus*, are widely grown in the tropical and subtropical areas. They are a good source of vitamin C and are usually eaten plain or in fruit salads. Among the citrus fruits,

mandarin has gained high popularity and is commercially cultivated for its processing quality, fresh consumption and aromatic flavor. The peel of C. *reticulata* exhibits antimutagenic , anti-inflammatory , antioxidant , antitumor and antiatherosclerosis functions. *Citrus aurantifolia* (family: Rutaceae) is mainly used in daily consumption, in many cultural cuisines, and in juice production. It is widely used because of its antibacterial, anticancer, antidiabetic, antifungal, anti-hypertensive, anti-inflammation, anti-lipidemia, and antioxidant properties; moreover, it can protect heart, liver, bone, and prevent urinary diseases. Its secondary metabolites are alkaloids, carotenoids, coumarins, essential oils, flavonoids, phenolic acids, and triterpenoids. The other important constituents are apigenin, hesperetin, kaempferol, limonoids, quercetin, naringenin, nobiletin, and rutin, all of these contribute to its remedial properties.

C. aurantium has a thick peel that is richer in pectin than the sweet orange peel and that contains higher amounts of essential oils when compared with other *Citrus* species. The essential oil content in *C. aurantium* peels ranged between 0.1 and 1.7% with limonene being the most abundant volatile component (Bendha *et al*., 2016). Details of compounds found in Italian *C. aurantium* peel were provided as follows: monoterpene hydrocarbons representing 72.5% while oxygenated monoterpenes representing 7% with the major component limonene (66%) (Ben Hsuano *et al*., 2019). Recently, a slightly different chemical composition of the essential oils of *C. aurantium* peel was reported. The major volatile components identified were monoterpene hydrocarbons (51%) and oxygenated monoterpenes (46%)—mainly: limonene (49%), linalool (32%), linalyl acetate (12%), myrcene (1.2%), geranial (1%), neral (0.5%), β-pinene (0.5%), γ-terpinene (0.4%), sabinene (0.3%), geranyl acetate (0.2%), and β-caryophyllene (0.1%) (Hosseini *et al*., 2019). *Citrus* genus is a prominent staple crop globally. Long-term breeding and much hybridization engendered a myriad of species, each characterized by a specific

metabolism generating different secondary metabolites. *Citrus aurantium* L., commonly recognized as sour or bitter orange, can exceptionally be distinguished from other *Citrus* species by unique characteristics. It is a fruit with distinctive flavor, rich in nutrients and phytochemicals which possess different health benefits. Hence this present study on phytochemical analysis, antioxidant, antioxidant activity, antibacterial, FTIR and GC- MS analysis of *Citrus* peel

INTRODUCTION

Scope and objectives

Citrus fruit is the most abundant fruit that grows worldwide and contains very rich amounts of phytochemicals and bioactive compound. *Citrus* fruit (Hesperidium) is a plant belonging to the Rutaceae family. They are a rich source of Vitamin C, A, E alkaloids and flavonoids, and other minerals. *Citrus* plants originated from tropical, subtropical and East Asia and it is consumed all over world as an rich source of Vitamin C and other minerals and good source of vitamin A and contains a powerful natural antioxidant antiviral, antifungal and antibacterial activity that builds the strong body immune system. Citrus fruit peels are recognized as being a healthful source of bioactive compounds polyphenols, dietary fibre, essential phenolics, and ascorbic acid. Citrus peels enhance the antimicrobial activity of harmful bacteria, fungi, and viruses. Citrus fruits are rich sources of useful phytochemicals, such as vitamins A, C, and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins and other compounds . These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions, including antioxidant, anti inflammatory, anti-mutagenicity, anti-carcinogenicity, and anti-aging. For this reason I have chosen of some of the selected Citrus peels. I hope this study is helpful and create awareness of our people. However it is essential to work on locally available resources to bring out their pharmaceutical values. Hence the present investigation was undertaken with following objectives:

- Evaluating the phytochemical analysis of selected citrus peels (*Citrus aurantifolia*, *Citrus reticulata* and *Citrus aurantium*).

- Elucidating the effectiveness of Citrus peel in controlling human pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Vibrio chlorae* and *Staphylococcus aureus*
- Quantification of antioxidant activity of selected Citrus peel (*Citrus aurantifolia*, *Citrus reticulata* and *Citrus aurantium*).
- To identify the functional groups present in the Citrus peels (FTIR and GC- MS)

REVIEW OF LITERATURE

LITERATURE REVIEW

Plants are a source of many potent and strong drugs that are used medicinally in various countries (Conner *et al*.,2017) . Plants play a significant role in human lives, especially in food and medicine. Many drugs are found and derived from plants. All parts of the plants can be used for medicinal purposes, but mostly the part of the plant extracted as a drug is the fruits. Fruits are known to be a source of nutrients, and it is recommended to consume them daily to get physiological benefits for the body.

Citrus aurantifolia is a well known fruit that is usually used in beverages and food. It is also known as 'Mexican lime'. Its use in medicinal have long been known. Contrary to its common name, this fruit is distributed and cultivated in tropical and subtropical countries, not limited to only one country (CABI, 2019). Usually, key lime is known for its rich vitamin content and its antioxidant properties (CABI, 2019). In fact, studies about key lime have been done, with topics surrounding various pharmacological effects, including antimicrobial, anti-cholesterol, antiinflammatory, insecticidal, and even anticancer properties . The plant is believed to have originated from East Asian origin, particularly in northern Malaysia, or northern India, next to Myanmar, which was then brought to North Africa, Palestine, and to Mediterranean Europe by Arabs for trading. Then, the fruit is cultivated in tropical and subtropical countries, because most limes are tolerant to drought and prefer warm temperatures to grow (Cyndi *et al*., 2017)

Citrus reticulata Blanco is a large species belonging to the family Rutaceae, with various varieties and hybrid . It includes popular citrus types such as Satsumas, Clementines, Tangerine and the Mediterranean mandarin . Tangerine is a group of orange-coloured citrus fruits consisting of mandarin hybrids–, although the term tangerine is used interchangeably with

mandarin.Mandarins, like other citrus species, are indigenous to the subtropical and tropical zones of Asia, particularly China and Cochin-China. Some researchers have reported that mandarins, alongside other citrus species, evolved in a region including Vietnam, South China, India and Japan. They are now widely cultivated around the world in the warm temperate and tropical areas. Mandarins account for 22–25 per cent of world citrus production among the commercially cultivated citrus species. The major citrus growing regions of the world and their estimated. The edible part of the raw mandarin fruit possesses antioxidants such as vitamin C, carotenoids and phenolic compounds. The fruit is also a rich source of amino acids, sugars, organic acids, amino acids, pectins, minerals and volatile organic compounds. These constituents are essential for the proper functioning of the body by protecting it against chronic diseases and providing basic nutrition. The dietary fibre and phenolic compounds in mandarins are useful in the formulation of functional food. Mandarin fruit also contains coumarins, for instance, bergapten which sensitizes the skin to sunlight.(*Khan et al .*, 2017*)*

The fruit has been reported to possess laxative, aphrodisiac, antiemetic, astringent and tonic properties. While the fruit peel regulates skin moisture, softens hard and rough skin and cleanses oily skin.Traditionally, it is also used as a stomachic and carminative. Both the pericarp and endocarp are anticholesterolemic, analgesic, antiseptic, antiasthmatic, anti-inflammatory, antiscorbutic, antitussive, carminative, expectorant and stomachic. Therefore, they are used in the treatment and management of dyspepsia, gastro-intestinal distension, cough with profuse phlegm, hiccup and vomitin. The unripened green exocarp is used in the treatment of chest pains and hypochondrium, gastro-intestinal distension, swelling of the liver and spleen and cirrhosis of the liver. The seed is analgesic and carminative, thus used in the treatment of hernia, lumbago, mastitis and pain or swellings of the test.

Citrus aurantium the primitive center of origin of citrus species has been a subject of speculation and discussion for some time. The most recent research indicates an origin in Australia, New Caledonia and New Guinea. Some researchers believe that the origin is in the part of Southeast Asia bordered by Northeast India, Burma (Myanmar) and the Yunnan province of China. Now, a worldwide cultivation and high demand production for citrus fruit make it stand high among fruit crops. Growth of the citrus industry, including rapid development of the processing technology of frozen concentrated orange juice after World War II, has greatly expanded with international trade and steadily increased consumption of citrus fruits and their products during the past several decades. Because of distinct aroma and delicious taste, citrus fruits have been recognized as an important food and integrated as part of our daily diet, playing key roles in supplying energy and nutrients and in health promotion. Citrus fruits are characterized by low protein and very little fat content, citrus fruits supply mainly carbohydrates, such as sucrose, glucose, and fructose. Fresh citrus fruits are also a good source of dietary fiber, which is associated with gastrointestinal disease prevention and lowered circulating cholesterol. In addition to vitamin C, which is the most abundant nutrient, the fruits are a source of B vitamins (thiamin, pyridoxine, niacin, riboflavin, pantothenic acid, and folate), and contribute phytochemicals such as carotenoids, flavonoids, and limonoids. These biological constituents are of vital importance in human health improvement due to their antioxidant properties, ability to be converted to vitamin A (for example, βcryptoxanthin), and purported protection from various chronic diseases. All these characteristics enhanced worldwide citrus fruit cultivation .

Onyeagba *et al*., 2004 studied the antimicrobial effect *in vitro* of aqueous and ethanolic extracts of lime (*Citrus aurantifolia* Linn.) juice were assayed against *Staphylococcus aureus*; *Bacillus* spp., *Escherichia coli* and *Salmonella* spp. All the test organisms were susceptible to undiluted

lime-juice. The aqueous and ethanolic extracts of garlic and ginger singly did not inhibit any of the test organisms. The highest inhibition zone of 19 mm was observed with an combination of extracts on Staphylococcus aureus. Salmonella spp were resistant to almost all the extracts except lime.

Akinnibosun *et al.*, 2015 investigated the comparatively study the phytochemical and antimicrobial properties of *Bryophyllum pinnatum* and *Citrus aurantifolia* leaf extracts and their synergy. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, *Aspergillus niger*, *Mucor mucedo*, *Penicillium notatum* and *Candida albicans*, were used as test organisms and the antimicrobial activity of the extracts was determined by the agar-well diffusion method. Extracts of *C. aurantifolia* were more effective against the test organisms than *B. pinnatum* extracts,except the aqueous extract. Synergistic antifungal activity of the aqueous extract was 0.0 ±0.0 mm for all the test fungi, the synergistic antifungal activity ranged from 8.7 ± 0.6 mm to 14.0 ± 0.9 mm, 10.0 ± 0.9 mm to 21.7 ± 0.6 mm and 0.0 ± 0.0 mm to 20.0 ± 0.6 mm for the ethanol, methanol and acetone extract respectively. Larger zones of inhibition were observed in the methanol extract of the synergy than the other extracting solvents. The synergy gave higher zones of inhibition neither B. pinnatum extract nor *C. aurantifolia* extract could give. It was also observed that the extracts compared well with the standard antimicrobial agents used as positive control. The phytochemical analysis of the extract

revealed the presence of phytochemical constituents which conferred antimicrobial property

on the plants. From the foregoing, the methanol extract of the synergy is considered the

most effective in the treatment of infections caused by the test organisms.

Oboh and abulu *et al* ., 1997 studied the antibacterial effects of extracts from *C. aurantifolia* (lime) leaves were demonstrated *in vitro* against some Gram positive and Gram negative bacteria. An ethanolic extract from *C. aurantifolia* exhibited mean zones of inhibition of l 4.2mm, 9.2mm, 8.5mm, 8.1 mm and 7 .2mm on *Bacillus subtilis*. *Salmonella* sp, Escher*ichia coli, Streptococcus faeralis*, and *Staphylococcus aureus* respectively. The water extracts exhibited mean zones of inhibition of 13.7m and 9.7mm on£. coli and P. guajava were bactericidal against Sal111011e/la sp and *E. Coli* with mean zones of inhibition of 15.2mm and 13, 9mm respectively. Results from this study provide evidence for the medicinal values of the tested plants and thus their therapeutic utilization singly or jointly by patients with fevers and gastrointestinal disorders.

Rupali ghosh and Nazia hoque *et al* ., 2020 investigated antioxidant, antimicrobial and cytotoxic effects of petroleum ether, chloroform and hydromethanol fraction of methanol extract of *Citrus aurantifolia* peel. Preliminary phytochemical screening of the fractions was done following the standard procedure. Antioxidant activity was measured using DPPH free radical scavenging assay besides measuring total phenolic and flavonoid content using Folin-Ciocalteu reagent and aluminum trichloride method, respectively. The antimicrobial activity was conducted by disc diffusion method and cytotoxic activity was determined by brine shrimp lethality bioassay. The results of phytochemical screening were indicative of the presence of steroids, alkaloids, saponins, glycosides, flavonoids in the fractions. A dose dependent scavenging activity was observed in DPPH radical scavenging assay where chloroform fraction demonstrated the highest activity with IC50 value of 153.68 ± 3.60 µg/ml. The highest phenolic content was observed in chloroform fraction (308.0 ± 6.55 mg/g gallic acid equivalent) and highest flavonoid

content was found in hydromethanol fraction (132.66 ± 2.36 mg/g quercetin equivalent). The chloroform fraction showed excellent antibacterial activity against all the tested bacteria where highest zone of inhibition (19 mm) was produced against Bacillus cereus. In brine shrimp lethality bioassay, LC50 values for petroleum ether, chloroform and hydromethanol fractions were 367.39 µg/ml, 228.14 µg/ml and 296.52 µg/ml, respectively. The present findings suggested that *C. aurantifolia* peel could be a potent source of medicinally important secondary metabolites and further investigations can be done to identify those active compounds responsible for such bioactivity.

Sameer and Mohamed, 2017 investigated antimicrobial activity of (*Syzygium aromati*-cum) and lemon (*Citrus* aurantifolia) against standard microorganism. performed by cup-plate agar diffusion method against Five standard bacteria species: two Gram-positive Bacteria *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), Three Gram-negative bacterial strains including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) a *Salmonella* ty-phi (6539), one standard fungal strains, *Candida albicans* (ATCC 7596). The oil solution results of *S. aromaticum* exhibited inhibitory effects against most of the tested organisms with the zone of inhibition ranging from (10 to 32 mm). The most active effect observed against *C. albicans* (ATCC 7596) (32 mm) on the other hand the lemon oil peels showed antimicrobial activities against all tested microbes (11 - 20 mm) the highest inhibition zone was observed against B. subtilis (NCTC 8236) (20 mm) in 100 mg/ml concentration for each test control positive Gentamicin and Nystatin with zone of inhibition ranging from (14 to 35 mm) against all strains tested. These findings provide scientific evidence to support the traditional medicinal uses of these extracts and indicate a promising potential of these plants for medicinal purposes.

Lucia galovicova and Petra borotova ., 2022 evaluated the biological activity of *Citrus aurantifolia* essential oil (CAEO) with emphasis on antioxidant, antimicrobial, and insecticidal activity, chemical composition, and the antimicrobial effect of its vapor phase in situ on various food models. We determined the main volatile components of CAEO as α-phellandrene (48.5%) and p-cymene (16.5%). The antioxidant activity was high and reached 74.5 ± 0.5%, which corresponds to 442 ± 2.3 TEAC. The antimicrobial activity in the contact phase was most pronounced against Gram-negative bacteria, with inhibition zones of 12.66–15.33 mm and a minimal inhibition concentration of 2.36–8.26 μL/mL. The antimicrobial activity of the CAEO vapor phase was high at the highest concentration tested (500 μL/mL), but the inhibitory effect was seen at all concentrations tested. The effect was observed on all types of microorganisms and all types of model foods. Based on the findings, CAEO could find use in storing and extending the shelf life of agricultural products. Insecticidal activity reached 10–90% depending on the concentration used. The significant insecticidal effect provides the possibility of using CAEO as a natural insecticidal, larvicidal, or repellent preparation.

Farhana Rumzum Bhuiyan and Mahmudul hasan, 2019 studied the three different species of Citrus (*Citrus limon*, *Citrus aurantifolia* and *Citrus macroptera*) fruits pulp extracts were allowed to antimicrobial screening against pathogenic bacteria viz., *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella sp*., *Pseudomonas sp*. and *Salmonella* sp. Ethanol extract of *Citrus macroptera* showed highest zone of inhibition (14±0.25mm) against *Klebsiella* sp. and methanol extract of *Citrus aurantifolia* showed highest zone of inhibition (8 ± 0.56 mm) against *Salmonella* Sp. Ethanol extract and methanol extracts of *Citrus* fruits are more effective against pathogenic bacteria. Among different extraction methods, water bath method showed better result than soaking method. So it could be assumed that *Citrus* fruit may release more bioactive

compound in water bath method rather than soaking method. Qualitative phytochemical screening revealed the presence of various active phytoconstituents in the ethanolic extract of three Citrus species.

Bukola and Olubusola, 2016 investigated the phytochemical composition and comparative evaluation of antimicrobial activities of the crude juice extract and biosynthesized Silver nanoparticle (SNPs) from Citrus aurantifolia juice. The juice extract contained bioactive compounds such as flavonoids (710mg/100g), tannins (525mg/100g), phenols (65mg/100g) and terpenes (56mg/100g). Changes in colour, UV-Vis Spectroscopy at 300-550nm ranges and

FTIR revealed the functional groups present in the biosynthesized SNPs. The crude extract and SNPs exhibited varying antimicrobial activities against some selected pathogens including Streptococcus pyogenes ATCC 19615, *Klebsiella pneumoniae* ATCC 10031, *Bacillus* sp, *Actinobacillus* sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The crude extract has more antibacterial potential against the tested pathogens than the biosynthesized SNPs. The crude extract also had higher antimicrobial activities against *Streptococcus pyogenes* which were resistant to ciprofloxacin. The result revealed that the crude extract was more effective than the SNPs produced and the Minimum Inhibitory concentration (MIC) also showed increasing activities with an increase in the concentration of the juice extract and SNPs. Crude extract of *Citrus aurantifolia* contain bioactive compound with potent antimicrobial potential and the

extract was more effective than the biosythesized SNPs.

Rajkumari et al ., 2016 studied Citrus plants belonging to the family Rutaceae which include fruits such as orange, mandarin, lime, lemon, sour orange and grape fruit appear as a well known promising source of multiple beneficial nutrients for human beings. Processing of citrus by-products potentially represents a rich source of phenolic compounds and dietary fibre, owing to

the large amount of peel produced. These citrus fruit residues, which are generally discarded as waste in the environment, can act as potential nutraceutical resources. Due to their low cost and easy availability such wastes are capable of offering significant low-cost nutritional dietary supplements. The utilization of these bioactive rich citrus residues can provide an efficient, inexpensive, and environment friendly platform for the production of novel nutraceuticals or for the improvement of older ones. This review systematically summarized the potential components present in citrus peel, which generally discarded as waste.

Balakrishnan shanmuga samy *et al* ., 2020 studied Citrus fruit and in particular flavonoid compounds from citrus peel have been identified as agents with utility in the treatment of cancer. This review provides a background and overview regarding the compounds found within citrus peel with putative anticancer potential as well as the associated in vitro and in vivo studies. Historical studies have identified a number of cellular processes that can be modulated by citrus peel flavonoids including cell proliferation, cell cycle regulation, apoptosis, metastasis, and angiogenesis. More recently, molecular studies have started to elucidate the underlying cell signaling pathways that are responsible for the flavonoids' mechanism of action. These growing data support further research into the chemopreventative potential of citrus peel extracts, and purified flavonoids in particular. This critical review highlights new research in the field and synthesizes the pathways modulated by flavonoids and other polyphenolic compounds into a generalized schema.

Hafizu *et al*., 2017 examined the composition and antimicrobial activities of citrus peel essential oils. Citrus peels are the waste product of fruit processing industry rich in essential oil (EO) with many phytochemical compounds of excellent antimicrobial properties against many bacteria and fungi. The citrus peel EOs has been widely used in pharmaceuticals, foods and other

industries as preservatives, and its generally regarded as safe (GRAS). The extract can be obtained through extraction different techniques such steam and hydro-distillation. The components of the EOs are important and their quality depends on the qualitative and quantitative characteristic of the oils. Equipment such as gas chromatography (GC)-flame ionization detector (FID) and GC-MS are widely used for their analysis. The growing concern on the serious microbial spoilage and the interest in shifting from synthetic to natural antimicrobial agents leads to research and screening of plant and other vegetable sources to identify new compounds for the manufacturing and industrial uses. Although the antimicrobial properties of EOs have been reviewed extensively, the antimicrobial properties of citrus peels oil have not been extensively discussed. The hydro-distillation methods appeared to be more widely used by many researchers, perhaps due to its efficiency, inexpensive and yield of excellent quality EOs.

Nadia bashir *et al* ., 2018 studied the processing of citrus by-products potentially represents a rich source of phenolic compounds and dietary fibre, owing to the large amount of peel produced. These citrus fruit residues, which are generally discarded as waste in the environment, can act as potential nutraceutical resources. Due to their low cost and easy availability such wastes are capable of offering significant low-cost nutritional dietary supplements. The utilization of these bioactive rich citrus residues can provide an efficient, inexpensive, and environment friendly platform for the production of novel nutraceuticals or for the improvement of older ones. This review systematically summarized the potential components present in citrus peel, which generally discarded as waste.

Amritpal kaur et al ., 2020 studied Citrus peel (CP) forms around 40–50% of the total fruit mass but is generally thought to be a waste. However, it is a substantial source of naturally occurring health enhancing compounds, particularly phenolic compounds and carotenoids. Phenolic

compounds in CP mainly comprise phenolic acids (primarily caffeic, p-coumaric, ferulic and sinapic acid), flavanones (generally naringin and hesperidin) and polymethoxylated flavones (notably nobiletin and tangeretin). It has also been noted that CP's contain more amounts of these compounds than corresponding edible parts of the fruits. Phenolic compounds present in CP act as antioxidants (by either donation of protons or electrons) and protect cells against free radical damage as well as help in reducing the risk of many chronic diseases. Owing to the more abundance of polyphenols in CP's, their antioxidant activity is also higher than other edible fruit parts. Therefore, peels from citrus fruits can be used as sources of functional compounds and preservatives for the development of newer food products, that are not only safe but also have health-promoting activities. The present review provides in-depth knowledge about the phenolic composition, antioxidant potential and health benefits of CP.

Ghoul *et al* ., 2016 studied citrus peel is rich in functional ingredients such as essential oils (0.6–1%), fibers (6.30–42.13 g/100 g db), phenols (0.67–19.62 g/100 g db), and vitamin C (0.109–1.150 g/100 g db). Flavanones (hesperidin: 0.002–80.90 mg/g db, neohesperidin: 0.05–11.70 mg/g db, narirutin: 0.03–26.90 mg/g db; naringin: 0.08–14.40 mg/g db), and polymethoxylated flavones (sinensetin: 0.08–0.29 mg/g db, nobiletin: 0.20–14.05 mg/g db, tangeretin: 0.16–7.99 mg/g db) are the main phenolic compounds (PCs) of citrus peel. Due to their antioxidant activity, PCs are used in various applications such as formulation of healthy food, cosmetic, and pharmaceutical products. PCs present sensitivity to process operating conditions (during juice processing and further thermal and nonthermal processing). This review summarizes the main publications dealing with the proximate chemical composition, the functional properties, and the potential applications of the main citrus peel compounds. The effects of conventional and nonconventional processing on PCs of citrus fruits and their derived and coproducts are

analyzed. The information provided in this review allows a better choice of appropriate processes and their optimal operating conditions for a better retention of antioxidants in citrus products.

Lorie Hamelin *et al* ., 2021 reported essential oil (EO) extractions from citrus peel wastes (CPW), including harmonized data on the various citrus species and cultivars. Harmonization is vital to enable sustainable management practices. The review only includes eco-efficient extraction techniques. In total, the review contains 66 quantified examples using i) mechanical cold press ii) thermal extraction with water or steam media iii) thermal microwave-assisted extraction iv) other innovative methods (such as ultrasound). The technologies were assessed for their potential use in cascading production to achieve economies of scope, particularly considering the use of extraction residues for subsequent fermentation to produce various products from energy carriers to enzymes. Two techniques were found insufficient for direct use in fermentation. Cold press extracts an inadequate amount of EO (average yield 2.85% DW) to ensure suitable fermentation, while solvent extraction contaminates the residues for its subsequent use. Extractions using water media, such as hydrodistillation and microwave-assisted hydrodistillation (average EO yield 2.87% DW), are feasible for the liquid-based fermentation processes, such as submerged fermentation. Steam extraction is feasible for any type of fermentation. Our review highlighted solvent-free microwave extraction (average EO yield 5.29% DW) as the most effective method, which provides a high yield in a short extraction time. We also uncovered and discussed several inconsistencies in existing yields and energy consumption.

Umar Garba *et al* ., 2017 examined the composition and antimicrobial activities of citrus peel essential oils. Citrus peels are the waste product of fruit processing industry rich in essential oil (EO) with many phytochemical compounds of excellent antimicrobial properties against many

bacteria and fungi. The citrus peel EOs has been widely used in pharmaceuticals, foods and other industries as preservatives, and its generally regarded as safe (GRAS). The extract can be obtained through extraction different techniques such steam and hydro-distillation. The components of the EOs are important and their quality depends on the qualitative and quantitative characteristic of the oils. Equipment such as gas chromatography (GC)-flame ionization detector (FID) and GC-MS are widely used for their analysis. The growing concern on the serious microbial spoilage and the interest in shifting from synthetic to natural antimicrobial agents leads to research and screening of plant and other vegetable sources to identify new compounds for the manufacturing and industrial uses. Although the antimicrobial properties of EOs have been reviewed extensively, the antimicrobial properties of citrus peels oil have not been extensively discussed. The hydro-distillation methods appeared to be more widely used by many researchers, perhaps due to its efficiency, inexpensive and yield of excellent quality EOs.

Nooshin koolagi *et al* ., 2020 studied Citrus fruit and in particular flavonoid compounds from citrus peel have been identified as agents with utility in the treatment of cancer. This review provides a background and overview regarding the compounds found within citrus peel with putative anticancer potential as well as the associated in vitro and in vivo studies. Historical studies have identified a number of cellular processes that can be modulated by citrus peel flavonoids including cell proliferation, cell cycle regulation, apoptosis, metastasis, and angiogenesis. More recently, molecular studies have started to elucidate the underlying cell signaling pathways that are responsible for the flavonoids' mechanism of action. These growing data support further research into the chemopreventative potential of citrus peel extracts, and purified flavonoids in particular. This critical review highlights new research in the field and

synthesizes the pathways modulated by flavonoids and other polyphenolic compounds into a generalized schema.

Loannou *et al* ., 2014 reported in the literature for the extraction of phenols from citrus peel. Extraction methods may cause a degradation of phenolic compounds due to high applied temperature and pressure or long extraction times (conventional solvent extraction, high-pressure extraction). However, other extraction methods are limited by the polarity of phenolic compounds (supercritical CO_2 extraction). Novel techniques of extraction of bioactive compounds have been developed in order to shorten the extraction time, increase the extraction yield, and prevent the degradation of the phenolic compounds. This review provides a critical comparison of the different extraction methods of citrus peel phenolic compounds. The review compiles valuable data that could be useful for the choice of an appropriate extraction method for bioactive compounds from vegetables sources. The main parameters influencing the extraction yield.

Liwen wang *et al* .,2014 studied Citrus is a kind of common fruit and contains multiple beneficial nutrients for human beings. Flavonoids, as a class of plant secondary metabolites, exist in citrus fruits abundantly. Due to their broad range of pharmacological properties, citrus flavonoids have gained increased attention. Accumulative in vitro and in vivo studies indicate protective effects of polymethoxyflavones (PMFs) against the occurrence of cancer. PMFs inhibit carcinogenesis by mechanisms like blocking the metastasis cascade, inhibition of cancer cell mobility in circulatory systems, proapoptosis, and antiangiogenesis. This review systematically summarized anticarcinogenic effect of citrus flavonoids in cancer therapy, together with the underlying important molecular mechanisms, in purpose of further exploring more effective use of citrus peel flavonoids.

Deokyeol jeong *et al* ., 2021 studied the citrus peel waste (CPW), have emerged as a promising and sustainable option for biorefinery without competing with human foods and animal feeds. CPW is largely produced and, as recent studies suggest, has the industrial potential of biological valorization into fuels and chemicals. In this review, the promising aspects of CPW as an alternative biomass were highlighted, focusing on its low lignin content. In addition, specific technical difficulties in fermenting CPW are described, highlighting that citrus peel is high in pectin that consist of non-fermentable sugars, mainly galacturonic acid. Last, recent advances in the metabolic engineering of yeast and other microbial strains that ferment CPW-derived sugars to produce value-added products, such as ethanol and mucic acid, are summarized. For industrially viable CPW-based biorefinery, more studies are needed to improve fermentation efficiency and to diversify product profiles.

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Phenolic compounds in CP mainly comprise phenolic acids (primarily caffeic, p-coumaric, ferulic and sinapic acid), flavanones (generally naringin and hesperidin) and polymethoxylated flavones (notably nobiletin and tangeretin). It has also been noted that CP's contain more amounts of these compounds than corresponding edible parts of the fruits. Phenolic compounds present in CP act as antioxidants (by either donation of protons or electrons) and protect cells against free radical damage as well as help in reducing the risk of many chronic diseases. Owing to the more abundance of polyphenols in CP's, their antioxidant activity is also higher than other edible fruit parts. Therefore, peels from citrus fruits can be used as sources of functional compounds and preservatives for the development of newer food products, that are not only safe but also have health-promoting activities.

Fiza nazir *et al* .,2021 studied to make value added products such as marmalade, jams, juice, jellies, etc. which leads to the generation of waste into peel (rind) and seeds. The main fruit waste i.e. peels and seeds. The peel (skin) can be used for the preparation of sweets.

Dexian zhi *et al*., 2014 studied Citrus is a kind of common fruit and contains multiple beneficial nutrients for human beings. Flavonoids, as a class of plant secondary metabolites, exist in citrus fruits abundantly. Due to their broad range of pharmacological properties, citrus flavonoids have gained increased attention. Accumulative in vitro and in vivo studies indicate protective effects of polymethoxyflavones (PMFs) against the occurrence of cancer. PMFs inhibit carcinogenesis by mechanisms like blocking the metastasis cascade, inhibition of cancer cell mobility in circulatory systems, proapoptosis, and antiangiogenesis.

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Guihua xu *et al* ., 2006 evaluated the samples of the Citrus fruits viz., lemon (*Citrus limon* (L.) Burm. f.), grapefruit (*Citrus paradisi* Macfayden), bergamot (*Citrus* bergamia Risso et Poit.), bitter orange (*Citrus* aurantium L.), sweet orange (*Citrus sinensis* (L.) Osbeck), mandarin (*Citrus reticulata* Blanco) were collected from southern Turkey (Antalya) in November 2006 and their peel oils were obtained by coldpressing process. The antimicrobial activities of Turkish Citrus peel oils were evaluated using the disk diffusion method toward 9 bacteria and the results compared with those for penicillin-g, ampicillin, cefotaxime, vancomycine, oflaxacin and tetracycline. Antifungal activities were reported for Kluyveromyces fragilis, Rhodotorula rubra, Candida albicans, Hanseniaspora guilliermondii and Debaryomyces hansenii yeasts, and the results were referenced against nystatin, ketaconazole and clotrimazole antifungal agents. The Citrus peel oils showed strong antimicrobial activity against the test organisms. Lemon and bergamot peel oils have a little higher activity than the other Citrus peel oils.

Mueen iqbal et al ., 2013 focused on unexplored Pakistani citrus species viz. sweet oranges (*Citrus sinensis* Vars. Jaffa, Blood Red and Mosambi), Mandarins (*Citrus reticulata* Var. kinnow) and grapefruits (*Citrus paradisi* Var. Shamber) for peel oil yield, chemical composition and antipathogen activities. The chemical composition of citrus peel oil was analyzed through

gas chromatographic - mass spectrometric analysis. Six compounds viz. D-limonene, d-carvone, Z-5-nonadecane, thujol, *trans*-P-mentha-2,8-dienol and heneicosane were commonly present in all cultivars. However, D-limonene (40.9–76 %) was a major compound in all citrus peel oils. The high amounts of phenolic compounds were recorded in the peel oil with a maximum amount in grapefruit (8.58 mg/g) and minimum in Kinnow mandarin (5.20 mg/g). Jaffa orange cultivar showed a highest radical scavenging activity (70.14 %). Furthermore, peel oils were tested for their antimicrobial activities against five pathogenic bacterial strains viz. *Staphylococcus aureus, Eschrichia coli, Salmonella typhi, Proteus vulgaris, Staphylococcus epidermidis*and two pathogenic fungal strains viz. *Aspergillus flavus* and *Trichophyton alba*. Citrus peel was found effective against all tested micro-organisms and in particular Jaffa orange essential oil was highly effective against all microbial strains growth and *Salmonella typhi* was the most inhibited strains among all.

Fernando asenjo *et al* . 2005 evaluated some functional properties of fibre concentrates from apple and citrus fruit residues, in order to use them as potential fibre sources in the enrichment of foods, was carried out. Fiber concentrates were analysed for their proximate content (moisture, lipids, protein and ash); caloric value; dietary fibre composition and functional properties (water retention capacity – WRC, swelling capacity – SW, fat adsorption capacity – FAC and texture). All the fibre concentrates had a high content of dietary fibre (between 44.2 and 89.2 g/100 g DM), with a high proportion of IDF. Protein and lipid contents ranged between 3.12 and 8.42 and between 0.89 and 4.46 g/100 g DM, respectively. The caloric values of concentrates were low (50.8–175 kcal/100 g or 213–901 kJ/100 g). Grapefruits had the highest WRC (2.09–2.26 g water/g DM) high SW and FAC. Texture was strongly dependent on the particle size and it was

increased by the heat treatment. Every concentrate studied had interesting characteristics, suggesting possible uses in the development of fibre enriched foods.

Jianchu chen *et al* ., 2007 studied peels of 2 citrus varieties, namely, Satsuma mandarin (*C. unshiu* Marc.) and Ponkan (*C. poonensis* Hort. ex Tanaka), which belong to *C. reticulata*, were selected. As a result, hot water extraction was efficient in extracting phenolic acids and some minerals. As for citrus flavonoids, narirutin, nobiletin, and tangeretin were easier to extract than hesperidin. The result of antioxidant capacity assays indicated that for citrus peels, hot water extract had almost the same capacity as the methanol extract. We suggested that Ponkan was more suitable as the source of *chenpi*, since its hot water extract had much higher content of phenolic acids, FGs and PMFs, and higher antioxidant capacity than those of Satsuma mandarin. Generally, to raise the extraction temperature or to prolong the time could not yield higher content of phenolic compounds and stronger antioxidant capacity, though the content of minerals increased to some extent. Furthermore, a 2nd-time extraction seemed necessary since considerable minerals and phenolic compounds could be obtained by doing so. Finally, we suggested that 2 times extraction at 100 °C for 30 min was proper to extract the minerals and phenolic compounds .

Dong hong liu et al ., 2009 studied seven phenolic compounds of two families including cinnamic acids (caffeic, *p*-coumaric, ferulic, sinapic acid), and benzoic acids (protocatechuic, *p*-hydroxybenzoic, vanillic acid) from citrus (*Citrus unshiu Marc*) peels were evaluated by UAE. The effects of ultrasonic variables including extraction time, temperature, and ultrasonic power on the yields of seven phenolic acids was investigated. Results showed that the yields of phenolic compounds increased with both ultrasonic time and temperature increased, whereas the opposite occurred with increasing time at higher temperature to some certain. In the case of 40 °C, the

decrease in the yields of some phenolic compounds was observed with increased time, whereas those of other compounds did not significantly declined. Ultrasonic power has a positive effect on the yields of phenolic acids under study. Among all ultrasound variables, temperature is the most sensitive on stability of phenolic compounds. Moreover, when phenolic compounds from citrus peel extracts were subjected to ultrasound process, the benzoic acids were more stable than the cinnamic acids. Meanwhile, the optimal ultrasound condition was different one compound from another. These were partly attributed to both the differently chemical structures of phenolic acids and the combination effects of ultrasonic variables.

Seok- moon jeong *et al* ., 2004 identified the antimicrobial compounds and demonstration of antimicrobial activity of lemon (*Citrus lemon* L.) peel against bacteria. As microorganism are becoming resistant to present day antibiotics, our study focuses on antimicrobial activity and future prophylactic potential of the lemon peel. Biologically active compounds present in the medicinal plants have always been of great interest to scientists. The peel of citrus fruits is a rich source of flavanones and many polymethoxylated flavones, which are very rare in other plants. These compounds, not only play an important physiological and ecological role, but are also of commercial interest because of their multitude of applications in the food and pharmaceutical industries. The citrus peel oils show strong antimicrobial activity. The antimicrobial activity has been checked in terms of MIC by using different solvents against microorganisms like Pseudomonas aeruginosa NCIM 2036 for which MIC was 1:20 in presence of methanol, for Salmonella typhimurium NCIM 5021 the observed MIC was 1:20 in presence of acetone. In case of Micrococcus aureus NCIM 5021 the observed MIC was 1:20 when ethanol was used as solvent. The compounds like coumarin and tetrazene were identified by GC/MS of lemon peel extract.

Smith et al ., 2006 evaluated the total phenolic contents of five citrus peels (Yen Ben lemon, Meyer lemon, grapefruit, mandarin and orange) extracted either by ethanol or by simple aqueous extraction were evaluated using the Folin-Ciocalteu assay and compared. The main parameters that affected the yield of phenolics included the condition of the peels, temperature of the extraction, solvent concentration and species of citrus. Generally, grapefruit peel had the highest total phenolic contents, followed by mandarin, Yen Ben lemon, orange and Meyer lemon peel. High extraction (about 74%) was obtained using ethanol as solvent and the percentage extraction could further be increased using a higher temperature of 80 °C. In addition, the total antioxidant activity of the phenolic contents extracted from different citrus peels were investigated using the FRAP assay. The phenolics in grapefruit peels had the highest total antioxidant activity, followed by Yen Ben lemon, mandarin, orange and Meyer lemon.

Hubert richard *et al* ., 1998 investigated the antioxidant properties of selected fresh and frozen peels of Citrus species. Frozen and fresh peels of lemon (*Citrus limon*), key lime (C. *aurantifolia*) and musk lime (*C. microcarpa*) were screened for their antioxidant properties such as total phenolic content and total flavonoid content. DPPH radical scavenging activity and ferric ion reducing antioxidant power (FRAP) assays were also determined. Among the three citrus peels, musk lime peel had the significantly highest total phenolic content and total flavonoid content. Frozen citrus peels showed significantly higher antioxidant content than the fresh peels. The frozen peels also showed promising antioxidant activity as indicated by their significantly higher FRAP value compared with fresh citrus peels. Moreover, frozen citrus peel possessed higher antioxidant activity as indicated by its lower EC50 values which ranged between 0.823 ± 0.1 and 3.16 ± 0.92 mg mL-1. A moderately high correlation was determined between FRAP value and total phenolic content (r=0.783), and between FRAP value and total flavonoid content.

This study shows that frozen peels of citrus are functional foods and sources of potent antioxidants.

Alberto martin *et al* 2015 studied the phenolic compounds, antioxidant capacity and antimicrobial activity of citrus peel extracts. Total phenolic contents (TPC) and antioxidant properties of extracts were determined as free radical-scavenging ability of DPPH and using the ABTS radical cation decolorization assay. Additionally, extracts were tested for antimicrobial activity against twenty different strains of bacteria representing both Gram-positive and Gram-negative types. Citrus peel extracts demonstrated antimicrobial activity against a wide range of bacteria. The maximum level of TPC as well as antioxidant capacity were observed at 300 MPa for 3 min. Citrus peels extracts demonstrated antimicrobial activity against a wide range of microorganisms. The antimicrobial activity of orange peel extract was the highest among the four citrus peels studied. Generally, bacteria *Acinetobacter* and the strain *Listeria innocua* were more sensitive to the peel extracts.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection and processing of the plant materials:

The peels of *Citrus aurantifolia* and *Citrus reticulata* and *Citrus aurantium* were collected from in and around Thoothukudi district in TamilNadu, during December 2021. The peel were collected, and then washed carefully with water to remove dust and foreign materials. The peels were dried under shade and coarsely powdered.

Preparation of plant extracts:

10 grams powdered sample was sequentially extracted with 200 ml of methanol, acetone, ethanol and aqueous solution using in soxhlet apparatus. The prepared extracts were tested for phytochemical screening and anti bacterial activities.

Phytochemical qualitative analysis:

The phytochemical tests were done for analyzing different chemical groups present in the extracts. These were done to find out the presence of bioactive chemical constituents such as alkaloid, flavonoids , tannins, phenol, terpenoids, glycosides, anthroqinone, steroids and saponins. Detection of achieve phytochemical constituents was carried out for all the extracts using the standard procedures (kokatte, 2005; Harborne, 1984)

Test for alkaloids:-

Mayers Test :-

3 ml of extracts was added to 1% HCl and then allowed to steam bath. Few drops of Mayer s reagent was added to the mixture. Turbidity indicates the presence of alkaloids.

Test for Flavonoids :-

Lead acetate Test:-

To 1 ml of extract , 1ml of lead acetate was added . Formation of yellow precipitate showed the presence of flavonoids.

Test for Tannins:-

Ferric chloride Test:-

To 1 ml of extract , 1 ml of distilled water was taken and stirred. Few drops of ferric chloride solution were added to the mixture bluish green colour precipitate showed the presence of tannins.

Detection of Phenols:-

Fecl3 Test :-

About 2 ml of plant extracts was taken and warmed at 45-50 □.Then 2 ml of 0.3% $Fecl_3$ was added. Formation of green or blue colour indicates the presence of phenols.

Test for Terpenoids:-

Salkowski Test :-

About 2 ml of chloroform was added to 1 ml of the extract. Then 3 ml of concentrated H2So4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Glycosides:-

2 ml of extract was dissolved in chloroform and 2 ml of acetic acid was added to the mixture. T he solutions were cooled and then add few drops of sulphuric acid. A colour change from blue to green indicates the presence of glycosides.

Test for Cardiac glycosides:-

Killer- killiani Test:-

1 ml of extract was dissolved in 5 ml of water . 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added .T his was under layer with 1 ml of concentrated sulphuric acid . A brown ring at the interface indicated the presence of a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring , while in the acetic acid layer a greenish ring may from just above the brown ring and gradually spread this layer.

Test for A nthraquinone;-

1 ml of the extract was boiled with 10 ml of sulphuric acid and filtered while hot.The filtered was shaken with added to 5 ml of chloroform .T he chloroform layer was pipetted into another test tube followed by addition of 1 ml of dilute ammonia. T he resulting solution was observed for colour change to violet indicates presence of anthroquinone.

Test for Steroids:-

Salkowski Test:

To 2 ml of extract,was dissolved in chloroform,2 ml of concentrated sulphuric acid was added to the mixture.Red colour formation indicates the presence of steroids.

Test for Saponins:-

Foam Test:-

2 ml of extract dilute with 5 ml of distilled water and warmed.The formation of stable foam indicates the presence of saponins.

Antioxidant activity :

Free radical scavenging assay (Hatano *et al*., 1988)

1ml aliquot of test sample was added to 3 ml of 0.004% DPPH (2, 2-diphenyl-1-picrylhydrazine) solution prepared in methanol. The mixture was vortexed for 1 minute and kept at room temperature for 30 minutes in darkness. The absorbance was read at 517 nm. A low absorbance of the reaction mixture indicated a high free radical scavenging activity.

DPPH scavenging activity (%) = (Acontrol – Atest /Acontrol) x 100

Where, Acontrol is the absorbance of the DPPH solution without test solution, Atest is the absorbance of DPPH with test solution. Methanol was used as blank. Ascorbic acid was used as control.

ANTIBACTERIAL ACTIVITY

Bacterial strains used

The test organisms were obtained from the Department of Microbiology, St. Marys College (Autonomous), Thoothukudi. The two gram positive bacteria viz, *Bacillus subtilis* G +ve , *Staphylococcus aureus* G +ve and two gram negative bacteria *Escherichia coli*, *Vibrio cholera* were used in the present study. *Bacillus subtilis* is responsible for causing food borne gastroententis. *E.coli*, *Staphylococcus aureus* cause diseases like *mastitis*, *abortion* and upper respiratory complications, while *Vibrio cholera* cause disease like cholera.

Disc diffusion assay (Bauer *et al*., 1966)

Antibacterial activity of each peel extract was analysed using human pathogens., Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus* and Gram negative bacteria *Escherichia coli* and *Vibrio cholerae* obtained from the Department of Microbiology; St. Mary's College (Autonomous), Thoothukudi. Each bacterial pathogen was subcultured in agar medium and maintained. What man No. l sterile filter paper discs (5mm) were impregnated with 2.5 mg/ ml and dried aseptically at room temperature. The spread plates were prepared by proper concentration of inocula. Each sample loaded disc was placed in the seeded agar plate. After 24-48 hours of ±37°C incubation, the diameter of the inhibition zone was measured. For positive control, streptomycin disc (100 µg/ ml) was used, whereas for negative control, respective solvents were loaded on sterile discs.

FT-IR (Fourier transforms infra-red spectroscopy) spectroscopic analysis

Ten milligram of *Citrus aurantifolia* , *Citrus reticulata* and *Citrus aurantium* peel powder was mixed with 100 mg of dry potassium bromide (FT-IR grade) and then compressed

into a pellet using hydraulic press (5000-1000 psi).T he pellet was immediately put into the sample holder and FT-IR (Systronics 166) spectra were recorded in the range of 400-4000 cm -1.

GC-MS Analysis (Gas chromatography – Mass spectrometry) (Hema *et al* ., 2010)

GC – MS analysis of the extracts were carried out with GC- MS Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) employing the following conditions : Column Elite -1 fused silica capillary column (30 mm x 0.25 mm ID x1 µmdf, composed of 100% Dimethyl poly silaxane), operating in electron impact mode at 70 eV; Helium (99.999%) was used as a carrier gas at a constant flow of 1 ml / min and an injection volume of 0.5 µl was employed (split ratio of 10:1); injector temperature 250□; Ion – source temperature 280□. The oven temperature was programmed from 110□ (isothermal for 2 min), with an increase of 10□/ min, to 200□ then 5□/ min to 280□ ending with a 9 minute, isothermal at 280□. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Total GC running time was 36 min.

Interpretation on mass spectra of GC- MS was conducted using the database of National Institute of Standard and Technology (NIST). The mass spectra of the unknown compounds were compared with that of the unknown components stored in the NIST library. The name, molecular weight and structure of the components of the best materials were ascertained.

Plate 1: Citrus aurantifolia

Class : Dicotyledons
Sub class : Polypetalae
Series : Disciflorae
Order : Geraniales
Family : Rutaceae
Genus : *Citrus*

Description :

C. aurantifolia is a perennial evergreen tree that can grow to a height of 3–5 m Stem: irregularly slender branched and possesses short and stiff sharp spines or thorns 1 cm or less. Leaves: alternate, elliptical to oval, 4.5–6.5 cm long, and 2.5–4.5 cm wide with small rounded teeth around the edge. Petioles are 1–2 cm long and narrowly winged. Flowers: short and axillary racemes, bearing few flowers which are white and fragrant. Petals are 5, oblong, and 10–12 mm long. Fruits: green, round, 3–5 cm in diameter, it is yellow when rip.

Plate 2: Citrus reticulata

Class : Dicotyledons

Suc class : Polypetalae

Series : Disciflorae

Order : Geraniales

Family : Rutaceae

Genus *:* *Citrus*

Description :

The mandarin orange (*Citrus reticulata*), also known as the mandarin or mandarine, is a small citrus tree fruit. Treated as a distinct species of orange, it is usually eaten plain or in fruit salads. Tangerines are a group of orange-coloured citrus fruit consisting of hybrids of mandarin orange with some pomelo contribution.

Mandarins are smaller and oblate, unlike the spherical common oranges (which are a mandarin–pomelo hybrid). The taste is considered sweeter and stronger than the common orange. A ripe mandarin is firm to slightly soft, heavy for its size, and pebbly-skinned. The peel is thin, loose,with little white mesocarp, so they are usually easier to peel and to split into segments.

Plate 3: Citrus aurantium

Class : Dicotyledons

Sub class : Polypetalae

Series : Disciflorae

Order : Geraniales

Family : Rutaceae

Genus : *Citrus*

Description

Evergreen tree with small thorns; broad, green leaves; and large, white, fragrant flowers. Its spherical fruit have a thick, orange or sometimes green peel. The juicy pulp is orange in colour and can taste sweet or sour.

RESULT AND DISCUSSION

Table 1: Preliminary phytochemical analysis of *Citrus aurantifolia*

(+ : present; - : absent)

S.NO	Phytochemicals Extracts	Ethanol	Acetone	Methanol	Aqueous
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	_
3	Tannins	+	+	+	_
4	Phenols	+	+	+	+
5	Terpenoids	+	+	+	_
6	Glycosides	_	+	_	_
7	Cardic glycosides	_	+	_	_
8	Anthroquinone	_	_	_	_
9	Steriods	+	+	+	_
10	Saponins	+	_	+	_

Table 2 :Preliminary phytochemical analysis of *Citrus reticulata*

(+ :present ; - : absent)

S.NO	Phytochemicals Extracts	Ethanol	Acetone	Methanol	Aqueous
1	Alkaloids	–	–	–	–
2	Flavonoids	–	+	+	+
3	Tannins	–	–	–	–
4	Phenols	–	–	–	–
5	Terpenoids	+	+	+	+
6	Glycosides	+	+	+	–
7	Cardic glycosides	–	–	–	–
8	Anthroquinone	–	–	–	–
9	Steriods	+	+	+	–
10	Saponins	+	–	–	–

Table 2 :Preliminary phytochemical analysis of *Citrus aurantium*

(+ :present ; - : absent)

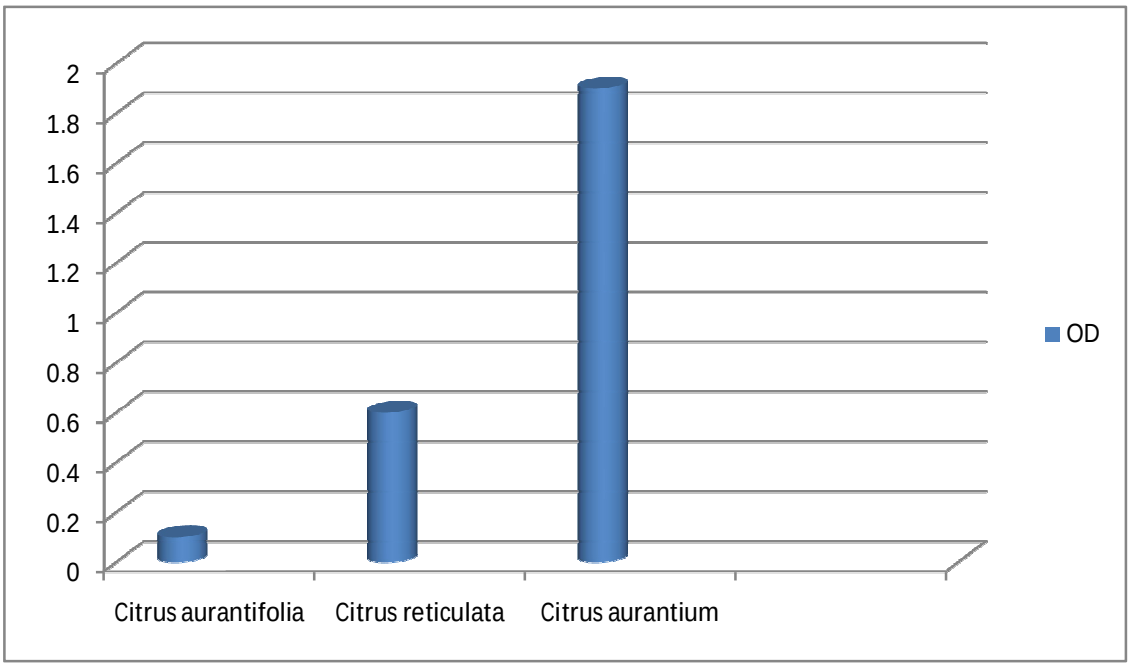
S.NO	Phytochemicals Extracts	Ethanol	Acetone	Methanol	Aqueous
1	Alkaloids	–	–	–	–
2	Flavonoids	–	+	+	+
3	Tannins	–	–	–	–
4	Phenols	–	–	–	–
5	Terpenoids	+	+	+	+
6	Glycosides	+	+	+	–
7	Cardic glycosides	–	–	–	–
8	Anthroquinone	–	–	–	–
9	Steriods	+	+	+	–
10	Saponins	+	–	–	–

Antioxidant activity

Exogenous chemicals and endogenous metabolic processes in human body produce free radicals especially oxygen derived radicals, which are capable ofoxidizing biomolecules, resulting in cell death. DPPH (2, 2-diphenyl-1-picrylhydrazylreagent) has been used extensively for investigating the free radical scavenging activities of compounds. The assay is based on the reduction of alcoholic DPPH (2, 2-diphenyl-1-picrylhydrazyl) in the presence of a hydrogen-donating antioxidant which resulted into the formation of non-radical form DPPH-H (diphenyl-1-picrylhydrazyl) (Shon *et al*., 2003). Dried *Citrus* peel extracts were potentially able to reduce stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical to yellow coloured diphenylpricrylhydrazine. The antioxidants present in soluble fraction of *Citrus* functioned as proton source to reduce DPPH and their reducing ability was compared with ascorbic acid (standard). The antioxidant activities of all the *Citrus* peel were invariably different. Among, three Citrus peel Acetone extract of *C. aurantium* exemplified higher DPPH radical scavenging activity (0.660%). This was followed by aqueous extract of *C. aurantifolia* (0.596%). Acetone extract of *C. reticulata* was noted for their lower radical scavenging ability. Sultana et al (2008) who reported 19.87% and 21.5% extract yield from *Citrus* peels, respectively. Methanol is generally employed for the extraction of antioxidants compounds from plant materials due to their polarity and good solubility with many antioxidant components.

Table 1: Anti-oxidant activity in plant of *in DPPH radical -scavenging activity*

S.No	Plant	DPPH free radical assay
1.	*Citrus aurantifolia*	0.1
2.	*Citrus reticulata*	0.6
3	*Citrus aurantium*	1.9



Antibacterial activity

In the present study, antibacterial activity of three citrus peel extracts (*Citrus aurantifolia and Citrus reticulata and Citrus aurantium)* of four different solvents (ethanol, acetone, methanol and aqueous) were tested against four human pathogenic bacteria (*Escherchia coli, Bacillus subtilis, Staphylococcus aureus* and *Vibrio cholerae).* Three species of Citrus peels were presented in table (4 to 6). The diameter of the inhibition zones against these species ranged from 6 to 17 mm. The study revealed that all extracts inhibited the growth of all the pathogens tested. As shown in Table 4 methanolic extract of *C. aurantifolia* exhibited maximum activity against *S. aureus* (13 mm). This was followed by acetone and aqueous extracts against *V. chlorae* (11 mm) and *E. coli* (9 mm). The minimum zone of inhibition was noted in ethanolic and methanolic extract against *E. coli* and *B. subtilis* with a diameter of 4 mm.

In *C. reticulata* the highest zone of clearance was obtained from acetone extract against *Staphylococcus aureus* (12 mm). Similarly acetone and ethanol extracts of inhibited the growth of *E.coli* and *V. cholerae* by showing 10 and 9 mm of inhibition zone. The moderate sensitivity was noted in ethanol extract against *E. coli*. The ethanol, methanol and aqueous extract of *Citrus reticulata* showed less sensitivity and resistant to *E. coli*, *V. chlorae* and *B. subtilis* (4 mm).

Staphylococcus aureus and *V. cholerae* was seemed to be more sensitive to methanol, and aqueous extract of *Citrus aurantium* with inhibition zone 8 mm. *E. coli*, *B. subtilis* and *S. aureus* showed 4mm inhibition zone against an methaolic, ethaolic acetone and aqueous extract of *C. aurantium.* Cowan (1999) reported that the potency of Citrus fruit peel is enhanced by the type of solvent used indicating that there are some active ingredients in orange

peel which have high antimicrobial effect but which would not be released except when orange fruit peel is used in conjunction with a particular solvent.

Phytochemical constituents of plants such as tannins, alkaloids, flavonoids, phenolic compounds and several other aromatic compounds are secondary metabolites which can be used in achieving a defence mechanism against plundering by many micro-organisms. Activity is elicited by these medicinally bioactive components through a variety of mechanisms. Tannins stop cell wall synthesis in its tracks by forming irreversible complexes with prolene-rich proteins . Saponins have the potential to induce protein leakage. Terpenoids cause the breakdown of a microorganism cell wall by compromising the membranous tissue . Flavonoids are known to be synthesized in response to microbial infection by plants and have been shown to be effective antimicrobial substances against a wide range of microorganisms *in vitro*. They can form complexes with extra cellular and soluble proteins, as well as bacterial cell walls . Steroids are also known for their antibacterial activity, which is related to membrane lipids and induces liposome leakage. It is observed that the leaf content has more phytochemicals as compared toother plant parts . Therefore it is concluded that peel extracts of *Citrus aurantifolia and Citrus reticulata and Citrus aurantium* used in the present study could be effectively processed to be utilized as a source for antibacterial therapeutic drug preparations.

Table 4: Antibacterial activity of *Citrus aurantifolia* extracted with different solvents against human pathogens

S.No.	Solvent	Inhibition zone (mm)			
		E.coli	*B.subtilis*	*V.cholerae*	*S.aureus*
1	Methanol	8mm	4mm	7mm	13mm
2	Ethanol	4mm	6mm	8mm	8mm
3	Streptomycin	17mm	17mm	17mm	17mm
4	Acetone	9mm	10mm	11mm	7mm
5	Distilled water	6mm	7mm	5mm	8mm

Control – Streptomycin (100µg/ ml) peel extract - (2.5 mg/ml)

Table 5: Antibacterial activity of *Citrus reticulata* extracted with different solvents against human pathogens

S.No.	Solvent	Inhibition zone (mm)			
		E.coli	*B.subtilis*	*V.cholerae*	S. aureus
1	Methanol	4mm	5mm	4mm	7mm
2	Streptomycin	15mm	15mm	15mm	15mm
3	Ethanol	8mm	4mm	9mm	5mm
4	Acetone	10mm	7mm	7mm	12mm
5	Distilled water	4mm	6mm	5mm	4mm

Control – Streptomycin (100µg/ml) peel extract - (2.5 mg/ml)

Table 6: Antibacterial activity of *Citrus aurantium* extracted with different solvents against human pathogens

S.No.	Solvent	Inhibition zone (mm)			
		E.coli	*B.subtilis*	*V.cholerae*	S.aureus
1	Methanol	4mm	6mm	5mm	8mm
2	Streptomycin	16mm	16mm	16mm	16mm
3	Ethanol	6mm	4mm	5mm	7mm
4	Acetone	7mm	4mm	5mm	4mm
5	Distilled water	4mm	6mm	8mm	7mm

Control – Streptomycin (100µg/ml) peel extract - (2.5 mg/ml)

Table 3: Antibacterial activity of *Citrus aurantifolia* extracted with different solvents against human pathogens

S.No.	Solvent	Inhibition zone (mm)			
		E.coli	*B.subtilis*	*V.cholerae*	*S.aureus*
1	Methanol	8mm	4mm	7mm	13mm
2	Ethanol	4mm	6mm	8mm	8mm
3	Streptomycin	17mm	17mm	17mm	17mm
4	Acetone	9mm	10mm	11mm	7mm
5	Distilled water	6mm	7mm	5mm	8mm

Control – Streptomycin (100µg/ ml) peel extract - (2.5 mg/ml)

Table 4: Antibacterial activity of *Citrus reticulata* extracted with different solvents against human pathogens

S.No.	Solvent	Inhibition zone (mm)			
		E.coli	*B.subtilis*	*V.cholerae*	S. aureus
1	Methanol	4mm	5mm	4mm	7mm
2	Streptomycin	15mm	15mm	15mm	15mm
3	Ethanol	8mm	4mm	9mm	5mm
4	Acetone	10mm	7mm	7mm	12mm
5	Distilled water	4mm	6mm	5mm	4mm

Control – Streptomycin (100µg/ml) peel extract - (2.5 mg/ml)

Table 5: Antibacterial activity of *Citrus aurantium* extracted with different solvents against human pathogens

S.No.	Solvent	Inhibition zone (mm)			
		E.coli	*B.subtilis*	*V.cholerae*	S.aureus
1	Methanol	4mm	6mm	5mm	8mm
2	Streptomycin	16mm	16mm	16mm	16mm
3	Ethanol	6mm	4mm	5mm	7mm
4	Acetone	7mm	4mm	5mm	4mm
5	Distilled water	4mm	6mm	8mm	7mm

Control – Streptomycin (100µg/ml) peel extract - (2.5 mg/ml)

FT-IR SPECTROSCOPY

The FTIR analysis was carried out to predict the functional groups present in the peel of *Citrus aurantifolia, C. reticulata* and *C. aurantium*. The results of FTIR spectral studies were presented in **Table 10 to 12** and **Figure 4 to 6.**

From the spectral data, presence of C-Br, C=C-H:C-H, C-Cl, C-N, N-O, C-C, C=C, O-H and C-H were identified. These bonding are responsible for the presence of alkyl halide, aromatic amines, nitro compounds, nitrite, cyctopentanone, esters, carboxylic acids, alkynes, alcohol and phenols in the peel of *C. aurantifolia*.

From the spectral data, presence of C-Br, C-Cl, O-B, =C-H, C-H, C-N, C-O, C-C, N-O and H-C=O:C-H were identified. These bonding are responsible for the presence of alkyl halides, carboxylic acids, alkanes, aliphatic amines, secondary alcohol, alcohol, aromatics, nitro compounds, aldehyde and phenols in the peel of *C. reticulata.*

From the spectral data, presence of C-Br, C-Cl, =C-H, C-H, C-N, C-O, C-C, N-O, H-C=O:C-H and O-H. these bonding are responsible for the presence of alkyl halides, carboxylic acids, alkanes, aliphatic amines, secondary alcohol, alcohol, aromatics, nitro compounds, aldehydes and phenols in the peel of *C. aurantium*.

Fourier transform infrared spectroscopy is a physicochemical analytical technique which provides a clear picture of the metabolic composition of peel at a given time. FTIR is employed to elucidate the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of respective chemical functional groups (Bobby *et al.,* 2012). It is possible to detect the minor changes in the primary and secondary metabolites in leaves by observing the IR spectra (Surewicz *et al.,* 1993). FTIR has been used to identify the complicated structures of plant secondary metabolites and in the characterization of bacterial, fungal and plant species (Hori and Sugiyama 2003; Yang and Yen 2002).

GC-MS

The acetone extraction of *Citrus aurantifolia* plays an important role on the herbal drug formulations. Hence the present study was aimed to find out the bioactive compounds present in the peel of *Citrus aurantifolia* by using Gas chromatography and Mass spectrometry. The active compounds with their CAS number are presented in **Table 13 and Fig. 7,** which shows the presence of 23 bioactive phytochemical compounds on acetone extract of *Citrus aurantifolia* peel. The GC-MS results showed the phytoconstituents such as Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene, 2-Ethylacridine, Propiophenone, 2'-(trimethylsiloxy)-, 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-, Tetrasiloxane, 1,7-diallyoctadecyl-, Silicic acid, diethyl bis(trimethylsilyl) ester, 1H-Indole, 1-methyl-2-phenyl-, Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, Tris(tert-butyldimethylsilyloxy)arsane, Benzo[h]quinoline, 2,4-dimethyl-, 1,1,1,3,5,5,5-Heptamethyltrisiloxane, 1,4-Bis(trimethylsilyl)benzene, 1,2-Bis(trimethylsilyl)benzene, Cyclotrisiloxane, hexamethyl-, Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane, 5-Methyl-2-phenylindolizine, 1,2-Benzisothiazol-3-amine tbdms, Benzo[h]quinoline, 2,4-dimethyl-, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester, 2-Methyl-7-phenylindole, Anthracene, 9,10-diethyl-9,10-dihydro-. The above mentioned bioactive phytocompounds posses the many biological activity including antioxidant activity, antibacterial, antifungal and antimicrobial activities, anticancer, antitumourous activity, etc. (Falowo *et al.*, 2016; Momin and Thomas, 2019) Hence further study is needed to prove the medicinal values of the *Citrus aurantifolia*.

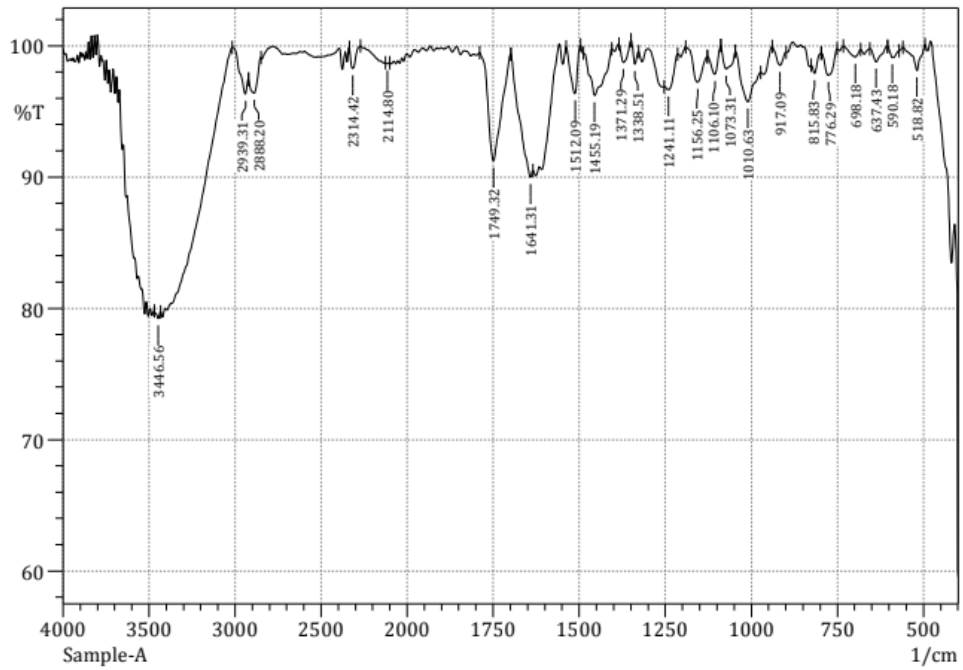


Figure 4. FT-IR SPECTRUM OF SAMPLE CITRUS AURANTIFOLIA PEEL

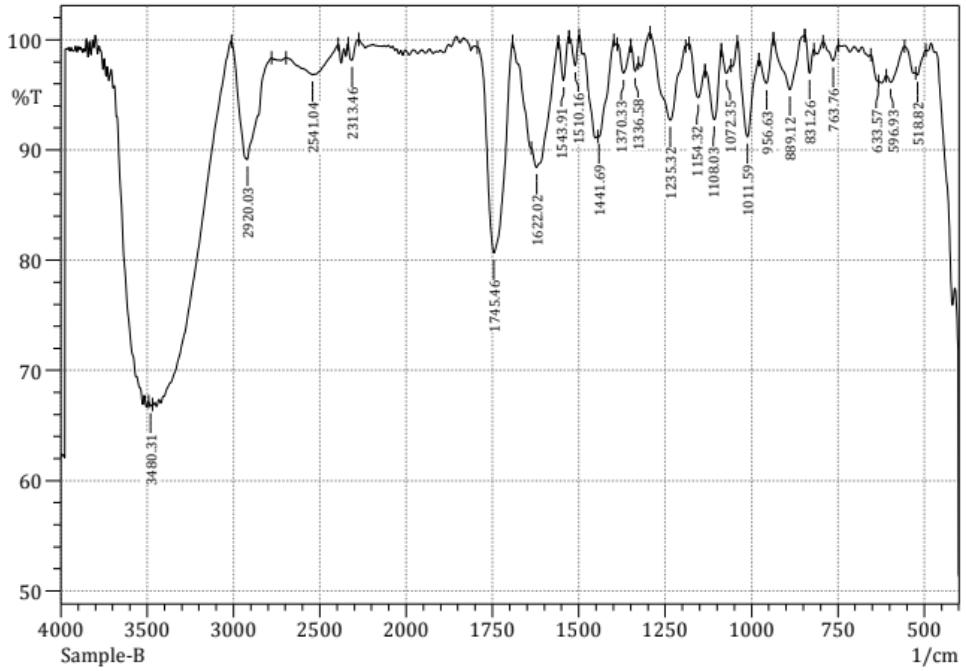


Figure 5. FT-IR SPECTRUM OF CITRUS RETICULATA PEEL

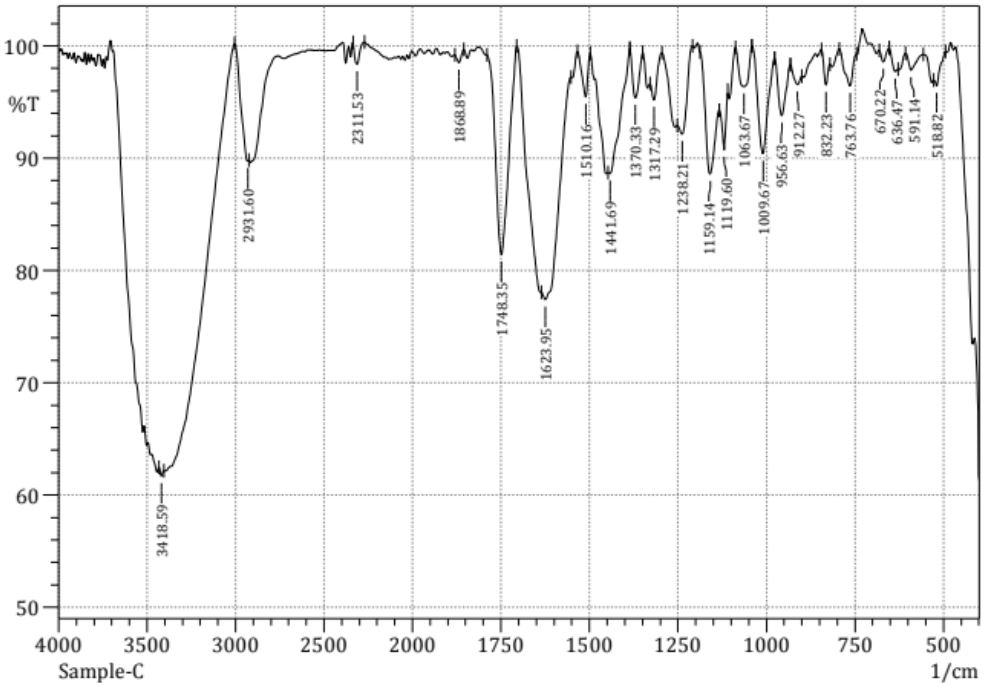


Figure 6. FT-IR SPECTRUM OF CITRUS AURANTIUM PEEL

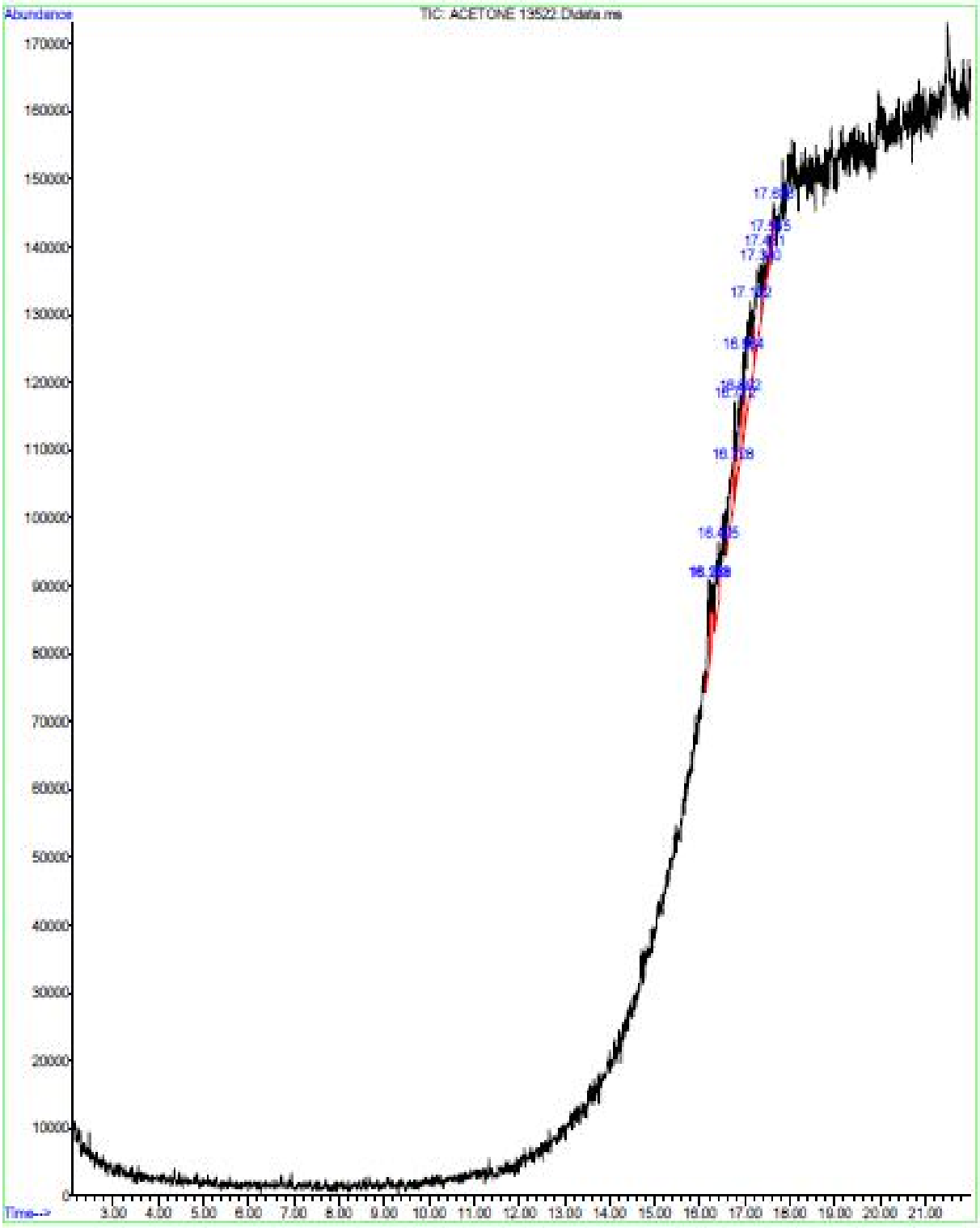


Figure 7. GC-MS analysis of acetone extract of *Citrus aurantifolia* peel

SUMMARY AND DISCUSSION

Summary and Conclusion

Citrus fruits are the biggest fruit sector production all over the world, at the same context, wastes the dominant byproduct of *Citrus* processing industries. These *Citrus* fruit residues, which are generally discarded as waste in the environment, can act as potential nutraceutical and pharmaceutical resources. Due to their low cost and easy availability such wastes are capable of offering significant low-cost nutritional dietary supplements. The utilization of these bioactive rich citrus residues can provide an efficient, inexpensive, and environment friendly platform for the production of novel nutraceuticals and pharmaceuticals.

Citrus by –products is a major source of phenolic compounds; flavonoids, These flavonoids belong to six classes and have different biological activities i.e. antioxidant, anticancer, antiviral and antinflammatory. Dimou et al. 2019 on their review concluded that by –product of fruits and vegetables have an important role to be used as functional activity in cosmotics, nutraceuticals and as functional foods either in their raw material for additive processes or as ingredients for new products. Citrus peel has limonoids and flavonoids as their anticancer constituents. The most abundant citrus flavonoids, generally known as the flavanones, include hesperidin, naringin, narirutin, and neohesperidin, and these compounds have been found to provide health benefits such as antioxidative, anticancer, antiinflammatory, and cardiovascular protective activities. The phytochemical analysis of different peel extracts (ethanol, acetone, methanol and aqueous) of *Citrus aurantifolia* and *Citrus reticulata and Citrus aurantium* were found to contain alkaloids, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and anthraquinone (Table 1, 2 and 3). They have a high therapeutic value and are commonly used in the pharmacy and drug industries.

In the present study, antibacterial activity of three citrus peel extracts (*Citrus aurantifolia and Citrus reticulata and Citrus aurantium)* of four different solvents (ethanol, acetone, methanol and aqueous) were tested against four human pathogenic bacteria (*Escherchia coli, Bacillus subtilis, Staphylococcus aureus* and *Vibrio cholerae)*. Three species of Citrus peels were presented in table (4 to 6). The diameter of the inhibition zones against these species ranged from 6 to 17 mm. The study revealed that all extracts inhibited the growth of all the pathogens tested. As shown in Table 4 methanolic extract of *C. aurantifolia* exhibited maximum activity against *S. aureus* (13 mm). This was followed by acetone and aqueous extracts against *V. chlorae* (11 mm) and *E. coli* (9 mm). The minimum zone of inhibition was noted in ethanolic and methanolic extract against *E. coli* and *B. subtilis* with a diameter of 4 mm.

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From the spectral data, presence of C-Br, C-Cl, O-B, =C-H, C-H, C-N, C-O, C-C, N-O and H-C=O:C-H were identified. These bonding are responsible for the presence of alkyl halides, carboxylic acids, alkanes, aliphatic amines, secondary alcohol, alcohol, aromatics, nitro compounds, aldehyde and phenols in the peel of *C. reticulata.*

From the spectral data, presence of C-Br, C-Cl, =C-H, C-H, C-N, C-O, C-C, N-O, H-C=O:C-H and O-H. these bonding are responsible for the presence of alkyl halides, carboxylic acids, alkanes, aliphatic amines, secondary alcohol, alcohol, aromatics, nitro compounds, aldehydes and phenols in the peel of *C. aurantium*.

The acetone extraction of *Citrus aurantifolia* plays an important role on the herbal drug formulations. Hence the present study was aimed to find out the bioactive compounds present in the peel of *Citrus aurantifolia* by using Gas chromatography and Mass spectrometry. The active compounds with their CAS number are presented in Table 13 and Fig. 7, which shows the presence of 23 bioactive phytochemical compounds on acetone extract of *Citrus aurantifolia* peel. The GC-MS results showed the phytoconstituents such as Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-,2-(Acetoxymethyl)-3-

(methoxycarbonyl)biphenylene, 2-Ethylacridine, Propiophenone, 2'-(trimethylsiloxy)-, 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-, Tetrasiloxane, 1,7-diallyoctadecyl-, Silicic acid, diethyl bis(trimethylsilyl) ester, 1H-Indole, 1-methyl-2-phenyl-, Thiocarbamic acid, N,N-dimethyl, S-1,3-diphenyl-2-butenyl ester, Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, Tris(tert-butyldimethylsilyloxy)arsane, Benzo[h]quinoline, 2,4-dimethyl-, 1,1,1,3,5,5,5-Heptamethyltrisiloxane, 1,4-Bis(trimethylsilyl)benzene, 1,2-Bis(trimethylsilyl)benzene, Cyclotrisiloxane, hexamethyl-, Trimethyl[4-(2-methyl-4-oxo-2-pentyl)phenoxy]silane, 5-Methyl-2-phenylindolizine, 1,2-Benzisothiazol-3-amine tbdms, Benzo[h]quinoline, 2,4-dimethyl-, 1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester, 2-Methyl-7-phenylindole, Anthracene, 9,10-diethyl-9,10-dihydro-. The above mentioned bioactive phytocompounds posses the many biological activity including antioxidant activity, antibacterial, antifungal and antimicrobial activities, anticancer, antitumourous activity, etc. (Falowo *et al.*, 2016; Momin and Thomas, 2019). I was concluded from the present study that *Citrus* peel has anticancer, antimicrobial, anti-inflammatory activity etc. In Future a drug which is prepared from this *Citrus* peel would save the human life from various diseases.

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A COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF CORCORUS SPECIES – TILIACEAE

A dissertation submitted to

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affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY**

in partial fulfilment of the requirements for the Degree of

Master of Science in Botany.

By

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CERTIFICATE

This is to certified that is this short term project work entitled dissertation entitled **A COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF CORCHORUS SPECIES – TILIACEAE** submitted to **St. Mary's College (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the Degree of Master of Science in Botany and is a record of work done in the department of Botany **ST. Mary's College (Autonomous)** THOOTHUKUDI during the year 2021-2022.

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I do here by declare that this dissertation entitled **A COMPARATIVE PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF CORCHORUS SPECIES – TILIACEAE** Submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr.Mrs. S. Beulah JerlinM.Sc, M.Phil., Ph.D.** Assistant Professor. Department of Botany, St.Mary's College (Autonomous) THOOTHUKUDI and it has not been submitted elsewhere for the award of any other degree.

C. Eruthaya Abisha

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C.ERUTHAYA ABISHA

CONTENT

INTRODUCTION

INTRODUCTION

Study on natural products is always an interesting target for scientists over decades, especially on plants. Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. The plant based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabiet al., 2007).

Unlike botanists, who make a clear and formal distinction between plant morphology and plant anatomy (the former referring to whole-plant form and the organization of parts, the latter to the fine structure, or histology, of parts), zoologists are uncharacteristically vague in their usage of these terms.

Morphology "deals with the form of living organisms, and with relationships between their structures" (from the Greek term morpho), whereas anatomy is "the science of the structure of the bodies of humans, animals, and plants" (derived from the Greek terms ana- and -tomy, meaning "repeated cutting") (Brown, 1993). Morphology is the study of "form," which can be generalized to all hierarchical levels, from organelle to whole organism. It is also concerned with the relationships among structures, hence it includes emergent features of form such as relative size, allometry, and even function and physiology.

Anatomy, a field in the biological sciences concerned with the identification and description of the body structures of living things. Gross anatomy involves the study of major body structures by dissection and observation. "Gross anatomy" customarily refers to the study of those body structures large enough to be examined without the help of magnifying devices, while microscopic anatomy is concerned with the study of structural units small

enough to be seen only with a light microscope. Dissection is basic to all anatomical research. The earliest record of its use was made by the Greeks, and Theophrastus called dissection "anatomy," from anatemnein, meaning "to cut up."

Comparative anatomy, the other major subdivision of the field, compares similar body structures in different species of animals in order to understand the adaptive changes they have undergone in the course of evolution.

Plants are the one of the most important sources of medicines. Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. These plants find application in pharmaceutical, cosmetic, agricultural and food industry.

Historically, plants, fruits, vegetables, medicinal herbs etc. have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoidsand some other secondary metabolites, which are rich in valuable bioactivities like antioxidant, anti– inflammatory, antitumor, antimutagenic, anti - carcinogenic, antibacterial, or antiviral activities (Maridass and Britto, 2008).

According to the World Health Organization (WHO), a variety of drugs are obtained from different medicinal plants and about 80% of the world's developing population depends on traditional medicines for their primary health care needs.

Plant-produced chemical compounds or phytochemicals like alkaloids, glycosides, flavonoids, volatile oils, tannins, and resins have been used in a wide range of commercial and industrial applications such as flavours, aromas and fragrances, enzymes, preservatives, cosmetics, bio based fuels and plastics, natural pigments and bioactive compounds. The research on phytochemicals and use of phytochemicals is increasing more because of the harmful side effects of the synthetic compounds.

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg,1999). They protect plants from disease and damage and contribute to the plant's colour, aroma and \flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson,1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

In the pharmaceutical landscape, plants with a long history of use in ethno medicine can be a rich source of substances for the treatment of various ailments and infectious diseases. Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. The vast array of therapeutic effects associated with medicinal plants includes anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic properties.

Phytochemicals provide plants with colour, flavour and natural protection against pests. Numerous epidemiological studies have indicated that a diet rich in fruit and vegetables offers considerable health benefits to humans. Among these benefits are

Reduction of the risk of developing many forms of cancer (lung, prostate, pancreas, bladder and breast.)

1. Reduction of the risk of cardiovascular disease.

The majority of these beneficial effects are at least in part due to the presence of phytochemicals in vegetable and fruits. In this context phytochemicals may be defined as "non-nutrient" chemicals found in plants that have biological activity against chronic diseases. Kushad.et al., (2003)

Pharmacological activities of plant extracts have revealed anticancer, hepatoprotective, anti-inflammatory, and antimicrobial properties. Boerhaaviadiffusa L. (Nyctaginaceae) fresh or dried is the source of the drug punarnava which is official in Indian Pharmacopoeia as a diuretic. The plant is bitter, astringent, cooling, anthelmintic, diuretic, aphrodisiac, cardiac stimulant, diaphoretic, emetic, expectorant, anti-inflammatory, febrifuge and laxative besides being an active ingredient as a tonic. It is useful in all types of inflammation, strangury, leucorrhoea, lumbago, myalgia, cardiac disorders, jaundice, anaemia, dyspepsia, constipation, cough, bronchitis and general debility, dyspepsia, oedema, jaundice, cough, haemorrhoids, pulmonary cavitations, anaemia, enlargement of spleen, abdominal pain, abdominal tumours, cancers and acts as an antistress agent. (Maya Verma and Ashwani Kumar 2017).

In modern science, the importance of medicinal plants and increasing Iordachescu and Dumitriu, (1988). Nowadays, pharmaceutical and cosmetic industries are increasingly using plant resources from rural or unpolluted areas. Phytochemical screening is helpful to detect the various important compounds which could be used as the base of modern drugs for curing various diseases. The genus *Corchous* is a fibre plant and also used as a vegetable. Consumption of the leaves is reported to be demulcent, deobstruent, diuretic, lactagogue, purgative, and tonic. It is also a folk remedy for aches and pains, dysentery, enteritis, fever, pectoral pains, and tumors. Ayurvedics use the leaves for ascites, pain, piles, and tumor

skeeping this in view the plants *Corchorus aestuens*L.and*Corchorus tridens*Lare selected for this study

SCOPE

AND

OBJECTIVES

SCOPE AND OBJECTIVES

Medicinal plants have been widely used for thousand years for the treatment of many diseases. Most of the medicinal plants are allelopathic in nature has been used a popular folk and an orient medicines treats against many diseases. The aim of the study was to evaluate pharmacognostical and phytochemical screening of *Corchorus aestuens* and *Corchorus tridens* folk remedy for aches and pains, dysentery, enteritis, fever, pectoral pains, and tumors. Ayurvedics use the leaves for ascites, pain, piles, and tumors.The plants were selected on the basis of their previous report and their uses.

- To perform qualitative analysis of phytochemical in aqueous extracts of *Corchorus aestuens*L. and *Corchorus tridens*L.
- To evaluate various pharmacognostical parameters such as microscopic and macroscopic characters of *Corchorus aestuens*L .and *Corchorus tridens*L Which can further lead to provide a beneficial information towards the quality of the drug.

REVIEW

OF

LITERATURE

LITRATURE REVIEW

Phytochemicals accumulate in different parts of the plants, such as in the roots, stem, leaves, flowers, fruits or seeds (Costa *et al*., 1999). Phytochemical studies on *Sopubiadelphinifolia* were carried out by Deokule and Patale (2001). Laily *et al*, (2002) worked out a preliminary phytochemical survey of plants in Crocker range sabbah Malaysia.

Phytochemicals are knows as secondary plant metabolites and have biological properties such as antioxidant activity antimicrobial effect, modulation of detoxification enzymes, stimulation of immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown Phytochemicals. It is well known that plants produce these to protect themselves, but recent researches demonstrate that many Phytochemicals can also protect human against diseases (Rao 2003).

The chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, Coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu,2004)

Falodun *et al*., (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbolestersin in the aqueous and methanol extracts of *Euphorbia heterophylla.*

In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent inconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al.,* 2007).

A large number of medicinal plant are used as alternate medicine disease of man and animals. Since most of them are without side effects when compared when synthetic drug. identification of the chemical nature of phytochemical compound present in the plants will

provide some information on the different functional groups responsible for their medicinal properties. While studying the *in-vitro*efficacy of bioactive extracts of 15 medicinal plants against ESBC-Producing multi drug resistant bacteria lqbal Ahamadand farrukhAqil.,(2007)detected major group of compound as the most active fraction of four plant extracts by infrared spectroscopy.

Kareru *et al*.,(2008)carried out the spectral analysis for saponins in the crude dry power of 11 plants and detected that *Albiziaanthelmintica, sennasinguaena, Mytenussenegalenisis, sennadidymomatryaTerminalia brownie* and *Prunus Africana* were likely to be bidesmosidic, oleanne type triterpenoids, while those detected in *Entadaleptostachya* and *Rapanearhododendroides* might be monodesmosidicsaponins.

Krishna *et al.,* (2009) conducted preliminary Phytochemicals, total phenolics and flavonoids content analysis in the methanol extract of *Justiciagendarussa*. The aerial parts of *Hypericumperforatum* were experimented to acquire knowledge about their composition of bioactive compounds (Gioti *et al.,* 2009).

Medicinal plants are known to produce certain bioactive molecules which inhibit bacterial or fungal growth (antimicrobial activity) Sharma and Kamar, 2009).

Choudhary. *et al.,* (2009) observed the studies on leaf epidermal micromorphology wood element character and phytochemical screening of three medicinally important taxa of the family convolvulaceae.

Ravirajisingh *et al*., (2009) took methanol extract from *Clerodendrong landulosum* to study its qualitative and quantitative phytochemical constituents.

Jitin Ahuja *et al.,* 2011 studied the phytochemicals present in various extract of aerial parts of *A. parviflora* and to determine the total phenolic and flavonoid content in ethanolic extract. Total phenol and flavonoid content was determined by folin-ciocalteu assay and aluminum chloride colorimetric assay respectively. Ethanolic extract showed the presence of

alkaloids, sterols/ triterpenoids, flavonoids, tannins and coumarins. The phenolic and flavonoid content of ethanolic extract using gallic acid and rutin as standards was found out to be 1.09 ± 0.007mmgGAE/g and 1.163 ± 0.0208 mgRE/g respectively. The study showed significant amount of gallic acid and rutin equivalents were present in extract which may be responsible for valuable pharmacological property of extract. As phenolics and flavonoids are responsible for antioxidant activity of plant, present data implies that *A. parviflora* is a perfect candidate for in-vitro antioxidant activity and isolation of phytochemicals.

The phytochemical analysis revealed of significant amount of polyphenols and flavonoids (90% and 80%respectively). These findings suggest that *Theprosia purpurea* root extract posses prominent medicinal properties and can be exploited as natural drug to treat the disease anociated with free radical formation oxidative stress and xanthine, oxidase activity Khobra.,(2011).

Farhat and Iqbal (2011) observed the phytochemical screening of some Pakistanian medicinal plants and Chandra shekar and Rao (2012) observed the phytochemical analysis of ethanolic extracts of leaves of *Clerodendronviscosum.*

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical or biological properties or by the manufacturing processes. For the quality control of a traditional medicine, the traditional methods are produced and study, documents and the traditional information about the identity and quality assessment are interpreted in terms if modern assessment, Vikrant,*et al*., (2012).

Vikrant *et al.*(2012) worked at a preliminary phytochemical analysis of *Psidium* leaves. Nilofer *et al* (2013) studied the qualitative phytochemical analysis was done in rat tubers of six species *Dioscorea*found Meghalaya. The test conform the presence of various phytochemicals like flavanoids, saponins, steroids, cardiac, glycosides and terpenoids in two aqueous extract of methanol and ethyl acetate.

Johnson *et al.,* (2012) reported the phytochemical constituents of methanol flower extracts of *Helictresisora, Spathodeacampunulata, Antigononleptopus and Thunbergiagrandiflora.* Kirubae*t al.,* (*2012*) studied the phytochemical analysis of various solvents extracts of the flower of *Rhododendron arboretum* spp. *nilagiricum.*

Abbas *et* al., 2012 studied the qualitative and quantitative phytochemical analysis of fifteen weed seed extracts. Alkaloids, saponins, glycosides, terpenoids, anthraquinine, steroids, flavonoids and tannins were detected from the weed seeds. Tannins and alkaloids were in high concentration. Tannins ranged from 7.97 to 24.17%, alkaloids 0.88 to 4.00%, saponins 0.54 to 1.29% and flavonoids 3.91 to 15.55%. Wheat weeds care medicianlly important but their phytochemical potential needs to be further investigated.

The different solvent extracts of *Kirganelia reticulata* leaves were screened for their phytochemical constituents by Shruthi *et al*. (2012).

AgnelRuba *et al*. (2013) carried out preliminary phytochemical analysis of *Arthocnemum fruticosum* leaf using five different solvents.

PackiaLincy *et al*. (2013) conducted the preliminary phytochemical study of *Ventilagomader aspatana* whole plant, using different solvents

Mehta *et al*., (2013) worked at aphytochemical survey of leaf extract of *Phyllanthus fraternus*. The plant extract contain alkaloids like morphine and boldine. Extracts also contains tannins, saponin, terpenoids and steroid.

Chede (2013) observed the phytochemical screening of fruit pulp of *Citrus sinensis.* The aqueous as well as the ethanolic extracts of the pulp revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, steroids and aminoacids where asterpenoids are present only in the ethanolic pulp extracts.

Oancea *et al.,* 2013 observed the phytochemical screening of the bio active compounds in the most wide spread medicinal plants from Calarasi country. The following

categories were identified during the study: amino acids, protein,polysacharidesvitamin A and E.

AtiqMehsud *et al*., 2013 studied the morphology and anatomy of seven most common weed species infesting agricultural and non-agricultural lands of rainfed area of Bannu region were investigated during 2012. The study included *Datura metel L., Euphorbia hirta L., Fagoniacretica L., Heliotropium europaeum L., Parthenium hysterophorus L., Solanum surattenseBurm f. and Withania somnifera (L.) Dunal.* Due to some special morphological and anatomical features, the capacity of rapid absorption of water along with minerals from the soil may be facilitated to compensate the rapid water loss, and thus can also be regarded as common xerophytes. Their morphological, anatomical and histological characteristics are suitable for their successful growth in rain-fed condition of the region.

Ajiboye*et* al., 2013 studied to found out the presence of phytochemicals in the aqueous extracts of *Seneciobiafrae* leaves of both qualitative and quantitative screening methods. In qualitative analysis, the phytochemical compounds such as alkaloids, saponin, tannin, phlobatannin, phenol, anthraquinoes, flavonoids, glycosides, steroids, terpenes, cardenolides, flavonoid and chalocones were determined in the sample aqueous extracts by using standard methods. Also, quantitative analysis of the important secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins and tannins were tested in the sample extracts. Results concluded that the presence of these active compounds may be responsible for the medicinal purposes of the plan.

Gilberto Ocampo *et al.*, 2013 studied not only the species with different C 4 biochemistry (NADP-ME and NAD-ME types) and C 3 -C 4 intermediacy, but also displays different leaf anatomical configurations. Photosynthetic pathways were assessed based on leaf anatomy and carbon isotope ratios. Information on the NADP- ME and NAD-ME C 4

variants was obtained Here we addressed the evolutionary history of leaf anatomy and photosynthetic pathways in Portulacaceae.

Monika Gupta *et* al., 2013 studied the qualitative analysis of *Emblicaofficinalis, Acacia catechu, Acacia concina* and *Hibiscusrosa-sinensis* showed that tannins, saponins, flavonoids, terpenoids and alkaloids are present in all the plants except phlobatannins that is only present in *Acaciacatechu*. The pet ether and chloroform extract of *Emblicaofficinalis* does not show potential for oil and fat components whereas all the extract of *Emblicaofficinalis* showed positive test for carbohydrates. The identification of colouring chemical constituents of natural products together with their therapeutic properties are discussed.

Shinde *et* al., 2014 studied some parameters such as morphological, microscopical, physicochemical evaluation, florescence analysis; preliminary phytochemical analysis, thin layer chromatographic study and antimicrobial potential of alcoholic extract of *P. grandiflora* were carried out. Macroscopically the leaves are fleshy leaves, watery, needle shape. Flowers are racemes form; fruits are ovoid with small black coloured seed. Chemomicroscopy revealed the presence of Rubiaceous stomata in leaf; Rosette calcium oxalate crystals and protoplast in mesophyll of leaf, cortex and pith of stem and root; pink colored cuticle of stem, collateral vascular bundle with lignified xylem, abundant of starch grains and mucilaginous cells in all aerial parts. Physicochemical evaluation used to determined standards showed a results with total ash, acid insoluble ash, water soluble ash, sulphated ash, ethanol soluble extractive, water soluble extractive, moisture content, swelling index and total crude fiber content in powder of stem. Preliminary phytochemical analysis revealed the presence of alkaloid, steroids, Triterpenoids, flavonoid, tannins, and carbohydrates. The total flavonoid content in alcoholic extract was found .The alcoholic extract of herbs showed significant inhibition of microorganisms.

Silvia Netala *et al*., 2014 compared the structural features and physicochemical properties of three species of *Portulaca.* Different Parts of *Portulaca*were examined for macroscopical, microscopical characters. Physicochemical, phytochemical and fluorescence were also analysed. The plants are succulent, prostrate herbs. Usually roots at the nodes of the stem. Leaves are opposite with paracytic stomata and characteristic Kranz tissue found in C-4 plants. Abundant calcium oxalate crystals are present in all vegetative parts of the plant. Quantitative determinations like stomatal number, stomatal index and vein islet number were performed on leaf tissue. Qualitative phytochemical screening revealed the presence of alkaloids, carbohydrates, saponins, steroids and triterpenoids.

InSun Kim, 2014 studied the vegetative and reproductive morphology and anatomy of two Hawaiian endemic *Portulaca*species were examined. Specifically, *P. molokiniensis*and *P. sclerocarpa* were compared to closely related species in the genus. The comparisons were both qualitative and quantitative, using characteristics of leaves, stems, roots, and fruits. Tissue organizations of vegetative and reproductive parts of the plants were assessed using microtechnique procedures, statistical analysis, and scanning electron microscopy. The most notable features of these two species were (1) the size and frequency of stomata in *P. molokiniensis,* and (2) the large number of sclerenchymatous cell layers in the thickest fruit walls of *P. sclerocarpa*. These findings may imply that stomata development in *P.molokiniensis*and thick fruit wall development in *P. sclerocarpa*are evolved features of survival.

Okafor and Ezejindu (2014) investigated its phytonutrients. The aerial parts of *Portulaca oleracea* were harvested, air dried and powdered for this study. Chemical tests were carried out on the aqueous extract and the powdered specimen to determine the phytoconstituents. The presence of Alkaloid, saponin, tannin, flavonoid, cardiac glycoside, terpenoid, steroid, phobatannin, protein and starch were accessed qualitatively while

flavonoid, tannin alkaloid and saponin were determined quantitatively and it was found not to contain steroid and phobatanin but containing 32% of saponin as its highest constituent with 26% alkaloid.

Phytochemical screening on bark extract of *Albizialebbeck* revealed the presence of tannins, phenols, steroids, triterpinoids, Saponins, anthroquinones and phlobotannins (Kumar *et al.*, 2014).

Paula de Oliveira Amorim *et al.*, 2014 studied the isolation and structural determination of four new compounds: one acrylamide and three new phaeophytins together with twelve known compounds, including four phaeophytins. The structures of the compounds were established on the basis of 1D and 2D NMR, IR, HRESI-MS spectra, including GC–MS, and HPLC–UV analysis, as well as comparisons with the literature data. The CD spectra data analysis were used to define the absolute configuration of phaeophytins.

Sohail Ahmad *et* al., 2014 studied the crude and numerous fractions of leaves, stem, and roots of the plant were investigated for phytochemical analysis and DPPH radical scavenging activity. Phytochemical analysis of crude and fractions of the plant revealed the presence of alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, glycosides, and phenols. The antioxidant activity of various extracts was resolute against DPPH radical with the avail of UV at 517 nm. The stock solution (1000mg/mL) and then several dilutions of the crude and fractions were prepared. Ascorbic acid was used as a standard. The plant leaves (52.59 ± 0.84 to 90.74 ± 1.00), stem (50.19 ± 0.92 to 89.42 ± 1.10), and roots extracts (49.19 ± 0.52 to 90.01 ± 1.02) divulged magnificent antioxidant activities. For the ascertainment of the fatty acid constituents a gas chromatograph hyphenated to mass spectrometer was used. The essential fatty acids for growth maintenance such as linoleic acid (65.70%), eicosadienoic acid (15.12%), oleic acid (8.72%), and palmitic acid (8.14%) were found in high percentage.

Chaitanya MVNL *et al*., 2015 investigated the microscopical, Phytochemical and *In-vitro* antioxidant studies of hydro alcohol, Aqueous, Ethyl acetate, Chloroform, Pet - ether and total saponin fractions of aerial parts of *Cestrum aurantiacum*and *Solanummauritianum*. In this study, they founded that the selected plants posses' good amount of phenolics, alkaloids, flavanoids and saponins, among all the fractions the total saponin fractions showed better antioxidant activity in compare to other fractions ,The fractions also showed a good *In-vitro* cytotoxic activity on MCF-7 cell lines and moderate against HCT-116 cell lines in comparison to standard Salvicine.

Yan-Xi Zhou*et al.*,2015isolated the compounds from *Portulaca oleracea*, such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals. *Portulaca oleracea* possesses a wide spectrum of pharmacological properties such as neuro protective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, and anticancer activities. However, few molecular mechanisms of action are known.

Vidhya and Uma Vandhana, 2016 reported the phytochemical analysis and GC-MS analysis of *Cleome viscose* leaf parts and the presence of various metabolites including alkaloids, flavanoids, glycosides, tannis, saponins, steroids, terpenoides and phenolic compounds was analysed. The GC-MS results were noticed in the methanolic extracts of *Cleome viscosa*. The compounds were identified by comparing their retention time and peak area with that literature and by interpretation of mass spectra. The plant *Cleome viscosa* was used in various industries and its application includes wound healing, antioxidant, anti-inflammatory, antimicrobial and cancer preventive.

SethupandianGeetha *et al*., 2016 carried out the qualitative phytochemical screening of *C. religiosa* leaves and stem were studied. Five solvents such as aqueous, methanol, ethyl acetate, chloroform and acetone were used to obtain extracts from powdered plant parts. The

extracts were subjected to qualitative phytochemical screening using standard procedures. Results showed that the nine phytochemicals such as alkaloids, flavonoids, phenols, tannis, steroids, terpenoids, coumarins, quinines and saponins were found. However, catachin was absent in leaves and stem extracts. The diversity of photochemical present suggests that *C. religiosa* leaves and stem could serve as a source of useful drugs.

Majgaine, Shweta and D.L Verma,2017 studied that *Boerhaaviadiffusa* have some pharmacological activities and used as a medicine with multi action such andiuretic, entra sthamatic, diaphoretic, anthelmintic, antiasthamatic,diaphoretic,antihepatotoxicantiurethritis febrifuge, antiscabies these pharmacological activities are due to the presence of chemical constituents.

Sushmita Negi, 2018, studied the quantitative phytochemical analysis of *Portulacaoleracea*was carried for four main parameters. In addition to this total protein estimation was also carried. The plant samples were collected from garden area which was unpolluted site, and roadside area facing air pollution generated from traffic exhaust. Plant samples from polluted site also faced nutrient stress, water stress and stress of extreme temperature. Phytochemical analysis was carried separately for leaf and stem samples. Their percent values were found to be relatively high in samples collected from polluted site than the samples collected from unpolluted site. It shows that *Portulacaoleracea*has potential to grow in wasteland under nutrient and water stress conditions on one hand and at the same time the phytoconstituents values remain relatively high under stress.

Trupti P Durgawale *et* al., 2018 analysedthe two plant species *Portulaca oleracea* and *Portulaca quadrifida*. Pharmacological investigations have revealed the importance of these plants as sources of antioxidants, essential fatty acids, and even antimicrobial agents.

Hemalatha K and Abirami P. 2018 studied the physicochemical and preliminary phytochemical analysis of *Talinum portulacifolium* leaf. Five different solvents like

Petroleum ether, Chloroform, Methanol, Ethanol, Aqueous were used to obtain the extract. These extracts were subjected for physicochemical and qualitative phytochemical analysis by using the standard procedures. Physico chemical parameters like moisture content, total ash, water soluble ash and sulphated ash values were calculated. Alkaloids, flavonoids, glycosides and phenols are present in the all the extracts. These bioactive compounds obtained from the phytochemical analysis may be the responsible for the pharmaceutical activity.

Maryam Sharif Shoushtari *et al*., 2018 investigated the phytochemical profiles for two models of aqueous (Aq) and methanolic (Me) pollen extracts of *F. excelsior* from three pollination periods from hermaphrodite flowers (H) of polygamous and male flowers of pure male (M) in order to identify their constituent compounds. There was a significant difference ($P < 0.001$) between the means of TPC for M and TFC for the H. Comparison of H and M antioxidant activities showed that DPPH (IC50) to be (2.977 ± 0.117 μM) during the second pollination period of M and (4.877 ± 0.021 μM) for first period of H. The majority of the compounds identified were linalool (35.42%) from the monoterpenoides in H and Delta-cadinene (43.22%) belonging to the sesquiterpenes in M. They concluded that there is a significant difference between the H and M compounds in pollen at different periods.

Deepak Kumar et al., 2018 studied anatomical and morphological features of different vegetative organs and reproductive organs were studied along with medicinal importance of the plant. *Portulaca oleracea*. In the morphological study it was observed that the plant have sessile leaves which were ovate, smooth, succulent, arranged in opposite manner, stem was aerial, weak and cylindrical, root consists of a long thick taproot as well as many fibrous lateral roots, flowers were single in leaf axils, fruit consists of round to egg-shaped capsules with glossy brown and black seeds. In anatomical studies, cross sections of the leaf, stem and root were examined. Purslane has better nutritional quality than the major cultivated

vegetables with higher beta-carotene, ascorbic acid, and alpha-linolenic acid along with high nutritive and antioxidant properties.

NassimaBoutaoui *et al*., 2018 evaluated the metabolite recovery from different extraction methods applied to *Thymus algeriensis* aerial parts. The experimental results show that microwave-assisted aqueous extraction for 15 min at 100 _C gave the most phenolics-enriched extract, reducing extraction time without degradation effects on bioactives. Sixteen compounds were identified in this extract, 11 phenolic compounds and five flavonoids, all known for their biological activities. Color analysis and determination of chlorophylls and carotenoids implemented the knowledge of the chemical profile of this plant.

Al-Newani, 2019 investigated the *Portulaca oleracea*is a succulent plant in Portulacaceae family distributed around different regions of Iraq as collected widely in the gardens of Baghdad governorate. The results of this study shown that a systematic significant of morphological and anatomical data. purslane with branched shoot stems. Stems and leaves are glabrous and leaves are alternate, the petiole is absent. Inflorescences were viewed as clustered in the form of small one carrying many male and female flowers as the inflorescences take the form of long stemmed. Anatomical techniques revealed two patterns of stomatal complex, paracytic which is the most common followed by tetracytic is limited distributed type and it is recorded for the first time in this species. Druses crystals have been found distributed in the stem with angular collenchyma alternating with xylem parenchyma cells with large intercellular space. As well as, root anatomy has been done and the results showed casperian strips cells clearly in section with xylem and phloem region

RohitaSingla and Saroj Kumar Pradhan, 2019, analysed the phenols, flavonoids content and antioxidant activity of common weeds growing around agriculture fieldsof Punjab plains. Maximum content of phenolics were reported in *Ageratum conyzoides*(flower, 9.51±0.00 mg CA/g DW), *Launaea procumbens*(stem, 7.94±0.01 mg CA/g DW), *Ranunculus*

muricatus(flower, 7.15±0.07 mg CA/g DW) and *Sonchus asper*(flower, 8.12±0.34 mg CA/g DW). The flavonoid content was measured high in case of *Silybum marianum*(stem, 4.83±0.00 mg Q/g DW), *Ranunculus muricatus*(leaves, 2.96±0.01 mg Q/g DW), *Solanum nigrum*(leaves, 2.45±0.03 mg Q/g DW) and *Ageratum conyzoides*(leaves, 2.15±0.01 mg Q/g DW). All the species of weeds having high phenol and flavonoid content, also have strong antioxidant potential in terms of DPPH radical scavenging activity and total antioxidant capacity.

Ahmed.*et* al.,2019 examined the total phenolic, flavonoids content and antioxidant activities of *Citrullus colocynthis*L. and *Cannabis sativa*L. Phytoconstituents except terpenoids from *C. sativa*and *C. Colocynthis* leaves were reported while, in contrast, steroids, tannins and phenols were absent in *C. colocynthis*roots. The methanol derived maximum phenolic contents from *C. sativa* and *C. Colocynthis* leaves were 36.42 and 37.69 mg gallic acid equivalent GAE/g respectively. However, total flavonoids registered from *C. Sativa*l eaves and *C. Colocynthis* leaves and roots were 59.03, 50.58 and 43.32 mg quercetin equivalent QE/g respectively. Interestingly, *C. Colocynthis* leaves produced the highest flavonoids 119.63 mg QE/g using ethyl acetate extract. DPPH inhibition (%) was high in acetone 55.57, hexane 45.98 and distilled water 35.5% from *C. sativa, C. Colocynthis* leaves and roots respectively. Our findings suggest that studied plants contain phytochemicals, reasonable quantity of phenol and flavonoids content confer to the potential antioxidant activity responsible for insecticidal properties as safer alternatives of synthetic pesticides.

Pranabesh Ghosh *et* al., 2019 studied five common medicinal herbaceous weeds of West Bengal, and India namely; *Heliotropium indicum*, *Tridax procumbens*, *Cleome rutidosperma*, *Commelina benghalensis*, and *Euphorbia hirta* have been chosen from five different families describe their phytochemical, and anti-oxidant properties. This investigations have concluded that *Euphorbia hirta* possesses a significant amount of

phytochemicals, and it exhibits the highest anti-oxidant activities in comparison with the other four medicinal weeds. In *Euphorbia hirta*leaves acetone extract highest amount of phytochemicals were detected by qualitative assays.

Saswade. 2019 studied the qualitatively preliminary analysis of some different weed species. Discovery of active compounds and their role in curing diseases from this plant leads its importance. The presence of these secondary bioactive phytochemicals signifies the importance of these medicinal plants as an efficient source of therapeutic agent.

Muhammad Aslam Tahir and Muhammad. Abbasi, 2020 studied the two members of Portulaca Family namely, *Portulacapilosa*and*Portulacaquadrifida Linn*. Dried twigs of *Portulacapilosa*and*Portulacaquadrifida* ground to fine powder and then analyzed using FTIR technique. Functional groups of phytochemicals were identified through FTIR spectral lines. Appropriate correlations of absorption peaks to medicinal compounds have been discussed. As a result, both herbs are found to be rich source of bioactive compounds like alkaloids, flavonoids, fatty acids, tannins, triterpenoids, amino acids and saponins.

Morpho-Anatomical and Physiological Responses Can Predict the Ideal Period for the Transplantation of Hydroponic Seedlings of *Hymenaeacourbaril*, a Neotropical Fruit Tree. (Daniele et.al.2020)

The smaller flow of nutrients and water from the culture medium influenced the morphogenesis of the parenchyma tissues. The palisade parenchyma thickness declined, mainly in the plants grown with 100 and 200 μM of Cd. The reduced thickness of this tissue can be related to the smaller translocation of water, which interferes with cell expansion (Silva-Cunha et al. 2021).

However, high-throughputmethods for assessing internal anatomy remain elusive, precluding the widespread inclusionof internal anatomy in many modern -omics- level studies (Yadav et al., 2021).

Morpho-Anatomical Traits and Soluble Sugar Concentration Largely Explain the Responses of Three Deciduous Tree Species to Progressive Water Stress.(Jonathan et.al.2021)

Morpho-Anatomical Feature and Phytochemical Assessments of Lasiaspinosa (L)Thwaites. (Arya Lakshmi et.al.2021)

Morphological, Phytochemical and FTIR spectroscopy.analysis of portulacaceae members were studied. The result ofpreliminary phytochemical screening indicated that leaf and stem of both species of *Portulacaceae* were free from steroids. Moreover, quantitative estimation of phytochemicals also exhibited that leaf and stem of both species. Secondary metabolites, which are abundant in plants and have fascinating biological activities, are an important source with a variety of structural arrangements and properties. They have a rich source of protein and have high antioxidant (Beulah and Santhiya 2022).
scavenging activity.

MATERIALS

AND

METHODS

MATERIALS AND MRTHODS

MATERIALS

Botanical Name :*Corchorus tridens* .L.

Family **:** Tiliaceae

Common name : Horn –Fruited Jute

***Corchorus tridens.*L**is a woody hairless herb in the Phalsa family , Tilliacae. It is found in tropical regions throughout the world.*C. tridens* is an upright plant, with simple leaves, alternate, stalked and stipulated. The blade is linear, with dentate margin. The first tooth of the base of the lamina is bent back and extended by a filament. The flowers are arranged in bundles, opposite to petioles. They are large, yellow, the sepals and petals free and with many stamens. The fruit is a capsule linear, surmounted by a trifide beak, and splitting into 3 valves, many polyhedral seeds. (Plate 1)

Botanical Name :*Corchorus aestuans* .L.

Family **:** Tiliaceae

Common name: East Indian Mallow

***Corchorus aestuans* .L.**is a common annual herb in waste land. Plant with erect, sparingly branched stems to about 40 cm tall. Ovate,acute, green leaves have serrated margins. Yellow flower 1-3 in clusters very shortly stalked, very small, leaf opposed.the flowers 5 sepals and 5 yellow petal. Capsule is hexagonal,(Plate 1)

METHODS

The fresh plant materials of *Corchorus aestuans* and *Corchorus tridens* were collected from the Sundaravelpuram and St.Marys'College Campus, Thoothukudi. The collected samples were cut into small fragments and shade dried until the fracture was uniform and

smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve NO.60.The final uniform powder was used for the extraction of active constituents of the plants.

Pharmacognostic study

Morphology of Plant

The morphological characters of stem, leaf, inflorescence and flowers of the selected taxa were examined by physical observation. Ten quantitative macro morphological characters viz. leaf area , petiole length, spike length, calyx length, corolla length, stamen length and pistil length were measured.

Microscopic Study

Transverse sections of leaf were taken, stained with safranin and mounted in glycerin. Semi-permanent slides were prepared and observed under compound microscope. Photographs ofthe sections were taken by photomicroscope under ×10 and ×45 magnifications.

Pattern and Distribution of Stomata

Pattern and distribution of stomata were studied by stomata peel method. The peels of the epidermis were stained with safranin and mounted in glycerin. Semi-permanent slides were prepared and observed under compound microscope.

Powder microscopy: ShehlaAkbar et al., (2014)

Shade dried leaves, stem, fruit were finely powdered and studied under microscope.

Micro chemical test

S.No	Reagents	Observation	Characteristics
1	Dil. Iodine Solution	Blue	Starch
2	Dil. Acetic acid	Insoluble	Calcium oxalate crystals.
3	Dil. Hcl	Soluble	Calcium oxalate crystals.
4	Aqueous extract + lead acetate reagent	White precipitate	Tannins
5	Aqueous extract+ potassium permanganate solution	Decolourisation	Tannins
6	Strong KOH solution	Needle shaped potassium eugenate crystals	Eugenol
7	Alcoholic picric acid	yellow	Aluerone grains

PREPARATION OF EXTRACTS- COLD MAXERATION METHOD

The coarse powder of sample was extracted successively with methanol, chloroform and benzene. All the extract through whatman no.41 filter paper. Aqueous solution is used to extracts, the extract were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure (Brinda*et al.*, Anonymous 1990, Lala, 1993).

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The chemical tests for various phytoconstituents in the extracts were carried out as described below

Test for alkaloids

Mayer test

To the powder, two ml of Mayer reagent was added; a dull white precipitate reveals the presence of alkaloids.

Test for Terpenoids

Noller's test

To 1 ml extract with tin (one bit) and thionylchoride (1 ml) were added. Appearance of pink colour indicates the presence of Terpenoids.

Test for steroids

Libermenn- Buurchard's test

The powder was dissolved in two ml of chloroform in a dry test tube. Ten drops of concentrated sulphuric acids were added. The solution became red, then blue and finally bluish green indicates the presence of steroid.

Test for Coumarins

To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

Test for Tannin

The test solution was mixed with basic lead acetate solution. Formation of a white precipitate indicates the presence of tannins.

Test for Saponins

The test solution was shaken with water. Copious lather formation indicates the presence of Saponins.

Test for Flavones (Shinadow' test)

To a few mg f the powder, magnesium turnings and 1-2 drops of concentrated hydrochloric acid (Hcl) were added.Formation of colour indicates the presence of flavonoids.

Test for Phenols

To 1ml of the extract, 2ml of distilled water was added followed of 5% aqueous ferric chloride. Appearance of blue or green colour indicate the presence of phenols.

Test for protein

Biuret test (Gahn, 1984)

To 1 ml of the extract, one drop of 2% $CUSO_4$and 1 ml of 95% of ethanol add KOH pellets. Appearance of pink colour indicates presence of protein.

Test for carbohydrate

Benedit's test

To 0.5 ml of extract (500 l) add 0.5 ml of Benedict's reagent, incubate in boiling water bath. Appearance of Brick red precipitate indicates presence of sugar.

Test for Quinones

To 1 ml of extract and 1 ml of con.H_2 SO_4 was added. Appearance of red indicates presence of Quinone.

Test for Gums

1 ml of extract was mixed with water. Thickening of substance shows the presence of gum.

RESULT

AND

DISCUSSION

RESULT AND DISCUSSION

Macroscopical characters

The morphological characters of the studied taxa are presented in *Corchorus aestuans.L.*is a herb and C*orchorus tridens L.* is a small herb.. The arrangement of the leaf was opposite in *Corchorus aestuans* and alternate in *Corchorus tridens*

Variations in leaf shape, margin, base and apex of the two species are noted. In *Ccorchorus aestuans* leaf was ovate with acute apex, Rounded base, leaf green, hairy. In*Corchorus tridens* leaves were lanceolate, acuminate with a denate margin.

In the selected species, the inflorescence position, corolla color, and calyx shape had shown some variation. The corolla colour is yellow in both C*orchorus aestuans* and *Corchorus tridens.*

The quantitative macro morphological characters were measured and summarized in (Table1. and Plate 2 and 3) Leaf area, Capsule length, petiole length, Nodal length, Inter nodal length is also measured. (Table:6)

Microscopic Observation:

Corchorus aestuans

T.S. of the stem was a circle in shape. The epidermis was a single layer covered within thin cuticle, transverse by glandular trichomes. 2-4 layer of collenchymatous cortex present. The endodermis was distinct. Pith was parenchymatous. The leaf was dorsiventral and amphistomatic. 4 vascular bundle present in the centre of midrib surrounded by parenchyma. Both the epidermis consisted of single-layered polygonal cells. The lower epidermis was covered by thick cuticle than the upper epidermis. Paracytic stomata are seen in both the upper and lower epidermis. The laminar portion of the leaf had a single layer of elongated palisade cells and 3-5 layers of spongy parenchyma. Trichomes were seen on epidermis. The lamina had many vascular bundles. (Plate 4 and 5)

Corchorus tridens

In cross-section, the stem was quadrangular in outline. Collenchymous tissues were present at the corner. The outermost layer epidermis is covered with a thick cuticle. Cortex consisted of parenchymatous cells. The endodermis was distinct.The leaf was dorsiventral and amphistomatic. T.S. of the leaf showed distinct midrib covered by a thin layer of epidermis, collenchymatous tissues present under the epidermis. The lamina consisted of a thin epidermis covered by a thick cuticle. Mesophyll was with 1 or 2 layers of palisade and spongy parenchyma.(Plate6 and 7)

QUALITATIVE PHYTOCHEMICAL SCREENING

The term 'phytochemical' is reserved for those plant chemical that have a beneficial effect on human- health but are not essential from the point of view of nutrition. A medicinal herb considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins,resins, oleoresins, oils. Antifungal activity of medicinal plants is mainly due to the presence ofphyto chemicals like alkaloids,glycosides, phenols,tannins and flavonoids (Sarojini et al., 2011). Moreover, phytochemical screening of the drug is signified for proper identification, which further exerts importance on therapeutic activity of the medicinal plant.

Therefore the current study was attempted to find out the presence of preliminary phytochemicals in water extract of leaf of *Corchorus aestuans* and leaf of *Corchorus tridens* Presence and absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes.

This procedure is a simple preliminary pre- requisite before going for detailed phytochemical investigation. Different chemical compounds were detected in extracts of leaf of *Corchorus aestuans* and leaf of *Corchorus tridens* and were presented in Table 2 and 3.

The result of preliminary phytochemical screening indicated that Steroids, Coumarin, Tannins, Phenols, Quinones, Gum extract of stem, leaf of *Corchorus aestuans.*

The result of preliminary phytochemical screening indicated that Steroids, Taninns, Flavones,Phenols, Quinones, Gum extract ofstem, leaf of *Corchorus tridens*

The phytochemical screening of plants were consist with the result found in Alaghazeer and EI-Saltani(2012)and partial agreement with previous studies Ramawat and Dass (2009)Ravishanker and Bhagyalakshmi(2007) Serrano and Puuponen (2009)Yadav and Kumar (2010).It is difficult to compare the data with the literature because several variables influence the results. According to some authours, the quantity and the composition of bio active compounds present in plant are influenced by the genotype,extraction,procedure, geographic and climatic conditions, and the growth phase of the plants. Ciulei and Istodor. (1995),Trease and Evans(2002).

The therapeutic benefits of secondary metabolism of plant origin have been researched in several recent studies,Nayak.et.,al(2006). The phytochemical screening results of plants are consistent with the results found in Alghzeer and Saltani, (2012) where authors mentioned the presence of tannins alkaloids, saponin and terpenoids in this plant. Similar analyses were conducted in areas that have a long tradition in the cultivation and utilization of medicinal plants, such as Pakistan Dai and Mumper (2010) and India Ravishankar and Bhayalakshmi(2007). Phytochemical screening results can be found in a database with the most important medicinal and aromatic plants in Calarasi-Silistra region.

POWDER MICROSCOPIC CHARACTERS OF SELECTED SPECIES OF TILIACEAE:

The current study was attempted to find out the presence ofPowder microscopic characters of *Corchorus aestuans* and*Corchorus tridens* .Presence and absence of certain important compounds in an extract is determined by colour reactions of the compounds with

specific chemicals which act as dyes.Different chemical compounds were detected in extracts of leaf and stem of *Corchorus aestuans* and leaf and stem of *Corchorus tridens* and were presented in Table 4 and 5.

The result of powder microscopic characters indicated that calcium oxalate crystals, Tannins, Aluerone grains of leaf and stem powder of *Corchorus aestuans.*

The result of powder microscopic characters indicated that Tannins,Eugenol, Aluerone grains of leaf and stem powder of *Corchorus tridens* .These results were supported by the work done by Maria Sumathi, 2014 and Sheela, 2014

Table 1: Morphological characters of selected species of Tiliaceae

Characters		***Corchorusaestuans***	***Corchorustridens***
Duration		Annual	Annual
Stem	Strength	Erect sparingly	Erect
	Internal	Solid	Solid
Leaf	Arrangement	Opposite	Alternate
	Composition	Simple	Simple
	Shape	Ovate	Lanceolate
	Margin	Serrated	Denate
	Apex	Acute	Acuminate
	Base	Rounded	Cuneate
Inflorescence	Position	Axillary	Terminal
	Calyx number	5	5
	Corolla number	5	5
	Corolla colour	Yellow	Yellow
	Stamen number	10	numerous

Table 2: Preliminary phytochemical screening of *Corchorus aestuans.*LWhole Plant Extract

Bioactive components	Aqueous Solution	
	Stem	Leaf
Alkaloids	_	_
Terpenoids	_	_
Steroids	+	+
Coumarin	+	+
Tannins	+	+
Saponin	_	_
Flavones	_	_
Phenols	+	+
Protein	_	_
Carbohydrate	_	_
Quinones	+	+
Gum	+	+

+: Present

-: Absent

Table 3: Preliminary phytochemical screening of *Corchorus tridens.*L*Whole* Plant Extract

Bioactive components	Aqueous	
	Stem	Leaf
Alkaloids	_	_
Terpenoids	_	_
Steroids	+	+
Coumarin	_	_
Tannins	+	+
Saponin	_	_
Flavones	+	+
Phenols	+	+
Protein	_	_
Carbohydrate	_	_
Quinones	+	+
Gum	+	+

+: Present

-: Absent

Table 4: Powder Microscopial characters

Micro chemical test *Corchorus aestuans* L.

S.No	Reagents	Observation	Characteristics	*Corchorus Aestuans*	
				Stem	leaf
1	Dil. Iodine Solution	Blue	Starch	_	_
2	Dil. Acetic acid	Insoluble	Calcium oxalate crystals.	+	+
3	Dil. Hcl	Soluble	Calcium oxalate crystals.	+	+
4	Aqueous extract + lead acetate reagent	White precipitate	Tannins	+	+
5	Aqueous extract+ potassium permanganate solution	Decolourisation	Tannins	+	+
6	Strong KOH solution	Needle shaped potassium eugenate crystals	Eugenol	_	_
7	Alcoholic picric acid	yellow	Aluerone grains	+	+

+: Present

-: Absent

Table 5: Powder Microscopial characters

Micro chemical test *Corchorus tridens* L.

S.No	Reagents	Observation	Characteristics	*Corchorus tridens*	
				Stem	Leaf
1	Dil. Iodine Solution	Blue	Starch	_	_
2	Dil. Acetic acid	Insoluble	Calcium oxalate crystals.	_	_
3	Dil. Hcl	Soluble	Calcium oxalate crystals.	_	_
4	Aqueous extract + lead acetate reagent	White precipitate	Tannins	+	+
5	Aqueous extract+ potassium permanganate solution	Decolourisation	Tannins	+	+
6	Strong KOH solution	Needle shaped potassium eugenate crystals	Eugenol	+	+
7	Alcoholic picric acid	yellow	Aluerone grains	+	+

+: Present

-: Absent

Table 6: Quantitative macro morphological measurements of studied taxa

Sl.No	Parts	*Corchorusaestuans*	*Corchorustridens*
1.	Leaf area	7.37 sq.cm	13.5 sq.cm
2.	Capsule length	1.82 cm	3.9 cm
3.	Petiole length	1.2 cm	0.73 cm
4.	Nodal diameter	0.77 cm	0.81 cm
5.	Inter nodal length	2.27 cm	4 cm

PLATE 1

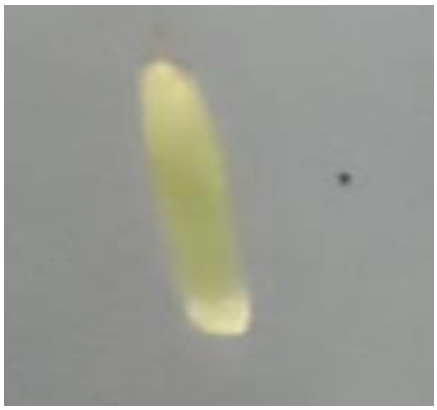
***Corchorustriden.* L.**

***Corchorusaestuans* .L.**

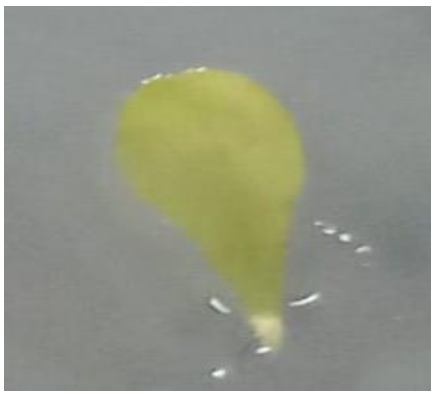
PLATE 2

A

B



C



D

E

F

***Corchorusaestuans* : Figure 3**: A-A twig with flower **B**-leaf **C**-calyx **D**-corolla **E**-stamen **F**-pistil

PLATE 3

A

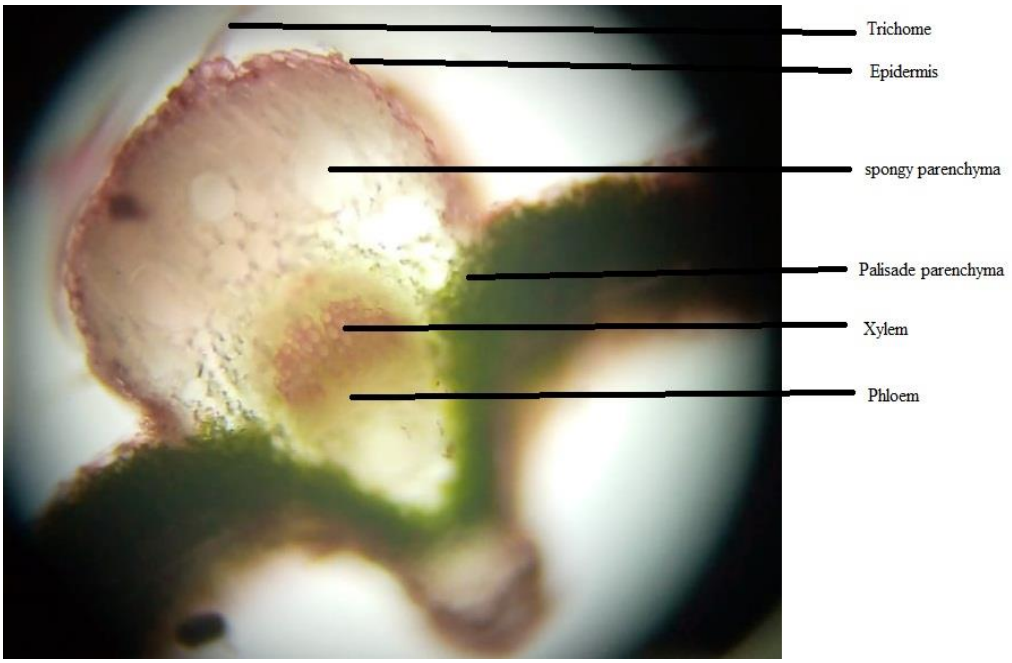
B

C

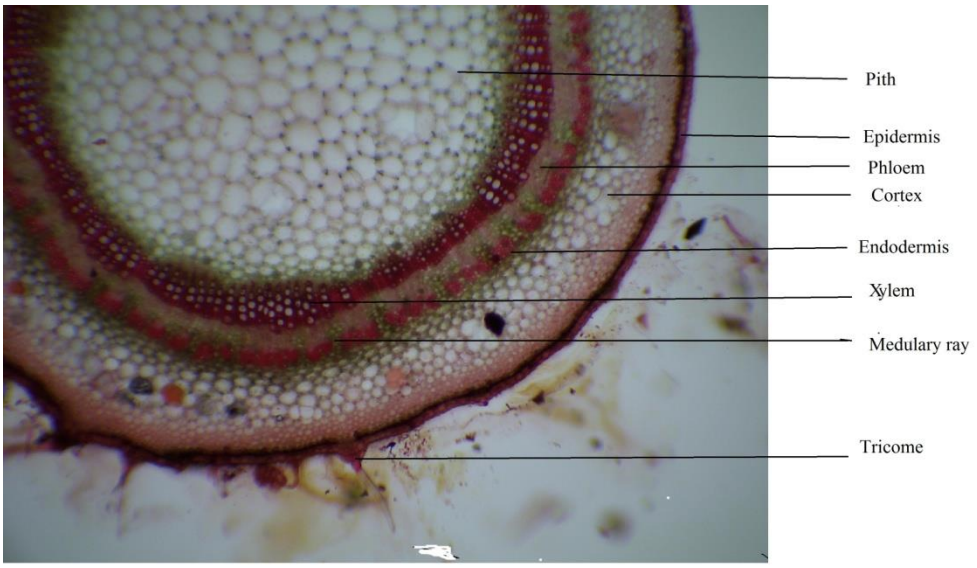
D

***Corchorustridens*: Figure 4**: A-A twig with flower **B**-leaf **C**-calyx **D**-corolla **E**-stamen **F**-pistil

PLATE 4

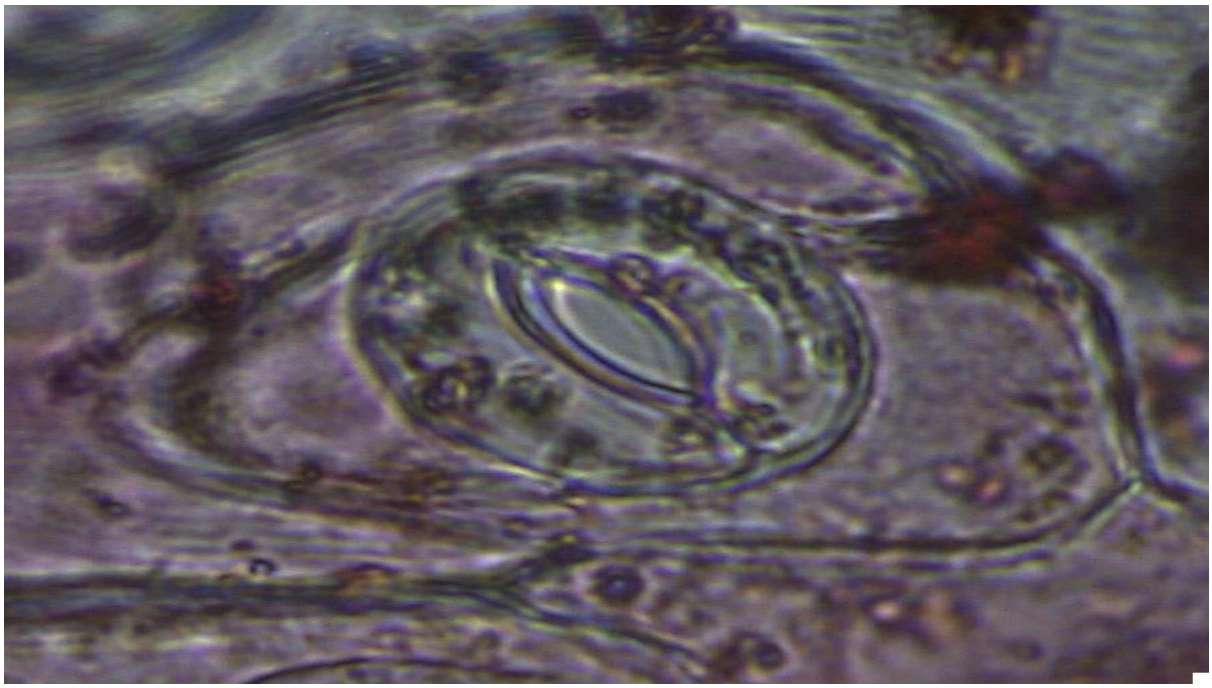


Corchorusaestuans:T.S of leaf

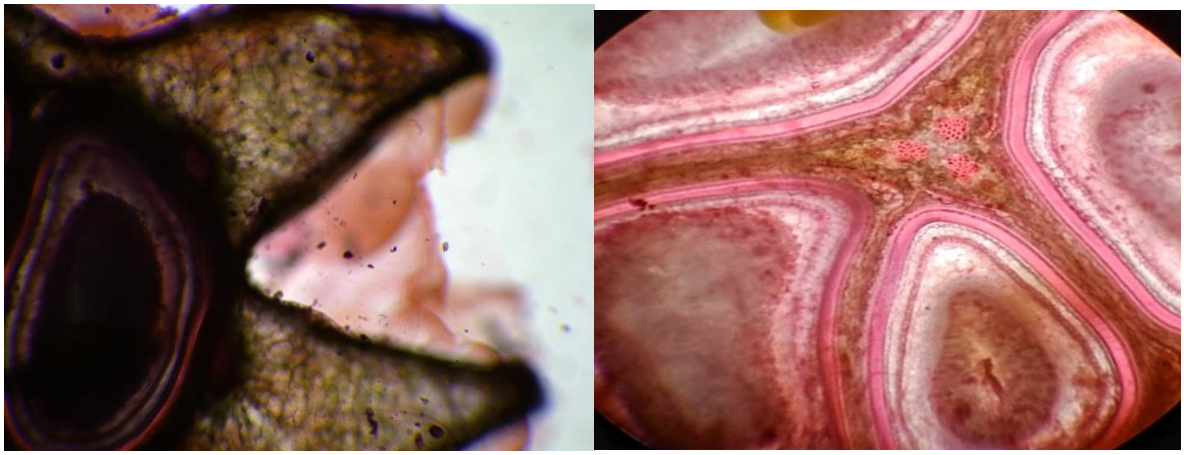


Corchorusaestuans:T.S of stem

PLATE 5

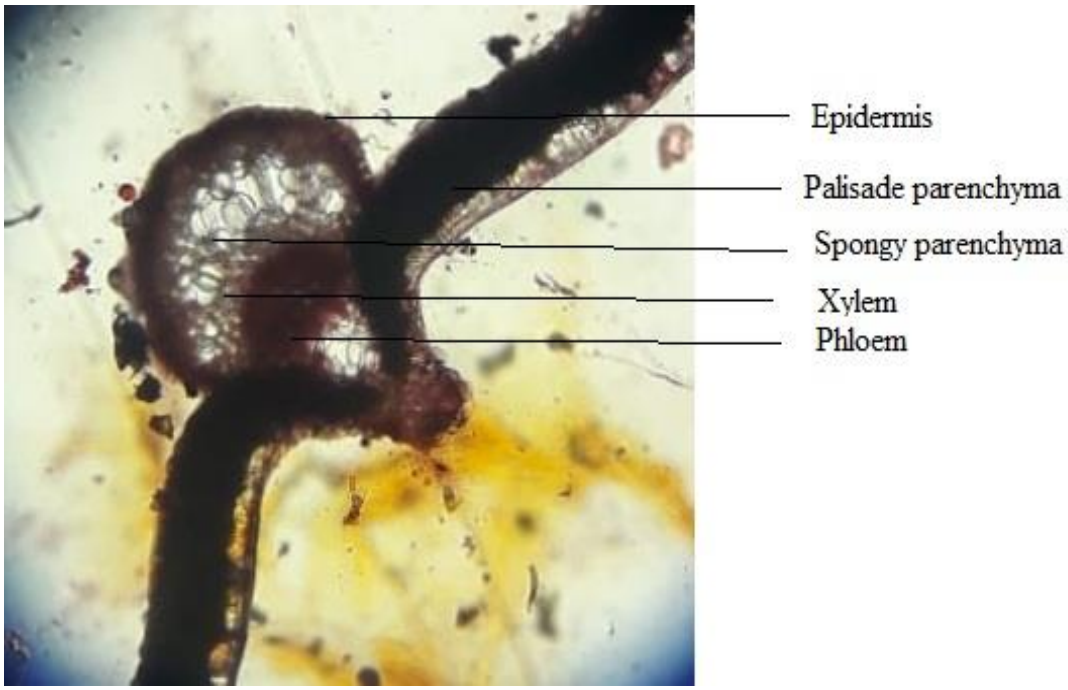


*Corchorusaestuans :*Stomata

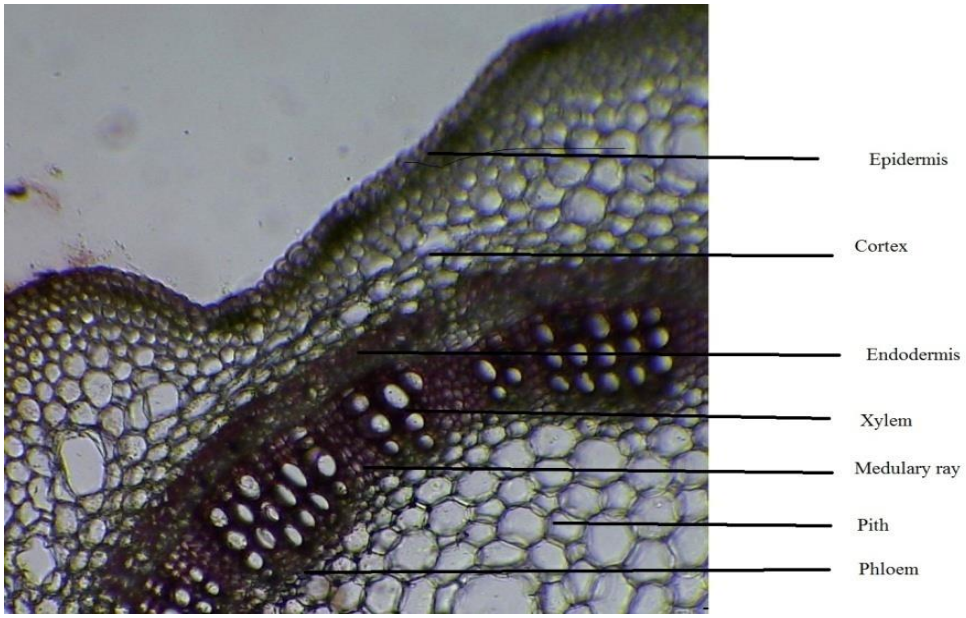


Corchorusaestuans : Capsule

PLATE 6

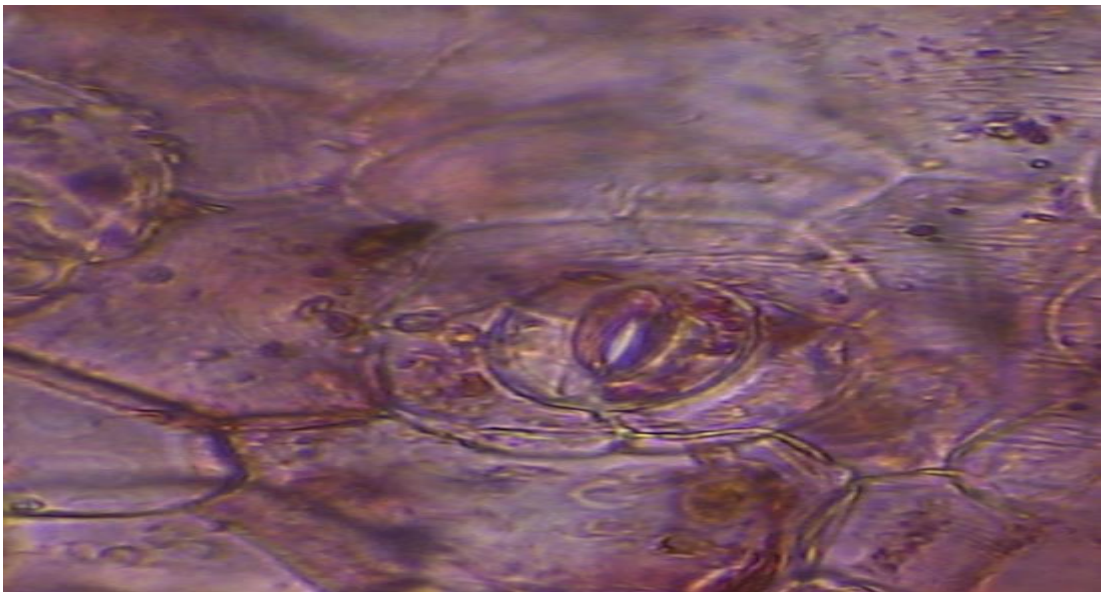


*Corchorustridens :*T.S. of leaf

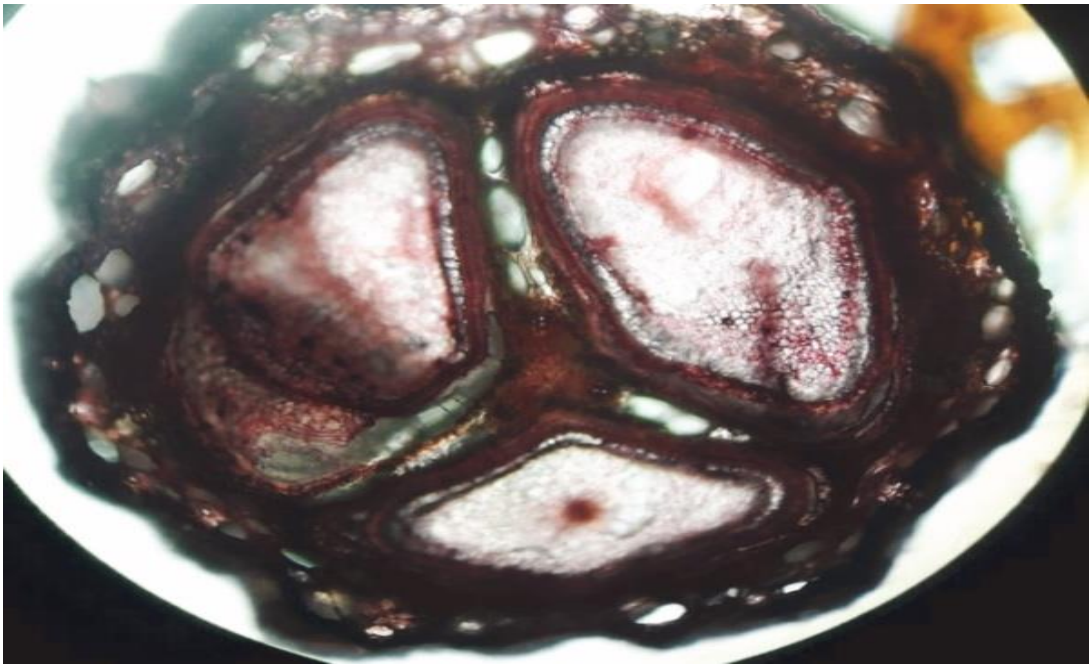


*Corchorustridens:*T.S. ofStem

Plate 7



Corchorus tridens : Stomata



Corchorus tridens : Capsule

SUMMARY

AND

CONCLUSION

SUMMARY AND CONCLUSION

In modern science, the importance of medicinal plants and increasing Iordachescu and Dumitriu, (1988). Nowadays, pharmaceutical and cosmetic industries are increasingly using plant resources from rural or unpolluted areas. Phytochemical screening is helpful to detect the various important compounds which could be used as the base of modern drugs for curing various diseases. The genus *Corchous* is a fibre plant and also used as a vegetable. Consumption of the leaves is reported to be demulcent, deobstruent, diuretic, lactagogue, purgative, and tonic. It is also a folk remedy for aches and pains, dysentery, enteritis, fever, pectoral pains, and tumors. Ayurvedics use the leaves for ascites, pain, piles, and tumorskeeping this in view the plants *Corchorusaestuens*L.and*Corchorus.tridens*L are selected for this study.

The stem, leaf and flower of *Corchorusaestuans* ,*Corchorustridens*were collected from Sundaravelpuram and St.Mary's College Campus,Thoothukudi for the current study.

From the present study, it was concluded that the morphologically they are dissimilar. Size of the leaf and shape of the stem is also vary. Morphological and anatomical features of plants are important in diagnosing the species. The results provide some pharmacognostic standards for the quality control of preparations from the plant in future. In both the species studied.

The epidermis in both species is with paracytic stomata on both surfaces. Trichomes are present in *Corchorusaestuans.* In both species. Epidermis have a thick cuticle.

Preliminary phytochemical like alkaloids, phenol, flavones, steroids, tannins, Coumarins and quinines are quantitatively screened.

The result of preliminary phytochemical screening indicates that stem, leaf of*Corchorusaestuans* is a rich source of Steroids, Coumarin, Tannins, Phenols, Quinones, Gum.

The result of preliminary phytochemical screening indicates that stem, leaf.*Corchorustrisens*of is a rich source of Steroids, Tannins, Flavones, Phenols, Quinones, Gum.

The result of micro chemical test indicate that stem, leaf of *Corchorusaestuans*is a rich source of Calcium oxalate crystals, Tannins , Aluerone grains.

The result of micro chemical test indicate that stem, leaf of *Corchorustrisens*is a rich source of Tannins, Eugenol, Aluerone grains.

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A COMPARATIVE STUDY ON CULTIVATION OF MUSHROOM USING DIFFERENT SUBSTRATES

A dissertation submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfillment of the requirement for the Degree of Master of science in Botany

By

P.ESAKKIAMMAL - 20APBO04

DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI - 628 001

2021 - 2022

CERTIFICATE

It is certified that this short term project work entitled "**A COMPARATIVE STUDY ON CULTIVATION OF MUSHROOM USING DIFFERENT SUBSTRATES**" submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfilment of the requirements for the degree of Master of science in Botany, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2021 – 2022 by the following student.

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DECLARATION

I do hereby declare that this work has been originally carried out by me under the guidance and supervision of **Ms. S. Pauline Jenifer**, Assistant Professor of Botany, St. Mary's College (Autonomous), Thoothukudi and this work has not been submitted elsewhere for the award of any other degree.

P. Esakkiammal

Place: Thoothukudi **Signature of the candidate**

Date: 26/05/2022 **(P.Esakkiammal)**

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P. Esakkiammal

CONTENT

INTRODUCTION

INTRODUCTION

Crop residues are largely abundant as agricultural waste after harvest. It is important to dispose agricultural waste in a green way which is environmentally friendly in this era of climate change. Extreme environmental conditions due to climate change are already being experienced **(Manyatsi *et al*., 2010)**. An alternative way of use of agricultural residues/wastes is in the use of the organic material in mushroom production (**Chang and Miles, 2004; Khare *et al*., 2010)**.

Mushrooms are a group of fleshy macroscopic fungi. They lack chlorophyll having heterotrophic mode of nutrition. They synthesize enzymes like cellulose and hemicellulose which bring the substrate to available forms. Mushrooms live on dead matter as they are saprophytes. **Chang and Miles (1992)** gave the definition that is now universally accepted. They defined mushroom as a "macro fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough to be seen with the naked eye and can be picked with hand." Mushroom cultivation has been reported as other effective way of alleviating poverty in developing countries (**Masarirambi *et al*., 2011**).

Human use of mushrooms extent as early to 5000 BC. About 2000 species of edible mushrooms are known all over the world. For centuries, some mushrooms have been used in religious ceremonies of many ancient people and primitive tribes. Mushrooms are believed by the Romans to have properties that could produce super human strength, help in finding lost objects and lead the soul to the realm of the gods **(Grube *et al.*, 2001).** Edible mushrooms are important sources of food. They form very nourishing meals

especially for invalids, for they are easily digestible. They are consumed not only for their innate flavour and taste, but also for their important nutritional value. On fresh weight basis mushrooms are superior in protein content (**Aremu *et al.,* 2009**) to all vegetables and fruits, but are inferior to meat and dairy products, which are the conventional protein sources. On dry-weight basis, however, mushrooms are similar with respect to dried yeast and superior to dried peas and beans.

Most mushroom derived preparation and substances find their use not as pharmaceutical but as a novel class of dietary supplements (DS) or ‚nutraceutical'. A mushroom nutraceuticals is a refined or partially refined extract or dried biomass from either the mycelium or the fruiting body of the mushroom, which is consumed in the form of capsule or tablets as a dietary supplement and which may enhance the immune response of human body, thereby increasing resistance to disease and in some cases causing regression of a disease state (**Wasser *et al.,* 2000**). Many pharmaceutical substances with potent and unique properties were isolated from mushroom and distributed worldwide (**Wasser and Weis, 1999**). Extensive clinical studies have explicitly illustrated that a number of mushroom species have medicinal and therapeutic value in the prevention /treatment of cancer, viral disease, hypercholesterolemia, blood platelet aggregation, and hypertension (**Jong *et al.,* 1991**, **Subramanian, 1995**).

Mushrooms are important sources of food. They are consumed not only for their innate flavor and taste, but also for their important nutritional value. The nutrient content varies from species and depends on their growth requirement. Mushrooms have a high percentage of water 93-95% as compared to learn beef (70%) and fresh vegetables (92%).

They also contain valuable minerals such as iron, potassium, phosphorus, calcium and copper, 56% carbohydrate, 30% protein, 2% fat and also 10% ash on dry weight basis. They are also rich in vitamin B and vitamin D. Mushrooms provide a high protein and low caloric diet and can thus be recommended to heart patients. They also contain all the essential amino-acid required by an adult (**Koyyalamudi *et al.*, 2009**). Mushrooms is reported to be an excellent source of riboflavin and nicotinic acid; a good source of pantothenic acid and ascorbic acid (**Ukpebor *et al.*, 2007**). The absence of starch in mushrooms makes it an ideal food for diabetic patients and for persons who wants to shed excess fat. **Kettawan *et al.* (2011)** and **Selvi *et al.* (2007)** have demonstrated that mushrooms contain antioxidants. Mushroom proteins contain all the essential amino acids and are especially rich in lysine and leucine, which are lacking in most staple cereal food. The low total fat content, and high proportion of polyunsaturated fatty acids (72-85%) relative to total fatty acids, is considered a significant contribution to the health value to mushrooms. Fresh mushrooms contain relatively large amount of carbohydrate and further range from 51-88% and 4- 20% mushroom appear to be a good source of vitamin including thiamine, riboflavin, niacin, biotin and ascorbic acid **(Andrae *et al.*, 1999).** They can be successfully used as appetizers in marinated form and also as an ingredient in soups, sauces, salads, stuffing and meat dishes **(Achremowicz *et al.*, 1983).** Mushrooms also contain many mineral salts and vitamins, particularly of the B and some D groups **(Mattila *et al.*, 2001).** 100 g of fresh mushrooms contains 5.3-14.8 g of dry matter, 1.5-6.7 g of carbohydrates, 1.5-3.0 g of protein and 0.3-0.4 g of fat. Recently, Dundar *et al*. (2009) have made a comprehensive study of effect of various substrate on the chemical composition and nutritional values of *P.ostreatus*.

Different varieties of mushrooms are seen in the world but all are not suitable for human consumption. Edible mushroom includes several wild and cultivated species. Most of the wild mushrooms are poisonous. Consumption of wild varieties without proper identification can cause mild symptoms to death. The colour and structure of mushrooms varies from species to species. Fruiting body of these macro fungi is made up of a network of fungal hyphae. Button mushroom (*Agaricus* spp.), shiitake mushroom (*Lentinus* spp.), oyster mushroom (*Pleurotus* spp.), wood ear mushroom (*Auricula* spp.), winter mushroom (*Flamulina* spp.) and straw mushroom (*Volvariella* spp.) are the most common cultivated species.

Oyster mushroom accounted for 14.2 % of the total world production of edible mushroom in 1997 **(Chang, 1999).** Oyster mushroom cultivation can play an important role in managing organic wastes whose disposal has become a problem **(Das and Mukherjee, 2007).** Oyster mushroom can be cultivated in any type of ligno cellulose materiallike straw, sawdust, rice hull, etc.

Malnutrition is a problem in developing third world countries. Mushrooms with their flavour, texture, nutritional value and high productivity per unit area have been identified as an excellent food source to alleviate malnutrition in developing countries **(Eswaran and Ramabadran, 2000).** Among the reasons for the quick acceptance of mushroom is its nutritive content. Mushrooms are eaten as meat substitutes and flavouring. In general edible mushrooms are low in fat and calories, rich in vitamin B and C, contain more protein than any other food of plant origin and are also a good source of mineral nutrients **(Bahl, 1998).**

Nanotechnology is a fast developing field which has now contributed to exciting transformation in biomedical and engineering in terms of effectiveness, health and economy. Nanobiotechnology is the descendant of nanotechnology and biology. Nanoparticles coated with molecules or bio-moieties are getting attention in nano drug delivery which has unique and targeted uses without affecting the cells in nearby areas of the target. Nanomaterials synthesized in laboratories can enter human body and cause potential health hazards. Therefore, there is a need to establish green chemistry approaches in the nanomaterials synthesis **(Albrecht *et al.*, 2006).** In this respect, synthetic methods based on naturally occurring biomaterials offer better alternative approaches. Silver nanoparticles have enormous capabilities other than its miniature size aspect, which will lead to excellent antimicrobial activity as compared with bulk Ag metal **(Mahendra *et al.*, 2009).** Hence, the presence of silver 5 nanoparticle producing efficiency of mushrooms is added to its pharmacological relevance.

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIVE

Oyster mushroom can be grown on different substrates containing lignin and cellulose. Demand of mushroom for consumers has been increasing day by day. As substrate plays an important role in determining yield of mushroom and it is necessary to evaluate different substrates for mushroom yield and also to find the best suitable substrate for its cultivation. The present research work was planned to protect the environment by utilizing the agro waste for mushroom cultivation because it is not only capable of bioremediation of waste but also provides a highly proteinaceous food. Therefore, the present study was carried out with the following objectives:

- Preparation of substrate for spawn production
- Cultivation of *Pleurotus florida*
- Study the effect of different substrates on the growth and morphological parameters of *Pleurotus florida*
- Qualitative analysis of the fruit body of *Pleurotus florida*
- Estimation of carbohydrate, protein, amino acid and lipid content of *Pleurotus florida*
- Study the antimicrobial efficiency of *Pleurotus florida.*
- Analyze the effectiveness of *Pleurotus florida* in green synthesis of silver nanoparticles.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Mushroom is a fruiting body of saprophytic fungi which is commonly found on decaying natural materials. Edible mushrooms have been a part of human diet since time immemorial. It serves as a good source of many macro and micro nutrients which helps to maintain healthy immune system and reduce the risk of nutrient deficiency. Mushroom cultivation also plays a key role in managing agricultural wastes. Mushrooms represent one of the world's greatest untapped resources of nutritious food. Cultivation of saprophytic edible mushrooms may be the only currently economical biotechnology for lignocelluloses organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution **(Obodai, 2003).**

Thousands of years ago, fructifications of higher fungi have been used as a source of food **(Mattila *et al*., 2001)** due to their chemical composition which is attractive from the nutrition point of view. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors. Edible mushrooms are regarded as a curative food having anti-carcinogenic, anti-cholesteromic, anti-viral properties and prophylactic properties with regard to hypertension and heart disease (**Mattila *et al*., 2000**). Some Asian communities use mushrooms for food and medicine traditionally. Generally, mushrooms have higher protein content than most vegetables. They are rich in minerals and vitamins. Fat content is low. The fat fraction is mainly composed of unsaturated fatty acids **(Manzi *et al*., 2001; Mattila *et al*., 2001).**

The nutritional and medicinal values of mushrooms have long been recognized. In recent times, mushrooms have assumed greater importance in the diets of both rural and

urban dwellers. For example, they are being marketed along major highways and urban centers where the trade now booms. It is conceivable that the increased demand for mushrooms is contingent upon the phenomenal rise in the unit costs of the conventional sources of meat (e.g. beef, pork, chicken, etc.) **(Abulude, 2017).**

CULTIVATION:

Agricultural waste disposal might be difficult for community farmers, however organic waste can be utilised as productive substrates for mushroom development. Wheat bran (WB) feeding to the substrate can increase mushroom yields. The goal of this research was to compare the effects of various factors. Banana leaves, sugarcane tops, maize stover, and maize stover were utilised as substrates for mushroom growth and yield. and cobs (1:1 dry mass/dry mass) with varying quantities of wheat bran amendments of 0%, 10%, and 15% Wet substrates were used. sterile, packaged in plastic bags, seeded with 2-4 percent spawn, and cultured for 3-3.5 months The bags were incubated to allow for full development. colonisation of numerous places **(Diana M. Earnshaw *et al,* 2012)**

Use of costly substrate for growing oyster mushroom increases their cost of production. So, there was need to search for certain alternative materials which should be available in sufficient quantity at relatively cheaper price (**Arya and Arya, 2003**). Rice straw, wheat straw, ragi straw, hulled maize cab, waste paper were tried in different studies. *Pleurotus* has been reported to grow readily on a number of non-conventional substrates **(Das *et al*., 2000; Mukherjee and Nandi, 2002; Nageswaran *et al*., 2003). Bandopadhyay and Chatterjee (2009)** reported that highest yield of *P.*

florida was obtained after three flushes in beds of combined substrates (paddy straw and water hyacinth) followed by paddy straw alone and water hyacinth alone.

Jeznabadi *et al*., (2016) evaluated the effect of different sources of Iranian agricultural wastes on the production parameters and protein content in the cultivation of *P. eryngii*. Wheat straw, wood chips, sawdust, sugar beet pulp, barley straw and maize stem residues were used as basal substrates, whereas wheat bran, rice bran, soybean powder and their combinations were used as supplement. Barley straw supplemented with rice bran gave the highest mushroom yield, followed by sugar beet pulp supplemented with rice bran, whereas the substrate with the worst performance was sawdust supplemented with rice bran. Protein content was differently affected by the various substrates, ranging between 4.64% (barley straw +wheat bran and wood chips + soybean powder + rice bran treatments) and 13.66% (wheat straw + wheat bran+ soybean powder treatment).

Yildiz *et al*. (2002) used different lignocellulosic wastes as raw materials for the cultivation of *P. ostreatus* and they reported that a mixture of sawdust with hazelnut leaves (50:50) was one of the substrates with the major biological efficiency. However, when the percentage of hazelnut leaves was increased over 50%, the mushrooms yields was decreased, so the authors concluded that leaf of hazelnut is not very appropriate, as the growth substrate in percentages higher than 50%. **Peksen and Kucukomuzlu (2004)** evaluated the effect of different substrates on the cultivation of different *Pleurotus* species (*P. ostreatus, P. sajor-caju,* and *P. sapidus*). The authors observed that total yield, biological efficiency and morphological parameters were statistically different in different species. They concluded that the optimum substrate for *Pleurotus* growth was the combination of hazelnut husk, wheat straw and wheat bran in the ratio 1.5:2:0.5. However,

the biological efficiencies of the substrates containing hazelnut husks were lower compared to the control composed of wheat straw + 5% wheat bran.

The viability of employing sugarcane bagasse (SGB) as an alternative to rubber tree sawdust for the production of *Pleurotus ostreatus* (oyster mushrooms) was investigated by (**Ahmad Zakil *et al*, 2020)**. The substrate was made entirely of different concentrations of rubber tree sawdust .They observed highest output in terms of stipe height, which was 7.75cm.

The influence of different types of spawning on oyster mushroom (*Pleurotus ostreatus*) production was investigated by (**Pathmashini *et al***, 2008) using sawdust. Kurakkan (Eleusine coracana), maize (broken) (Zea mays), sorghum (Sorghum bicolor), and paddy (Oryza sativa) grains were utilised to produce the spawn. Different sorts of sawdust spawned spawn running (mycelia development), pinhead formation and fruit body formation, mean yield, and biological efficiency were all evaluated in *P. ostreatus* spawns. They observed fastest spawning time was 21 days in sorghum grain based spawn.

Mushroom requires nutritional source like carbon, nitrogen and inorganic compounds during their life cycle. Their growth is mainly depended on some specific carbon sources such as cellulose, hemicellulose and lignin. *Pleurotus* species requires high carbon and less nitrogen source. However, requirement of nutritional source differs from species to species **(Sharma *et al.*, 2013).**

Dey *et al.* (2008) examined the effect of different substrates such as paddy straw, sugarcane bagasse and mustard straw on the production of oyster mushroom by following cylindrical block method. Specific substrates greatly influenced the count 11 of primordia,

fruiting bodies and the yield. The highest number of primordia, fruiting bodies and maximum yield was obtained with sugarcane bagasse while the lowest with mustard straw.

Islam *et al.* (2017) cultivated *Pleurotus ostreatus* upon a number of substrates that specifically influence the pattern of pin head and fruiting body formations. Cotton waste performed as a better substrate and it took minimum days (7.5) to begin pinhead formation followed by Chenopodium album and mixture of cotton waste + Chenopodium album. More fruiting bodies were developed when cultivation was based on cotton waste, Chenopodium album and mixture of cotton waste + Chenopodium.

PHYTOCHEMICAL SCREENING:

Edible mushroom has been recognized for a long time not only as a delicacy, but also for their use as food in man's diets. Mushrooms have been found to be rich sources of protein, lipids, amino acids, glycogen, vitamins and mineral elements **(Okhuoya *et al.*, 2010).** Mushrooms are used as possible treatments for diseases. *Lentinula edodes* (Shiitake), *Grifola frondosa* (Maitake) and *Ganoderma lucidum* (Reishi), have a history of medicinal use spanning millennia in parts of Asia. Medicinal mushroom possesses cardiovascular, anti-cancer, anti-viral, anti-bacterial, anti-parasitic, anti-inflammatory, hepato protective, and anti-diabetic activities **(Lentinan, 2009).**

The species that have been properly analyzed for medicinal value are: *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Grifola frondosa* (Maitake), *Agaricus blazei* (Hime-matsutake), *Cordyceps militaris* (Caterpillar fungus), *Pleurotus ostreatus* (Oyster mushroom) and *Hericium erinaceous* (Lions mane). There are many more species of cultivated and wild edible and non-edible mushrooms that have been analyzed for both

their nutritional and nutraceutical components **(Lakhanpal and Rana, 2005).** The active constituents found in mushrooms are polysaccharides, dietary fibres, oligosaccharides, triterpenoids, peptides and proteins, alcohols and phenols and mineral elements **(Pardeshi and Pardeshi, 2009)**

Bioactive proteins are an important part of functional components in mushrooms and also have great value for their pharmaceutical potential. Mushrooms produce a large number of proteins and peptides with interesting biological activities such as lectins, fungal immune modulatory proteins, ribosome inactivating proteins, antimicrobial proteins, ribonucleases and laccases **(Xu, *et al.*, 2011).** Polyunsaturated fatty acids are mostly present in edible mushrooms; thus, they may contribute to the reduction of serum cholesterol. It is noteworthy that trans isomers of unsaturated fatty acids have not been detected in mushrooms (**Guillamon *et al.*, 2010; Barros *et al.*, 2007**).

Khan *et al.* (2009) investigated the total protein, carbohydrate, lipid, crude fiber and mineral (ash) contents of *Agaricus bisporous, Pleurotus citrinopleatus, Pleurotus eryngii, Auricularia polytricha, Ganoderma lucidum, Hypsizygous ulmarius, Agrocybe aegerita, Volveriella volvacea, Lentinus edodes, Coprinus comatus and Coriolus versicolor*. The protein, carbohydrate, total lipid, crude fiber, and total mineral contents ranged from 18g to 38g, 9g to 50g; 1g to 12g; 8g to 52g and 5g to 12g per 100g of the mushroom species respectively. The metabolizable energy content of these selected mushrooms ranged from 150 Kcal /100 kg to 300 Kcal /100 kg of mushroom. Mshandete and Cuff (2007) analyzed the proximate content of three wild edible mushrooms namely *Coprinus cinereus, Pleurotus flabellatus, Volvariella volvaceae* using dried sample. Amount of protein, vitamin C, minerals, crude fiber, carbohydrate, fat content and energy

values ranged from 17-28 %, 33-55 mg/100 g, 5.2-3232 mg/100 g, 6.6-11 %, 50-62 %, 1-3.3%, 302-313 kcal/100 g respectively. Research conducted by **Banik and Nandi (2004)** informed that supplementation of rice straw with biogas residual slurry manure has significant effect on the improvement of the yield capacity, protein and mineral nutrient contents of *Pleurotus sajorcaju* mushroom. The antioxidant activity of two organic extracts of *Pleurotus osteratus* mushroom employing the solvents ethyl acetate and methanol resulted in a greater total phenolic content **(Anjana Shree K.G and colleagues, 2016)**. Secondary metabolites, antioxidant capabilities, and mineral element and heavy metal concentrations in macrofungi methanolic extracts were investigated by **Mary Obodai *et al.*, 2014**. The 12 strains differ statistically from one another.

ANTIBACTERIAL ACTIVITY

Pleurotus ostreatus was gathered in the wild and tested for antibacterial and antioxidant properties. The antibacterial activity was next tested against certain microorganisms using methanolic and aqueous extracts. In different organisms, the inhibitory zone for methanolic extract varies. Except for *Pseudomonas aerungiosa*, no inhibitory zone was seen in the aqueous extract. *Pleurotus ostreatus* inhibitory effect can thus be linked to the presence of phenolic and flavonoid chemicals in the extracts. **(Fakoya and colleagues, 2020)**

Two mushroom organic extracts were found to be efficient against certain bacteria when tested using the agar well diffusion method. The anti-gram negative bacterial activity of petroleum ether extract was higher than that of acetate extract, and the total phenolic content of acetone extract was similarly higher. Some chemicals had low and moderate

levels of phytochemical analysis, while others were not discovered. The findings suggest that this mushroom has antibacterial and antioxidant properties. **(Iwalokum *et al.*, 2007)**

Methanolic and aqueous extracts of dried mushrooms were analysed for phytochemical screening, antibacterial activity, antioxidant activity (DPPH), and total phenolic content. The phytochemical analysis revealed the presence of active substances. *Pleurotus ostreatus* had excellent antibacterial efficacy against all microorganisms studied. The antioxidant activity of mushroom extracts was shown to be favourable. The findings of this investigation revealed mushroom extracts' potential as an effective medicinal agent and food supplement. **(Sanjay Parihar *et al.*, 2015)**

QUANTITATIVE ANALYSIS:

The nutritional and therapeutic value of mushrooms is not widely recognised. The nutritional properties of dietary mushrooms *Pleurotus ostreatus, Pleurotus sajorcaju, Pleurotus florida,* and *Calocybe indica,* which are common among grown mushrooms in Bangladesh, were investigated in this study determined. These mushrooms were high in protein (20-25%) and fibre (13-24%) in dry samples, but had a reduced fat content lipid content (4 to 5 percent). Carbohydrate content varied between 37 and 48 percent (on the basis of dry weight). These had been Mineral content is also high (total ash content is 81.3%). The pileus and gills were high in protein and lipids, and the stripe was yellow high in carbohydrates and fibre Mushroom moisture content ranged from 86 to 87.5 percent. According to the findings of this investigation, mushrooms are edible **(Nuhu Alam *et al,* 2008).**

The mushroom's nutrient profile backs up assertions that it can be regarded as a functional food because it has health benefits for people of all ages. We came to the conclusion that rubber wood sawdust was a great base substrate for growing oysters. Mushrooms were grown on a commercial basis, and the quality of the oyster mushrooms harvested met national standards. particularly with regulatory norms and international quality standards (EU defined limitations) in terms of heavy metal contamination, and that CRC should market their product outside of Nigeria's boundaries. (**Igilu G.O *et al*, 2020**)

The nutrients and anti nutrients present in *Pleurotus ostreatus* (Jacq.) P. Kumm, an edible fungus discovered in Akwa Ibom state, Nigeria, were evaluated by **Okon *et al*, 2015**. Tannins, Phytate, Oxalate, and Hydrogen Cyanin are antinutrients that, when consumed in large amounts, can harm humans by impairing protein digestion and stunting growth. However, this research revealed that anti nutrients are present in modest levels in *Pleurotus ostreatus*. The structural variances between these mushrooms are due to differences in environmental circumstances and substrate.

Large fruiting bodies (LFB), small fruiting bodies (SFB), and the base were separated from king oyster mushrooms (*Pleurotus eryngii*). The LFB made up 79.90% of the overall weight, while the base made up 15.47 percent. 3-octanone, 1-octen-3-one, 3-octanol, and 1-octen-one were among the volatile chemicals discovered. The primary compounds in LFB and SFB were 3-ol, benzaldehyde, 1-octanol, and 2-octen-1-ol.benzaldehyde. Total free amino acids were abundant in both LFB and SFB. Sweet and delicious In all three portions, bitter components were comparable, while monosodium glutamate-like components were not. LFB and SFB had high levels of components. Six 5-nucleotides were discovered in three sections, the first of which was The highest

concentration was 5-cytosine monophosphate. The amounts of flavour 5'-nucleotides in LFB and SFB were similar. Those at the base are comparable and higher. LFB and SFB were shown to be similar in this investigation.(**Jeng-Leun Mau *et al*, 1998**)

The composition and sensory evaluation of chicken patties containing various quantities of grey oyster mushroom (*Pleurotus sajor-caju*, PSC) were investigated. Fresh PSC was used in the formulation of the chicken patties, which ranged from 0% to 50%. The results showed that a chicken patty prepared with 25% PSC had protein. Although 17.46 percent less than the control patty (18.13 percent), the difference was not significant. Protein, on the other hand, When chicken meat is replaced with 50% PSC, the content drops dramatically. Meanwhile, both were prepared. The fat content of chicken patties using 25% and 50% PSC was much reduced, at 10.67 percent and 10%, respectively. 7.15 percent and 7.15 percent, respectively. Furthermore, a patty containing 25% PSC had a moisture level of 56.91 percent, which is high. Significantly lower than the moisture content of the 50 percent patty, which was 58.80%. (**Wan Rosli W.I *et al*, 2011**)

The nutrient contents of oyster mushrooms grown on two substrates, wheat straw and brassica straw, were evaluated in this study. On a fresh weight basis, the moisture content of both species of mushroom was 89.68 and 88.98 percent, respectively, with no significant differences. Similarly, there were no significant differences in crude fibre, crude fat, ash, and energy. whereas there was a substantial difference in crude protein (25.30 and 26.99 percent) and carbs in total (52.34 and 50.52 percent). Both species of mushrooms had a lot of it. Vitamins, amino acids, and dietary fibre are all important. The readings for thiamine (1.18 and 1.13 respectively) were reported. lysine (6.00 and 6.25 g/100 g protein)

and riboflavin (3.89 and 3.52 mg/100 g).On a dry matter basis, wheat has 1.80 and 1.75 mg of methionine per 100 g.(**Vimla Dunkwal *et al*, 2009**)

SILVER NANO PARTICLES:

For thousands of years, mushrooms have been part of the human diet, and in recent years, the amount consumed has increased dramatically, involving a wide range of species. Mushrooms are utilised for both nutritional and medicinal purposes. Silver nanoparticles were synthesised in this study.A wild mushroom (*Agaricus bisporus*) and an edible mushroom (*Agaricus bisporus*) (*Ganoderma lucidum*) UV–vis spectroscopy, FTIR, and XPS were used to characterise the synthesised nanoparticles.SEM and powder XRD Silver nanoparticles were made at ambient temperature and at high temperatures.60 °C. The presence of bioactive functional groups is recognised by FTIR findings.Silver nitrate is reduced to silver nanoparticles. The XRD revealed the following:Silver nanoparticles with an average size of 10–80 nm are used. The nanoparticles of silver are Photocatalytic activity and biological activity were investigated.(**Mohana srimulu *et al*, 2017**)

Due to its unique qualities, such as low cost, simple synthesis methods, high water solubility, and eco-friendly nature, research and innovation in nanoparticles (NPs) synthesis produced from biomaterials has gotten a lot of interest. macrofungal NPs, which include a variety of *Agaricus bisporus, Pleurotus spp*., *Lentinus spp*., and *Ganoderma spp*. are examples of mushroom species. high nutritive, immune-modulatory, and antimicrobial (antibacterial, antifungal) value characteristics (antiviral, antioxidant, and anticancer). Metal uptake occurs intracellularly in fungi. They have excellent metal tolerance due to their ability and maximal wall binding capacity. bioaccumulation capacity To begin, two

methods have been identified in the literature. create metal NPs from macrofungi (intracellular technique), which refers to NP synthesis ions are transported inside fungal cells in the presence of enzymes;(**Kanchan Bhardwaj *et al*., 2020**)

Twelve extracts from four edible oyster mushroom species were evaluated for their chemical value (proteins, carbohydrates, and phenols) as well as their ability to biosynthesize silver nanoparticles (AgNPs). Three forms of growth were seen in limited conditions of dark incubation and temperature of 25 °C. Preparation of extracts has been developed. The properties of silver nanoparticles made from extracts Color and UV-Visible spectroscopy were used to confirm silver nanoparticles in 1 mM AgNO3 formation. *Pleurotus cornucopiae* var. citrinopileatus was discovered as a bright yellow oyster mushroom. a possible contender for the production of AgNPs The brown colour of the aqueous solution indicated formation of AgNPs The absorption band of AgNPs was found to peak at 440 nm for P, according to the results. citrinopileatus var. cornucopiae Others, however, P. ostreatus (**Mustafa Nadhim Owaid *et al*, 2017**)

The current research focuses on a simple, low-cost, quick bio-reduction of silver nitrate to silver nanoparticles (AgNPs) utilising hot water extracted from *Pleurotus cornucopiae* var. citrinopileatus basidiocarps. *P.cornucopiae* is a medicinal mushroom that can be eaten. UV–visible (UV–vis) light. The spectra revealed high absorption peaks between 400 and 500 nm, which are characteristic absorption bands of Fourier transform infrared (FT-IR) spectroscopy verified the presence of spherical AgNPs. For the reduction and capping of AgNPs, functional groups contained in biomolecules are used. The spherical shaped Field emissions scanning validated the morphology and o100nmparticlesizeofthesampleAgNPs.High-resolution transmission electron microscopy

(FESEM) and electron microscopy (FESEM) (HRTEM). Energy dispersiveX-rayanalysis(EDX)illustratedthattheAgNPswerecrystallineinnature. Mycosynthesized AgNPs decreased the growth of all Candida species examined considerably (po0.05). (**Mustafa Nadhim Owaid *et al*., 2015**)

The efficacy of silver generated at different concentrations (100, 200, 300, 400, 500, and 600) g/mL against Candida albicans isolates was examined, as well as the quality of silver nanoparticles AgNPs biosynthesized from a silver nitrate solution utilising the oyster *Pleurotus ostreatus.* The results showed that the *P. ostreatus* fungal filtrate was effective in the synthesis of silver nanoparticles. Characterization of bio produced AgNPs depending on the physical properties also proved product quality. UV Spectrophotometer Analysis, Scanning Electron Microscopy Test (SEM), and Energy Dispersive Spectroscopy are some of the characteristics. X-Ray Diffraction Technology (EDS) (XRD). The findings revealed that all of the different silver nanoparticle concentrations were effective (100-600) None of the Candida albicans isolates were inhibited by g/ml. In general, higher Nano-silver concentrations may be harmful.(**Wifaq Ahmed Mahmood *et al*, 2020**)

It is well known that silver has strong toxicity effect against wide range of microorganism and silver nanoparticles showed to be promising antimicrobial materials (**Jo DH *et al.*, 2015 and Jain *et al*.,2009**).

Using oyster mushroom *Pleurotus citrinopileatus* extract as a reducing agent and aqueous silver nitrate as the precursor, silver nanoparticles (AgNPs) were created. The development of AgNPs was noticed when the mixture's colour changed from colourless to dark-brownish. Energy Dispersive Spectroscopy (EDS) and Transmission Electron

Microscopy (TEM) The size, form, and content of manufactured materials were confirmed using X-Ray spectroscopy (EDX). In addition, UVVIS The reducing agent is identified as ethylene groups via spectroscopy and Fourier Transform Infrared (FTIR). for the production of AgNPs, as well as a capping agent This green synthesis is both cost-effective and environmentally benign. A simple way to make AgNPs. Colloid AgNPs have a bactericidal impact on harmful microorganisms like *E. coli. Staphylococcus aureus* and *Escherichia coli*.(**Maurya *et al*, 2016**)

Silver and silver salts have been used since the dawn of civilisation, but silver nanoparticles (Ag NPs) have just lately been discovered. They've been employed as antibacterial, antifungal, and antioxidants in agriculture and medicine. Ag NPs have been shown to stop the growth and multiplication of numerous bacteria, including *Bacillus cereus, Staphylococcus aureus, Citrobacter koseri, Salmonella typhii, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhii, Pseudomonas aeruginosa*, Escheria coli By binding Ag/Ag+ with biomolecules*, Klebsiella pneumoniae, Vibrio parahaemolyticus,* and *Candida albicans* are able to survive. present in the cells of microbes Ag NPs are thought to form reactive oxygen species and free radicals. which trigger apoptosis (cell death) and hinder cell multiplication Because Ag NPs are smaller than microbes, they are more effective. They enter the cell and rupture it.(**Khwaja Salahuddin Siddiqui *et al,* 2018**)

The aqueous extract of edible oyster mushroom (*Pleurotus florida*) was used as a reducing agent in the photo-irradiated extracellular production of silver nanoparticles. UV-visible spectroscopy, field emission scanning electron microscopy, transmission electron microscopy, and atomic force microscopy are used to study the appearance, size, and form

of silver nanoparticles. microscopy. Particles are crystalline in nature, according to X-ray diffraction investigations and energy dispersive X-ray analyses. Fourier The nanoparticles' surface is covered with biomoieties, according to an examination using transform infrared spectroscopy. As can be According to our findings, the biofunctionalized silver nanoparticles so created have excellent antibacterial properties, and Because the synthetic technique is both eco-friendly and simple, high-volume manufacture of the same can be explored. many applications(**Ravishankar Bhat *et al*, 2011**)

Silver nanoparticles have important applications in biology and medicine.Using *Agaricus bisporus*, *Calocybe indica*, *Pleurotus florida*, and *P. platypus*, silver nanoparticles were produced quickly within 72 hours of incubation. The mushroom extract incubated in deionized water did not lose its potency. The mushroom extract treated with silver nitrate turned brown. Due to the formation of silver nano particles after 72 hours UV visible research indicated the creation of the surface Plasmon resonance around 300nm, which nanoparticles of silver Functional groups were discovered in the FTIR studies. Silver nitrate is reduced to silver ions by this enzyme. Scanning electron microscope Microscopy has revealed more about the morphology and size of the cells. (**Sujatha *et al*, 2013**)

A wild strain of L. edodes was exposed to UV irradiation at 254 nm, a physical mutagen. The wild and irradiated strains were then tested for the generation of EPS and the subsequent use of the crude EPSs for AgNP biosynthesis. Color pattern and UV-visible spectroscopy were used to identify the particles. Superior EPS synthesis and nanoparticle characteristics Scanning Electron Microscopy was used to examine the nanoparticles produced by the UV irradiation method (SEM). EPS was created. The phenol-sulphuric

acid method was used to measure it, and GC-MS was used to investigate it. (**Adeyemi ojutalayo Adeeyo *et al*, 2018**)

The biosynthesis of silver nanoparticles is a straightforward, cost-effective, and environmentally benign method. The current study revealed the biosynthesis of silver nanoparticles utilising certain commonly available edible mushroom extracts and their antibacterial properties. UV, FTIR, and SEM were used to validate the creation of silver nanoparticles, and disc diffusion was used to investigate antibacterial activity. The successful synthesis of silver nanoparticles using mushroom extracts was validated by the results; they served as a reducing and capping agent and also had robust antibacterial action against S. aureus (gram positive bacteria). As a result, the manufacture of silver nanoparticles utilising edible mushroom extract merits consideration as a potential antibacterial agent (**Sankaran Mirunalini *et al*, 2012**).

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Collection of Agricultural Waste

Agro waste materials such as coir and sugarcane bagasse were used as the substrates for the present study. coir was collected from farmers and sugarcane bagasse was purchased from sugarcane vendors. These substrates were dried and stored. They were used for the experiments.

Spawn

Sorghum grain-based spawn of *Pleurotus florida* was procured for the present study from certified cultivation centre, MSM Mushroom Corner, Mushroom Cultivation Training and Seed Sale, Rediyarpatti, Tirunelveli.

METHODS

CULTIVATION OF MUSHROOM:

In the present study, the edible oyster mushroom *Pleurotus florida* was cultivated using the standard procedure given by Tamil Nadu Agricultural University.

Experimental Design:

In the present study, *Pleurotus florida* was cultivated by bag method using three different agro waste materials as substrates. The composition of the treatments was given below.

- **Treatment 1:** Sugarcane bagasse

- **Treatment 2:** Coir waste

Substrate Preparation:

Soaking:

The selected agro wastes (sugarcane bagasse and Coir) were cut into small pieces (6 -10cm) and soaked in water for 12 – 14 hrs

Sterilization:

Soaked substrates were sterilized at 121ºC for 20-30 minutes by using pressure cooker. After sterilization, the excess amount of water content was removed and cooled down by shade drying in the room temperature.

Bag Preparation:

Before starting the packing, hands were washed thoroughly with the help of antiseptic lotion. Polypropylene bags with the size of 60 x 30 cm and with a thickness of 80 gauges were used for the cultivation. The bottom end of the bag was tied with the help of thread and turned toward the inside.

Layering of Substrate:

The sterilized substrate was filled in the bag to a height of 3 inches. Handful of grain-based spawn was sprinkled over the layer. Likewise, few layers were placed on the bag. Finally, the bag was pressed gently and tied with a thread. Few holes were made on the bags to facilitate ventilation and for the removal of excess water.

Spawn Running:

The spawned bags were kept in a dark room for 1 week to facilitate the spawn running and colonization. Then the bags were transferred to cropping room.

Temperature and Humidity:

The optimum temperature (22ºC - 25ºC) and required humidity (85%) were maintained by spraying water on the walls of the mushroom unit four to five times in a day.

Harvesting:

After colonization, the mycelium starts to produce its reproductive structure called fruiting bodies. Initially, it looks like a pin head and it was transformed to a full matured fruiting body within two days. After maturity, edges of the pileus start to shrink towards inside. At this stage the fruiting bodies were collected manually and used for further experiments.

MORPHOLOGICAL PARAMETERS:

Length and width of stipe and pileus were measured immediately after harvesting with the help of thread and measuring scale.

Biological Efficiency:

Biological efficiency was calculated with the help of the following formula

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of the substrate}} \times 100$$

Moisture Content:

Known amount of sample was dried in shade for 12 hours and then moisture content was calculated by using the following formula

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

SAMPLE PREPARATION FOR BIOCHEMICAL ANALYSIS:

The fruiting bodies were shade dried and powdered with the help of mixer grinder. The powdered sample was sieved to get uniform size particle and stored in an airtight container.

QUALITATIVE ANALYSIS

Phytochemical constituents were analyzed using different extracts of *Pleurotus florida*. Standard procedures were followed for the same **(Horbone, 1984, Harborne, 1998, Kokate *et al*., 1995)**

Phytochemical Analysis:

Ethanol Extraction

Ethanolic extract of *P. florida* was prepared using 75% ethanol. Exactly 10 g of the powdered wild oyster mushroom was weighed out into a sterile beaker container containing 100 mL of 75% ethanol, stirred, wrapped with aluminum foil and allowed to stay for 72 hours at room temperature (25°C). After 72 hours, it was filtered and the solvent was heated in a water bath to evaporate completely. The slurry left behind was then stored in McCartney bottles and kept at 4°C until required for use

Aqueous Extraction

A portion (5 g) of powdered mushroom material was soaked in 50 mL of sterile distilled water, stirred and left overnight. After 24 hours, the suspension was filtered using Whatman No.1 filter paper and the filtrate was heated in a water bath at 70°C to allow the solvent to evaporate to dryness to eliminate the water. The extract was labeled and stored in the refrigerator until required for further analysis

Test for alkaloids

Three tests, Dragendoff's, Mayer's and Wagner's tests were performed for the presence of alkaloids. A 2 mL portion of each extract was stirred with 5 mL of 1% aqueous HCl in water bath. 1 mL of the filtrate of each sample was treated with few drops of Dragendoff's reagent and a second 1 mL with Mayer's reagent. Turbidity and white creamy precipitate was observed in either of those reagents as evidence for the presence of alkaloid. For Wagner's test, a few drop of Wagner's reagent was added to 1 mL of the sample. An orange precipitate appeared indicating the presence of alkaloids.

Test for glycosides

Legal's Test:

Few drops of 10% NaOH was added to the extract to make it alkaline before the addition of a freshly prepared sodium nitroprusside. Development of blue color indicates the presence of glycosides.

Keller-Killiani Test:

To 5 mL of the extract, 2 mL of glacial acetic acid was added followed by 1 drop of 5% FeCl3 and then con. H2SO4. The appearance of a reddish brown ring at the junction of the two liquid layers indicates the presence of glycosides in the extract.

Test for saponins (Frothing test)

A 2 mL portion of each extract was diluted with 10 mL of distilled water and heated in a water bath. After heating, this was shaken vigorously and left undisturbed for 20 min. A formation of stable froth indicated the presence of saponins.

Test for tannins

Two millimeters of wild edible oyster extract were stirred with 10mL of distilled water and heated in the water bath. A portion of 1 mL of 1% Fecl3 was added. Blue-black precipitate or coloration was an indication for the presence of tannins.

Test for reducing compounds

Fehling's Test:

Two millimeters of wild edible oyster mushroom extracts were put in test tubes and 5 mL of Fehling solution added and heated in the water bath for 5 min. The formation of brick-red precipitation or coloration indicated the presence of reducing sugar.

Molisch's Test:

To 5 mL of each extract, 2 drops of alcoholic solution of α-naphthol was added and the mixture well shaken. This was followed by the addition of 1mL of conc. H2SO4.

The formation of a violet ring in the test tube within few minutes indicated the presence of carbohydrates.

Benedict's Test:

A portion of 1 mL of Benedict's reagent was added to 2 mL of the extract and heated on a water bath for 2 minutes. The development of a characteristic colored precipitate indicated the presence of sugar.

Test for flavonoids (Magnessium hydrochloride reduction test)

A portion of 2 mL of each extract was added to a few pieces of aluminum metal and concentrated HCl added. The formation of orange, red, crimson or magnetic colour after few minutes showed the presence of flavonoids.

Test for polyphenol

Two millimeters of wild edible oyster mushroom extract were treated with 5 mL of distilled water and heated for 30 min in a water bath containing 1 mL of 1% Potassium ferrocyanide solution. The formation of green-blue colouration indicated the presence of polyphenol.

Test for anthraquinones

A portion of 2 mL of wild oyster mushroom extract was shaken with 10 mL benzene. This was filtered and 5 mL of 10% NH3OH was added. The mixture was shaken and the presence of pink/red or violet coloration in ammonical (lower) phase indicated the presence of free anthraquinones.

Test for steroids and triterpenoids

Salkowski Test:

Two millimeters of each extract was treated with few drops of conc. Sulfuric acid, shaken and allowed to stand for few minutes. Formation of a red color at the lower layer indicates the presence of steroids while the formation of yellow colored layer at the interface indicated the presence of triterpenoids.

Libermann Butchard's Test:

The extracts were treated differently with few drops of acetic anhydride, heated and allowed to cool to a temperature of <40°C in test tubes. The formation of brown ring and green color at the junction of two layers and upper layer respectively on addition of conc.

Sulfuric acid indicated the presence of steroids while deep red color indicated the presence of triterpenoids.

Test for proteins and amino acids

Biuret's Test:

An aliquot of 2 mL of the extracts were first treated with 1 drop of 2% CUSO4 solution. To this, 1 mL of 95% ethanol followed by excess KOH pellets was added. The formation of pink color in the ethanolic layers indicated the presence of proteins.

QUANTITATIVE ESTIMATION OF NUTRIENT CONTENT

TOTAL SOLUBLE PROTEIN (Lowry *et.al*., 1951)

Reagents:

Alkaline Copper Reagent

Solution A: 20% Sodium Carbonate in 0.1 N Sodium Hydroxide

Solution B: 1% Sodium Potassium Tartarate

Solution C: 0.5% Copper Sulphate

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1ml of solution B and 1 ml of solution C were mixed together freshly.

Folin-Ciacalteau Reagent (commercial reagent was diluted in the ratio of 1:1 with distilled water at the time of use).

Procedure:

100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant, 10%trichloro acetic acid (TCA) was added in 1:1 ratio (equal volume of supernatant and trichloro acetic acid) and left in an ice bath for 30 minutes to precipitate protein. Then

Procedure

1 ml of the sample was mixed with 1 ml of Ninhydrin reagent in a test tube. Tubes were kept in water bath for 20 minutes and then added 5 ml of diluents (equal volume of water and n-propanol) incubated at room temperature for 15 minutes and absorbance was read at 570 nm against a blank. The analysis was performed in triplicates and the results were expressed as mg/g.

DETERMINATION OF TOTAL LIPID (Folch *et al*., 1957)

500 mg of dried sample was taken in a screw capped test tube, 10 ml of 2:1solvent mixture (chloroform: methanol) was added. The tube was loosely capped and heated in a water bath at 60ºC for 30 minutes. After cooling the solution, the volume was made up to 10ml with the solvent mixture. 0.4 ml of the extract was pipetted in a separate test tube, allowed to dry completely and digested with 0.4 ml of concentrated H_2SO_4 by boiling in a water bath for 10 minutes. After cooling the tube, 5 ml of phosphovanillin reagent was added and allowed to stand for 30 minutes for colour development. The absorbance was then measured at 520 nm against a blank using spectrophotometer (Model No: UV 2371). Cholesterol was used as standard.

SYBTHESIS OF SILVER NANO PARTICLE:

Preparation of mushroom extract:

The mushrooms were kept in shade region for 4 days and then powdered. 1 g of powdered mushrooms was taken and 100 ml of distilled water was added and kept in stirrer for 60 min. The extract solution was filtered using Whatman filter paper. The aqueous extract was collected and used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles at room temperature:

In the synthesis of silver nanoparticles, 10 ml of the mushroom extract was added to 90 ml of 1 mM silver nitrate solution. This solution was kept at room temperature for 12 h. After keeping for 12 h silver nanoparticles obtained as a solid precipitate. The colour change from colourless to brown colour is observed. After the centrifugation the precipitated silver nanoparticles are collected.

ANTIBACTERIAL ACTIVITY:

The antibacterial activity of mushroom synthesised silver nanoparticles was carried out by the well diffusion method against *Escherichia coli* (gram negative bacteria), *Staphylococcus aureus* (gram-positive bacteria). Muller Hinton agar plates were prepared and solidified. After solidification, the bacterial culture such as *E. coli*, *S. aureus* were spread on the plate by using a sterilised cotton swab. A Whatman filter paper disc is dipped in AgNPs and another disc dipped in Amphicilin (Control). The dipped discs are kept in culture plates. These plates were incubated at 37 °C for 24 h incubation. The clear zone formation indicates the antimicrobial activity.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Two types of substrates were compared with respect to the production of oyster mushroom. The various substrates used in this study showed variations in spawn run, duration of first fruiting, days to harvest, length of stipe, diameter of pileus, total yield and weight of final substrate.

DAYS FOR THE COMPLETION OF SPAWN RUNNING

Time required for the completion of spawn running varied on different substrates ranged from 22 to 37 days **(Figure 1a).** As per the findings of this study, the growth of *P. florida* mycelia was relatively faster on coir (22 days). Our findings in the present experiment are almost similar to the findings of **Lalithadevi and Many (2014)** who reported that spawn running day was between 16 – 25 days on coir. The longest spawn running was observed in the case of sugarcane bagasse (37 days). The findings of the spawn run on sugarcane bagasse did not agree with the report of **Hossain (2017)** who stated that *P. ostreatus* completed the spawn run in 17 days on sugarcane bagasse. Increase in number of days for spawn running on lignocellulosic waste materials might be due to slow hyphal growth of mushroom on substrates **(Mandeel *et al*., 2005).**

The difference in days for full mycelia running on different substrates might be due to variation in their chemical composition and C:N ratio as reported by **Bhatti *et al*. (1987). Tan (1981)** reported that the spawn running took 16 – 25 days after inoculation. The variation in the number of days taken for a spawn to complete colonization of a given substrate depends on the function of the fungal strain, growth condition and substrate type.

NUMBER OF PINHEADS

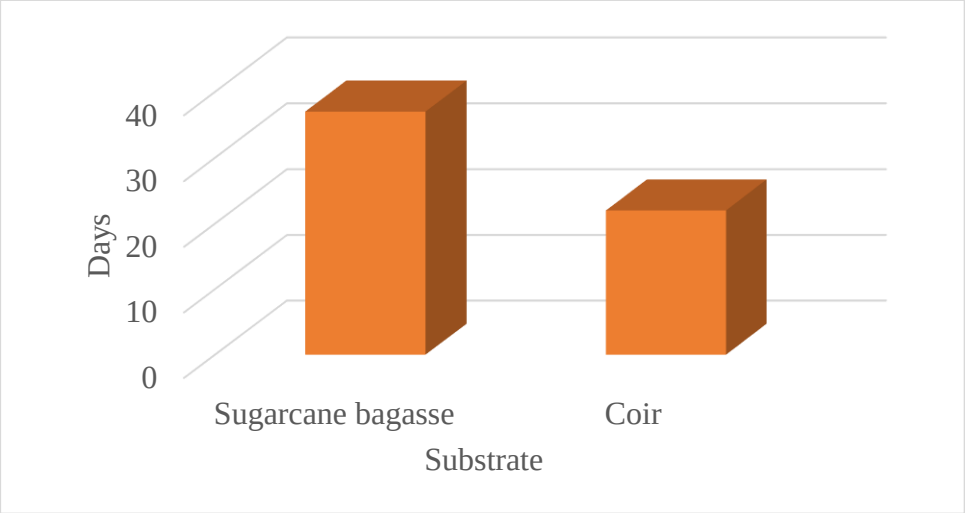

Figure 1a: Effect of Different Substrates on Spawn Running Days of *Pleurotus ostreatus*

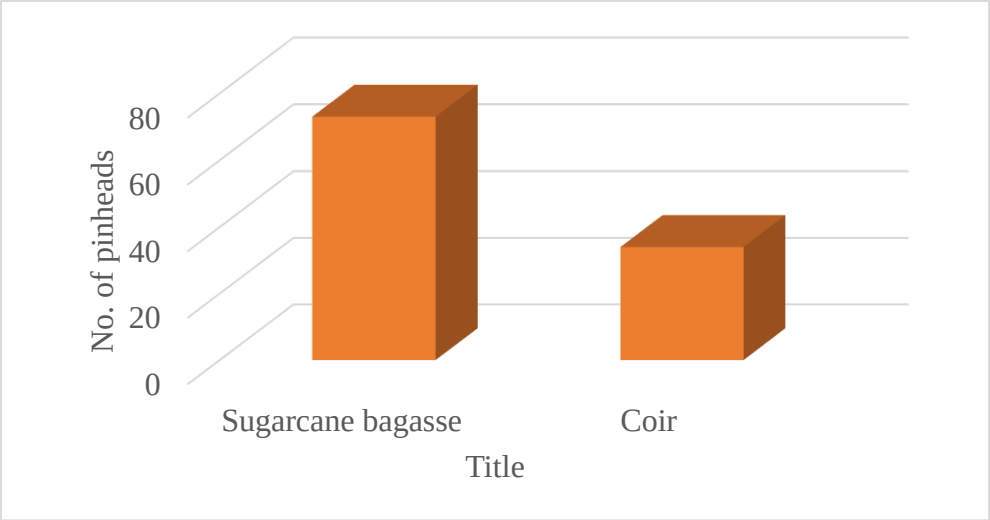

Figure 1b: Effect of Different Substrates on Number of Pinheads of *Pleurotus ostreatus*

P. florida produced different number of pinheads on different substrates as shown in **Figure 1b.** Maximum numbers of pinheads were recorded on coir followed by sugarcane leaves. Our findings are further supported by **Hague (2004)** and **Al Amin (2004)** who reported that highest number of pinheads of Oyster mushroom was found on coir. Minimum numbers of pinheads were observed on sugarcane baggase. Almost similar results are reported by **Hasan *et al*. (2015)** who observed minimum number of pinheads of Oyster mushroom on sugarcane bagasse.

YIELD AND BIOLOGICAL EFFICIENCY

Total yield and biological efficiency of *P. florida* cultivated on various substrates are presented in **Table 1**. Among all the two substrates, coir showed highest percentage of biological efficiency and yield (78.4% and 588 g). The lowest biological efficiency and yield (14% and 101 g) of *P. florida* were obtained on sugarcane baggase. Our results agree with the result of **Sardar *et al.* (2016)** who reported that lowest biological efficiency was obtained on sugarcane baggase. Higher the biological efficiency of different substrates represents its higher suitability for the cultivation of mushroom.

ORGANIC MASS LOSS

The mushroom has the ability to degrade lignocellulosic materials during the idiophase stage following severe nitrogen and carbon depletion **(Manson *et al.*, 1989).** Coir biomass loss was 26% which shows more degradation and solubilization than sugarcane bagasse **(Table 1)**

QUALITATIVE ANALYSIS

TABLE 1: EFFECT OF DIFFERENT SUBSTRATES ON THE PERFORMANCE OF PLEUROTUS FLORIDA

S.No	Substrate	Yield (g)	Biological Efficiency (%)	Organic Mass Loss (%)
1.	Coir	587.7	78.4	26.3
2.	Sugarcane baggase	101.7	13.6	12.5

The present study was carried out in preliminary phytochemical analysis of *P. florida*. The phytochemical characteristics of P. ostreatus of various extracts investigated were summarized in **Table 2**. The extracts of *P. florida* revealed the presence of medicinally important bioactive ingredients. The alcoholic extracts of *P. florida* showed the presence of alkaloid, glycosides, Saponin, reducing compounds, steroids, terpinoids and proteins. These phytochemicals have also been observed in mushrooms by others workers. Some phytochemicals such as steroid, glycoside, quinone and phytosterol was found of absence in all the extracts investigated in the present study.

NUTRITIONAL PARAMETERS

The results of moisture, protein, carbohydrate, lipid and amino acid contents are depicted in **Table 3**. The freshly cultivated *P. florida* contains high moisture content. Moisture percentage in mushroom depends on the maturity of fruiting bodies, species and storage conditions and during packaging or processing. The present study revealed that the highest moisture content was observed in coir (91%) followed by sugarcane bagasse (89.8%).

Ash content of tested mushroom was recorded between 84 mg/g DW to 98 mg/g DW in *Pleurotus florida*. The highest ash content was ascertained on sugarcane bagasse whereas the lowest was found on coir. Ash contents can affect human mineral intake and these minerals of mushrooms were bioavailable (**Dikeman *et al*., 2005**).

The carbohydrate content of mushrooms represents the bulk of fruiting bodies ranged from 3 to 11.6 mg/g on dry weight in the ethanolic extract followed by aqueous extract. Carbohydrate content was found to be maximum in dry thallus cultivated on

TABLE 2: QUALITATIVE ANALYSIS OF PLEUROUS FLORIDA

S.No.	Phytochemical constituents	Test	Observation	
			Ethanol	Aqueous
1.	Alkaloids	Dragendoff Test Mayer's Test Wagner's Test	++ ++ ++	- - -
2.	Glycosides	Legal's Test Keller-kiliani Test	- +	- -
3.	Saponin	Frothing Test	++	++
4.	Flavanoid	Magnesium hydrochloride reduction Test	-	-
5.	Tannin		-	-
6.	Anthroquinones		-	-
7.	Reducing compounds	Fehling's Test Molisch's Test Benedict's Test	+ ++ ++	++ - -
8.	Polyphenol		-	-
9.	Steroid Triterpenoid	Salkowski Test Libermann butchard's Test	+ ++	++ ++
10.	Protein	Biuret's Test	+	++

'+' Indicates Presence '–' Indicates Absence

TABLE 3: EFFECT OF DIFFERENT SUBSTRATES ON THE NUTRIENT CONTENT OF PLEUROTUS FLORIDA

Substrate	Moisture content (%)	Carbohydrate (mg/g DW)		Protein (mg/g DW)		Aminoacid (mg/g DW)		Lipid (mg/g DW)	
		Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Sugarcane baggase	89.8	3.02	5.6	80.5	134.2	136.6	206.5	1.8	5.3
Coir	91	3.7	11.6	144.1	194.1	155.9	220.3	4.7	8.6

sugarcane baggase (11.6 mg/g) followed by fruit bodies developed on coir (8.6 mg/g) in the ethanolic extract followed by 13.7mg/g and 6.72 mg/g respectively in the aqueous extract **(Figure 2a).**

Protein is an important constituent of dry matter of mushrooms. Protein content of mushrooms depends on the composition of substratum, size of pileus, harvest time and species of mushrooms **(Bano and Rajarathnam, 1982).** Protein content *of P. florida* was found maximum in sugarcane baggase treated (194 mg/g) coir (184 Protein content of the mushroom mycelium can be controlled by the amount of nitrogen supplied in the growth media. The Carbon: Nitrogen influences the protein and the fat content in the mushroom mycelium **(Shah *et al.*, 2004).** They differ according to the species but this difference depends on the substratum, atmospheric conditions, age and part of the fruitification **(Figure 2b).**

The mushroom protein is known to contain almost all the essential amino acids. Apart from essential amino acids, considerable amount of alanine, arginine, glycine, histidine, glutamic acid, aspartic acid, proline and serine can be found in mushroom. The free amino acid content of *P. florida* ranged from 80 mg /g to 194 mg / g **(Figure 2c).** The highest amount of amino acids was recorded in ethanolic extract of dry thallus cultivated on sugarcane baggase while the lowest amount of amino acids was registered in ethanolic extract of dry thallus cultivated on coir. The quantitative spectrum of essential amino acids has served as the basis to calculate biological value, nutritional value and protein score **(Haque, 1989).**

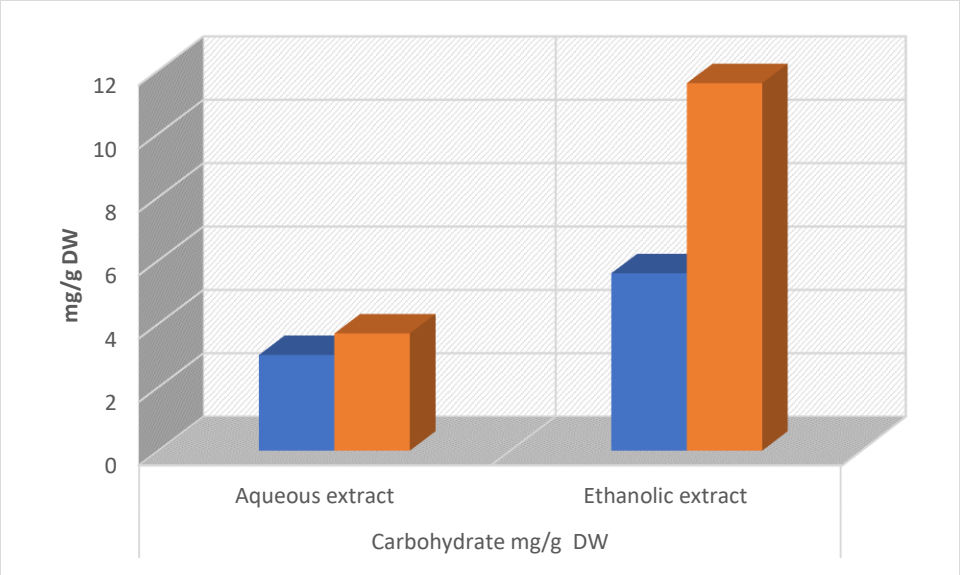

Figure 2a: Effect of Different Substrates on Carbohydrate Content of *Pleurotus ostreatus*

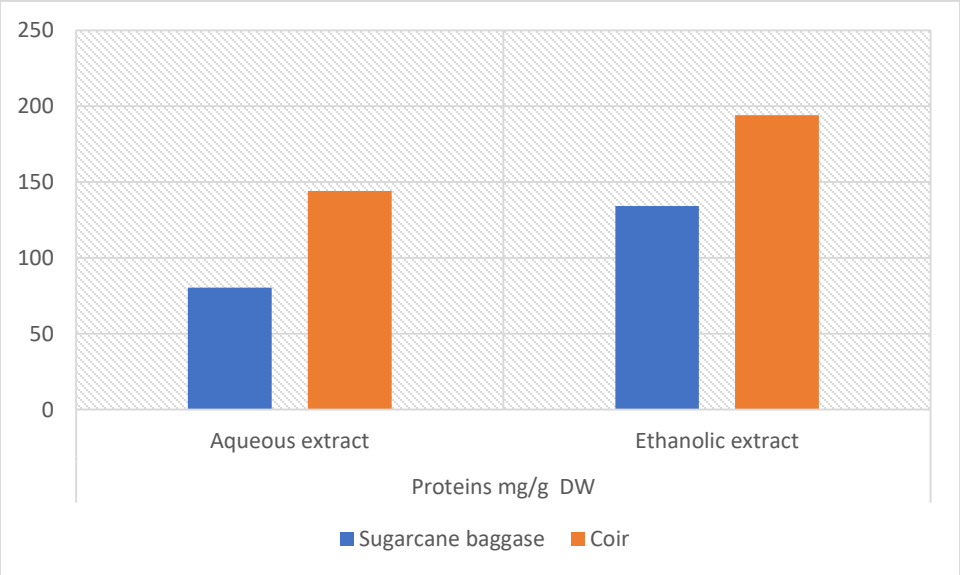

Figure 2b: Effect of Different Substrates on Protein Content of *Pleurotus ostreatus*

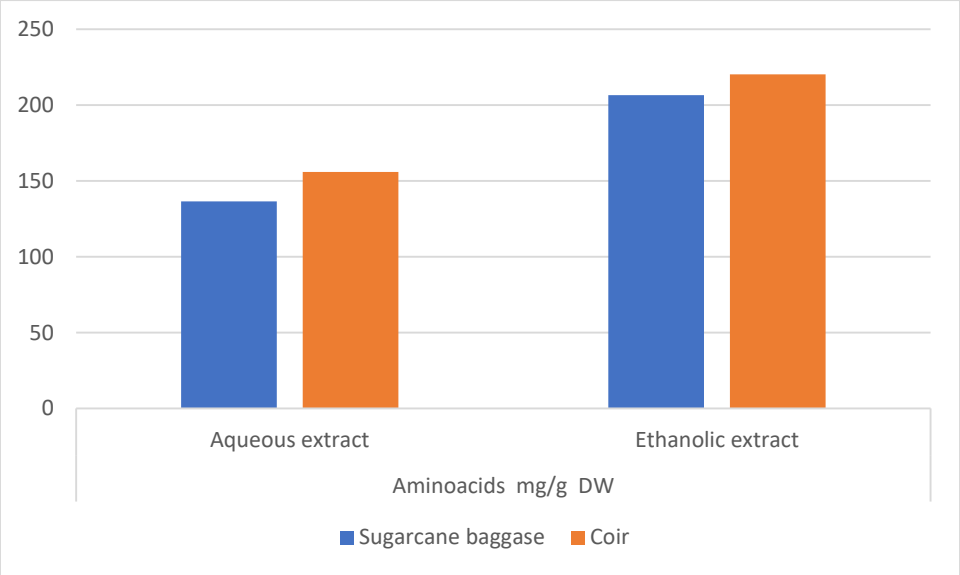

Figure 2c: Effect of Different Substrates on Aminoacid Content of *Pleurotus ostreatus*

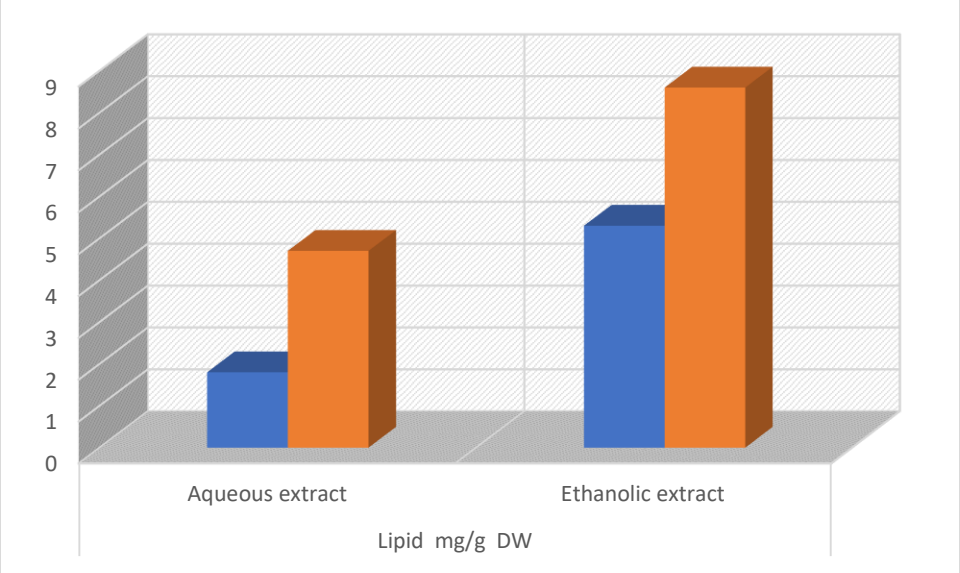

Figure 2d: Effect of Different Substrates on Lipd Content of *Pleurotus ostreatus*

Lipid content of all the four investigated substrates showed variation. In mushrooms, the fat content is very low as compared to carbohydrates and proteins. The highest lipid content was found in fruit bodies developed on sugarcane baggase (8.6 mg/g) followed by coir **(Figure 2d). Yilmaz *et al.* (2006)** and **Pedneault *et al.* (2006)** reported that fat fraction in mushrooms is mainly composed of unsaturated fatty acids.

SYNTHESIS OF SILVER NANOPARTICLES

Nanoparticles have a wide range of application as in combating microbes, biolabeling and in the treatment of cancer **(Travan *et al.*, 2009).** As mushroom extracts were mixed into aqueous solution of silver nitrate, it started to change the colour from watery to brown due to the reduction of silver ions, which indicated the formation of silver nanoparticles. UV – Vis spectroscopy is the significant method which gives the preliminary confirmation of silver nanoparticles. The fabricated silver nanoparticles showed absorption spectra ranging from 420 to 450 nm. in *Pleurotus florida.* Biologically synthesized silver nanoparticles could have many applications. Further studies are needed for synthesis and characterization of silver nanoparticles using mushroom extracts and also to explore the mechanism.

ANTIBACTERIAL ASSAY

The knowledge of drug has developed together with the evolution of scientific and social progress. Drugs derived from macro fungi are effective, easily available and less expensive and rarely have side effects. Initial screening for the potential antibacterial and antifungal compounds from mushroom may be performed by using the crude extracts. In the present study AgNPs synthesized from *Pleurotus florida* was tested against two human bacterial pathogens such as *Staphylo cocci* and *Escherichia coli.* The specific zone of inhibition

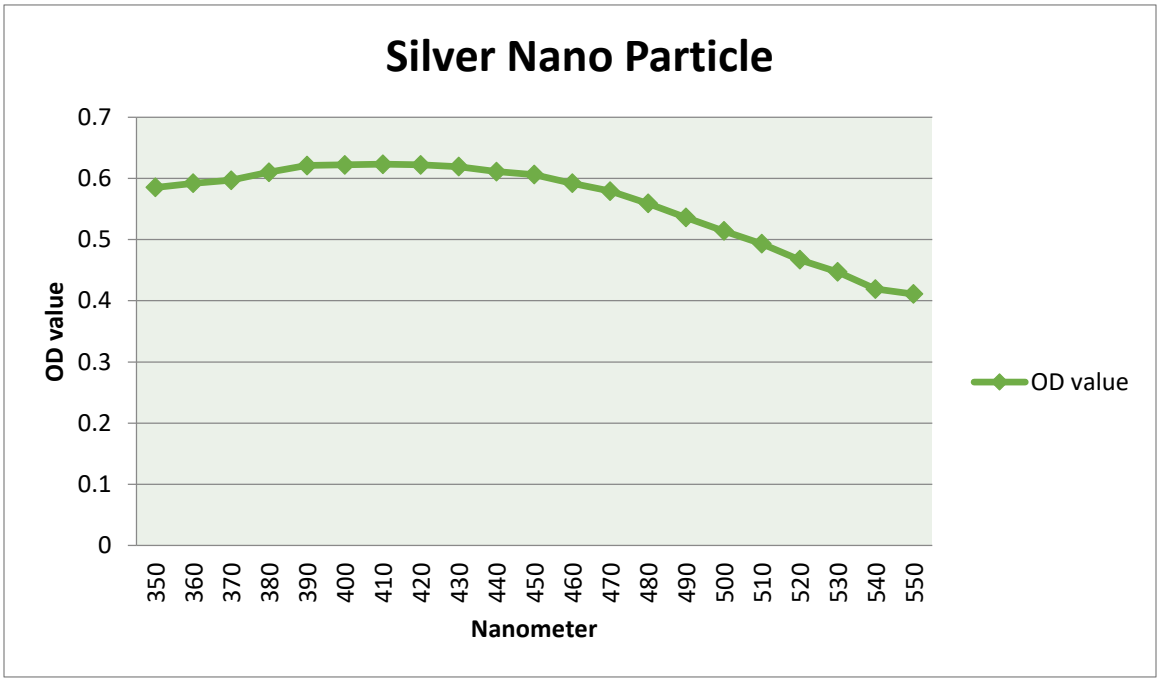

Figure 3: Green Synthesis of Silver nanoparticles in *Pleurotus ostreatus*

against different types of pathogenic bacteria is shown in **Table 4**. The silvernano particles synthesized from *Pleurotus florida* revealed highest antibacterial activity against *Escherichia coli*. The ethanol extract of lyophilized mycelium of *Pleurotus ostreatus* had a strong antibacterial activity of some bacterial strains **(Wolff *et al.*, 2008).** *Pleurotus* species particularly *Pleurotus florida* had a narrow antibacterial spectrum against Gram-negative bacteria and strongly inhibited the growth of Gram-positive bacteria tested including *Bacillus subtilis* **(Loganathan *et al.*, 2008).**

TABLE 4: ANTIBACTERIAL ACTIVITY OF SILVER NANO PARTICLE SYNTHESISED FROM PLEUROTUS FLORIDA

Bacteria	Zone of Inhibition (mm)	
	Control	Silver Nano Particle
Escherichia coli	7	13
Staphylococcus	3	10

SUMMARY AND CONCLUSION

centrifuged at 3000 rpm for 5 minutes and discarded the supernatant. The precipitate was dissolved in 0.1 N sodium hydroxide and diluted to a known volume.

5 ml of alkaline copper reagent was added to 0.5 ml of protein extract. After thorough mixing, 0.5 ml of folin ciocalteau reagent was added and allowed to stand for 30 minutes; the blue colour appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

ESTIMATION OF CARBOHYDRATE (Dubois *et al.*, 1956)

Reagents:

5% Phenol (5 ml phenol + 95 ml distilled water)

96% Sulphuric acid (96 ml sulphuric acid + 4 ml distilled water)

Procedure:

100 mg of sample was grounded with 10 ml distilled water. It was then filtered and centrifuged. The filtrate was collected. To 0.1 ml of the filtrate, 0.9 ml of distilled water, 1 ml of 5% phenol and 5 ml of 96% H_2SO_4 were added. After 30 minutes, absorbance was measured at 490 nm using UV visible spectrophotometer (Model No: UV 2371).The analysis was performed in triplicates and the results were expressed as mg/g DW. Glucose was used as standard.

ESTIMATION OF FREE AMINO ACID (Moore and Stein, 1948)

Total free amino acids (Ninhydrin method) were determined according to the procedure given by Moore and Stein.

SUMMARY AND CONCLUSION

Edible mushrooms have been eaten and appreciated for their flavor, economic and ecological values and medicinal properties. They are able to grow under climatic conditions on cheap, readily available waste materials. These mushrooms are a clear example of how low value waste which is produced primarily through the activities of the agricultural, forest and food processing industries, can be converted to higher value material useful to mankind. For many reasons, the fungus *Pleurotus* genus had been intensely studied and cultivated in many different parts of the world. This mushroom demands environmental controls for cultivation and its fruiting bodies are not often attacked by diseases and pest and it can be cultivated in a simple and cheap way. Another advantage of growing oyster mushroom is that a high percentage of the substrate is converted to fruiting bodies, increasing profitability as compared to other mushrooms making *P. florida* an excellent choice for mushroom cultivation.

1. The results of the present study support the efficient production of mushroom on substrates.
2. Utilizing these waste products as substrates for the production of mushrooms would reduce the adverse environmental effects of theses waste products.
3. An economical strategy for converting waste products into nutritious food source is represented in this study

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BIBLIOGRAPHY

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DISCOVER THE MICRO AND NANO-PLASTICS DEGRADING ENZYME CODING GENE FROM THE SELECTED MICROBES OF PLASTIC POLLUTED AREA

A dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

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MASTER OF SCIENCE IN BOTANY

By

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May - 2022

CERTIFICATE

This is to certify that this dissertation entitled, **"Discover the micro and nano-plastics degrading enzyme coding gene from the selected microbes of plastic polluted area"** submitted by **J. Jeya Ranchini Reg.No. 20APBO05** to St. Mary's College (Autonomous), Thoothukudi in partial fulfillment for the award of the degree of **"Master of Science in Botany"** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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DECLARATION

I do here by declare that this dissertation entitled, **"Discover the micro and nano-plastics degrading enzyme coding gene from the selected microbes of plastic polluted area"** submitted by me in partial fulfilment for the award of the degree of **'Master of Science in Botany'**, in the result of my original and independent work carried out under the guidance of **Dr. G. Flora M.Sc., M.Phil., Ph.D.**, Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

J. Jeya Ranchini.

Station: Thoothukudi **(J. Jeya Ranchini)**

Date: 26.05.2022.

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Table of Contents

Contents **Page**

INTRODUCTION

INTRODUCTION

Plastic is a matter that is hard to destroy and degrade once manufactured that goes in contradiction to natures rule; consequently, it creates a catastrophe for the complete world (Gilmore *et al.,* 2018). Plastic pollution has become one of the most widespread recalcitrant environmental contaminants (Stabnikova *et al.,* 2021). Plastic particles are ubiquitous pollutants in the living environment and even in the blood stream of human beings. Polyethylene terephthalate, polyethylene and polymers of styrene were widely encountered in the blood stream (Leslie *et al.,* 2022). In 2019 annual plastic production was 368 million tons, and it is expected that its production will increase up to 33 billion tons by 2050 (Bellasi *et al.,* 2020; Plastics Europe 2020). It is estimated that 76% of total plastics produced are land filled or spread in the natural environment (Geyer 2020). The total quantity of waste plastics in the marine environment per year is approximately 8 million tons (Rodrigues *et al.,* 2019) and according to Bowley with co-authors (2021) the number of plastic pieces in the ocean was between 15 and 51 trillion. Small sized particles of plastic wastes (microplastics, nanoplastics) are considered as a group of environmental pollutants with significant adverse impacts on the environment. The term "microplastics" first appeared in a study of small plastic particles in 2004 (Thompson *et al.,* 2004). The definition by Frias and Nash (2019) covers major aspects that could potentially describe what a microplastics, "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water". Another classification approach of small-sized particles in the environment is based on the categorization by the size of particles as macroplastics (>2 cm), mesoplastics (5 mm-2 cm), microplastics (<5 mm), and nanoplastics (<1 μm) (Blettler *et al.,* 2017). Small-sized plastic particles can be further classified

as primary microplastics (plastics directly released into the environment in the form of small particulates) and secondary microplastics (plastics originating from the degradation of larger plastic items into smaller plastic fragments during mechanical, chemical and biological degradation in the environment) (Boucher *et al.,* 2017). The source of environmental pollution with small plastic particles is non-sustainable use of plastics discarding of plastic wastes in the environment and relatively lean implementation of recycling and use of biodegradable plastics (European Commission 2018) as well as high stability of several types of plastics, especially hydrocarbon and halogen atoms containing plastics (Avio *et al.,* 2016). It was reported that the quantity of microplastic particles in marine aquatic systems can reach 140 particles/m3 and 8766 particles/m3 in water and sediment, respectively (Thushari *et al.,* 2020). A lot of microplastics are produced due to physical disintegration and chemical or biological destruction of plastic materials, arising mainly from plastic tableware, single-use beverage bottles and cosmetics which disintegrate into fibers and microspheres that enter the environment through discarding on land or water. The main polymer constituents of microplastics found in waters have been identified as polyethylene, polypropylene, polystyrene, and polyethylene terephthalate, accounting for 70% of the total, but polyvinylchloride, polyacrylonitrile, rubber, different copolymers are also common (Li *et al.,* 2020), however significant differences exist between studied environments (inland and marine waters, soils, sediments and others) as well as different world regions (Akdogan *et al*., 2019). MPs are constantly present in fresh and marine water systems. A lot of microplastics debris was found even in oceanic surface waters of the Antarctic Peninsula (Audrezet *et al.,* 2020). It is considered that microplastics have become a main source of anthropogenic pollution of the oceans (Bowley *et al.,* 2021) and MPs concentration in highly contaminated rivers could be up to 100 mg/L. It is evident that the quantity of microplastics will

increase over the next decade, so the fate and biological impact on the environment of this contaminant are in focus of scientific research. Microplastics have been found also in freshwater (Wagner *et al.,* 2014), drinking water (Eerkes-Medrano *et al.,* 2019), soil (Guo *et al.,* 2020) as well as in food particles (Rainieri *et al.,* 2019). Microplastics have been shown to have negative effects on aquatic and soil living organisms, including impaired reproduction, malnutrition, internal abrasions, and blockages (Kumar *et al.,* 2020; Gola *et al.,* 2021; Sun *et al.,* 2021), as well as adverse human health effects (Smith *et al.,* 2018; Prata *et al.,* 2020; Yee *et al.,* 2021). Microplastics can act as sorbents for other environmental pollutants such as, persistent organic pollutants (Bakir *et al.,* 2012), hydrocarbons (Song *et al.,* 2021), pharmaceuticals (Xu *et al.,* 2018) and other pollutants (Yu *et al.,* 2019) due to their high surface area and hydrophobicity of their surfaces. One of the microplastic toxicity processes is desorption of pollutants if the particles penetrate the live organisms (Sun *et al.,* 2021).

When plastic garbage is disposed in landfills, it appears to eliminate waste from the upper surface of land but it actually diminishes agricultural land (Zhang *et al.,* 2004). Because natural decomposition takes so long, the landfill area cannot be used for other purposes (Tansel and Yildiz 2001). In comparison to landfills, incineration is a superior solution because as it requires less space and provides better energy recovery (Sinha *et al*., 2010). However, it is not without restrictions because it produces greenhouse gases, as well as PCBs and free radical exposure (Astrup *et al.,* 2009). Regardless, landfills and incinerator constraints can be addressed through recycling, though this procedure is costly and end product quality is poor (Yamada-Onodora *et al.,* 2001). In compare to other approaches, biodegradation is the most effective one. It is inexpensive and does not emit hazardous pollutants into the atmosphere. Microorganisms are capable of degrading plastic. As a result, Microorganisms play a key part in the degradation

process (Head and Swannell 1999). Both natural and manmade plastics are degraded by microorganisms such as bacteria and fungi (Gu *et al.,* 2000). Biodegradation is influenced by a variety of parameters, including polymer properties, organism type, and pretreatment method. During degradation the polymer is first broken down into its constituent monomers, which are subsequently mineralized. Because Most polymers are too big to enter through cellular membranes, they must first be depolymerized into smaller monomers before being ingested and biodegraded by bacteria. (Shah *et al.,* 2008). As a result, biodegradation of microplastics is one of the most effective methods for reducing microplastic pollution without the use of plastics and a proven method for micro and nanoplastic pollution mitigation in the environment.

SCOPE AND OBJECTIVE

SCOPE AND OBJECTIVES

Plastic pollution cause detrimental effects on environment. The incarnation of macroplastics into micro and nanoplastics are the uncontrollable one due to the climatic influence and the microbial contribution. These consequences lead to allot an inevitable place for micro and nanoplastic pollution in aquatic and terrestrial environs. The degradation of micro and nanoplastics are needed one while it's not possible to completely degrade a plastic without any residues through chemical and physical approaches. But the biodegradation approach will completely dismantle the macroplastics, micro and nanoplastic contamination on environment and release the organic matter. Hence the current investigation is needed one to get a positive result on plastic pollution.

The main objectives of this study are as follows;

- To collect the samples from terrestrial environment (Pudhukottai and Mullakkadu) and freshwater bodies (Pudhukottai and Mullakkadu) of Thoothukudi District.
- Serially dilute the samples and isolate the pure culture (bacterial and fungal culture) using pour plate method.
- To study the degradation of micro and nanoplastics (Polyethylene) using isolated microorganisms (bacteria and fungi).
- To identify the micro and nano-plastic degrading microorganisms (bacteria and fungi) and sequence its genome.
- Identify the functional groups present in Polyethylene sheets using FT-IR analysis.

REVIEW OF LITERATURE

Microplastic pollution is a difficult issue (Windsor *et al.* 2019) with significant environmental and public health repercussions. This pollution problem is a classic transboundary of how land-based pollution can spread rapidly, even into remote areas such as virgin mountainous regions, wilderness areas, and the Arctic (Bergmann *et al.* 2019; Brahney *et al.* 2020),as well as the ocean's deepest trenches (Jamieson *et al.* 2019). Because plastic pollution is physically apparent, it has piqued the interest of a diverse group of stakeholders including scientists, policy makers, the media, and the general public. This subject has gotton a lot attention, possibly more than any other pollution concern in history of science (Sedlak 2017).

Food, medicines, cosmetics, detergents and chemicals all employ synthetic polymers in their packaging. Plastics are utilized for packaging applications in about 30% of the world's population. The usage rate is still growing at a rapid rate of 12% each year. Because they offer greater physical and chemical qualities, such as strength, lightness, resistance to water and most water-borne germs, they have supplanted paper and other cellulose- based products for packaging. Polyethylene (LDPE, MDPE, HDPE and, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), poly(ethylene terephthalate) (PET), poly(butylene terephthalate) (PBT), nylons are among the most commonly used plastics in packaging. Plastics are widely used not just because of their advantageous mechanical and thermal qualities, but also because of their stability and durability (Rivard *et al.,* 1995). Because of their durability and visibility in litter, Plastics (Polymers) have gotten more public and media attention than any other component of the solid waste stream. In 1993, the global demand for plastics was around 107 million tones, and in 2000, it was anticipated to be above 146 million tonnes.

The dramatic increase in production and lack of biodegradability of commercial polymers, mainly commodity plastics used in packaging (e.g. fast food), industry and agriculture, has focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries (Albertsson *et al.,* 1987). Plastic waste is disposed off through the process such as landfilling, incineration and recycling. Because of the persistent presence of wasted plastic in our environment, several communities have become more aware of its negative impacts on animals and the aesthetic characteristics of towns and forests. Plastic that has been improperly disposed of has the potential to damage people by polluting the environment. Furthermore, the combustion of polyvinylchloride (PVC) polymers releases persistent organic pollutants (POPs) such as furans and dioxins (Jayasekara *et al.,* 2005).

Micro and nanoplastic pollution

Microplastics were found in sediments collected at four sites along the River Thames in the United Kingdom, according to Horton *et al.* (2017). The average number of particles 100 g−1 in downstream samples was 66, with fragments dominating (91%). PP, PES and polyarylsulphone polymers were discovered. Storm drains and road-surface marking paints are two reasons for greater contamination in downstream samples. Fresh water, according to the study, is also susceptible to microplastic contamination.

Ambrosini *et al.,* (2019) found 74.4 ± 28.3 pieces kg-1 of microplastic debris in the supraglacial Italian Alps. Polyesters, polyamide, polyethylene and polypropylene made up the plastic composition. Human activities in the mountain may have released microplastic, which was then carried by the wind. They determined that microplastics can be transmitted by wind and that microplastic contamination can occur even at high altitudes.

To analyse the state of microplastic pollution in the sandy beaches of Lima, Peru, (De-la-Torre *et al.,* 2020) conducted a sampling campaign in the intertidal and supralittoral zones. Foams were the most dominant (78.3%) type of microplastic. FTIR spectroscopy identified the dominant polymer as PS. The study concluded that alarming concentrations of microplastics are present on Peruvian beaches however information on sources and impacts on living organisms is lacking.

Microplastics concentration in the Manas River, China, was explored by a group of researchers (Wang *et al.,* 2020). They observed a range of 21 ± 3 to 49 ± 3 items/L in which fibrous microplastics were dominant at all sites with a size range of 0.1 and 1.0 mm, while black and white were the dominant colours. IR spectral analysis showed that the dominant polymers were PP and PET. The study can help to understand the contamination features of microplastics in inland rivers.

Microplastic fibres constitute the largest component of plastics in aquatic environments. Sait *et al.,* (2021) investigated the photodegradation of PET, PA, PAN and their respective chemical profile, along with their potential for additive leaching. PET and PA fibers showed significant morphological changes upon exposure to UV. Chemicals identified in fibers and aqueous leachates include monomers, UV stabilizers and degradated polymers. Bisphenol A, bisphenol S and benzophenone-3 were quantified in all fibers and wool at concentrations between 4.3 and 501 mg/g, with wool displaying maximum concentration of BPs and BzPs at 863 and 27 mg/g, respectively.

Kosuth *et al.,* (2018) investigated microplastic particles in tap water, beer, and commercial sea salt. Microplastics were present in 81% of the tap water samples which composed of fibers (98.3%) of 0.1 ± 5 mm. The abundance ranged at 0 to 61 particles/L.

Likewise microplastics were present in beer and salt. The composition was dominated by fibers (99%). Beer was contaminated with 0 to 14.3 particles/L and sea salts contaminated with 46.7 to 806 particles/kg. It was estimated that an average person consumes more than 5,800 particles of micropalstics from tap water, beer and salt. In another study, high (HC) and low (LC) cost commercial were purchased by Renzi and Blašković (2018) to analyse for the presence of microplastics. The microplastics ranged between 1.57 (HC) –31.68 (LC) items/g. The sizes of particles ranged within 4–4628 μm. The samples were purchase from Italy and Croatia and it was found that all samples from both the countries had microplastic contamination but varied in abundance which is dependent on several factors.

Piñon-Colin et al., (2018) reported the microplastic pollution in sandy beaches of the Baja California Peninsula, Mexico. To reach the objective of the work, twenty-one sandy beach samples were collected from the sandy beach environment of the study region. The microplastics were separated using the density separation method and the composition of the plastic grain was confirmed by Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR). The spectroscopic and morphology characterization of the microplastics were studied using the SEM-EDAX facility. The average value of the microplastics in the sediment was 135 ± 92 particles kg−1. The sediment-associated microplastic composition was identified as polyacrylic, polyacrylamide, polyethylene terephthalate, polyesters, and nylon.

Gündoğdu and Çevik, (2017) reported the distribution of micro-and meso plastics in the Northeast Levantine coast of Turkey. The average of micro-and meso plastic was determined to be 0.376 items/m2. The highest value was determined in Mersin Bay at the mouth of the Seyhan river (906 items), and the lowest level was found in Station no. 4 in Iskenderun Bay (78 items).

Karlsson *et al.,* (2017) developed the screening method for microplastics in sediment, water, marine invertebrates, and fish. For separation of microplastics, they adapted enzymatic digestion protocol using proteinase K. Based on the number of plastic particles/ kg, the microplastic concentrations found in mussels were approximately a thousand-fold higher compared to those in sediment and surface water samples of the study region. Naidu *et al.,* (2018) reported the microplastics in the benthic invertebrates (Sternaspisscutata, Magelonacinta (deposit feeders), and Tellina sp. (suspension feeder) from the surface sediments and coastal waters of Kochi, Southeastern Arabian Sea. The thread-like fibres (polystyrene) were noticed in these organisms. The surface morphological characteristics of the plastic suggesting that the particle is degraded/weathered by intensive action of the marine environment.

ZhiluFu and JunWang (2019) findings revealed that the microplastics like PP and PE were abundant in examined freshwater ecosystems, with a high abundance seen in estuaries and inland waters near crowded urban areas of China. The plastic associated microorganisms was studied by Carson (2013) and established that the bacterial abundance was patchy but increases on foamed polystyrene while diatom abundance increased on rough surfaced materials. Sullivan *et al.* (2020) detected the nanoplastics in water samples using pyrolysis-gas chromatography time of flight masss pectrometry (PY-GCToF).

Rodrigues *et al.,* 2018 studied the microplastics abundances and distribution in Antuã River (Portugal) by applying the isolation method of wet peroxide oxidation with addition of zinc chloride to water and sediment samples collected in March and October 2016, in three sampling sites. The abundance of microplastics in water ranged from 5 to 8.3 mg m−3 or 58–193 itemsm−3 in March and from5.8–51.7 mgm−3 or 71–1265 itemsm−3 in October. In sediments, the abundance ranged from 13.5–52.7 mg kg−1 or 100–629 items kg−1 in March and from 2.6–

71.4 mg kg−1 or 18– 514 items kg−1 in October. The water and sediment samples with the greatest abundances were from São João da Madeira and Aguincheira, respectively. Spatio-temporal distribution showed different pattern according tomethodological approaches, seasonal and hydrodynamic conditions and the proximity to urban/industry areas. Analysis of plastics by Fourier transform infrared spectroscopy underline polyethylene and polypropylene as the most common polymer types identified in this work. The low medium high oxidation ratio was 56:22:22 (%) in March and 61:31:8 (%) in October. Foams and fibers were the most abundant type in São João daMadeira, while fibers and fragments were the most abundant in Aguincheira and Estarreja inwater and sediment samples, respectively. This study emphasizes the importance of rivers as carriage systems of microplastics.

Prajapati *et al.,* 2021 investigated the contamination of the environment with microplastics. They found that the wastewater treatment plants (WWTPs) are considered important emitters of microparticles into aquatic systems. Among these microparticles are microplastics from, e.g., cosmetic products and microfibers that are released during laundry of textiles made from synthetic fibers. The purpose of this study was to qualitatively and quantitatively characterize microplastic and microfiber contamination in effluents of the City of Saskatoon WWTP, Saskatchewan, Canada. The microplastics were determined using Raman and Fourier-transform infrared (FTIR) spectra of neat plastic standards.

Li *et al.,* 2021 studied the efficiency for sequential isolation of micro and nano-plastics by membrane filtration and cloud-point extraction methods. The plastics filtered through 1μm pore sized glass membrane, over 90.7% microplastics were trapped on the membrane whereas above 93.0% of nanoplastics remained in the filtrate.

Bharath *et al.,* 2021 identified the microplastics in Veeranam Lake; the collected water and sediment samples deposited with polymer type of plastic particles were nylon (39%), polyethylene (23%), polystyrene (19%), polypropylene (15%), and polyvinyl chloride (4%). The microplastics were isolated by an oxidative procedure and subsequent density separation using ZnCl2 solution (Naji *et al.,* 2021).

Liu *et al.,* 2021 described a method to detect, identify, and quantify microplastics in marine mussels (*Mytilus edulis*) using thermal gravimetric analysis – Fourier Transform infrared spectroscopy – gas chromatography mass spectrometry (TGA-FTIR-GC/MS) after extracting and isolating the microplastics using chemical digestion, density separation, and filtration. This technique is readily determined the type and amount of microplastics in the sample.

To understand the distribution of microplastics Peng *et al.,* (2018) investigated river sediments in Shanghai, China. Density separation, microscopic inspection and μFTIR analysis were performed to analyze the type of polymers. The microplastics average in the samples was 802 items per kilogram of dry weight. White spheres were the most abundant in river sediments. Seven types of microplastics were identified, of which polypropylene was the most prevailing polymers presented. Their study concluded that in situ data and legitimate estimations should be practiced while developing environmental policies.

Piperagkas *et al.,* (2019) aimed to compare the different sampling techniques to assess microplastics in sediments. Their study showed that microplastics can be noticed in deeper sediment layers with varying concentrations to the surface layer. Therefore they claimed that sampling methods that sample different sediment layers produce different results. Additionally, classic categorizations of microplastics as microfibers, membranes, etc., are insufficient hence a

classification based on the specificities of each region and microplastic properties is recommended.

The existence and source of microplastics were investigated by observing 2539 particles in 349 sediments off nine different tourist beaches along the southeast coast of South Africa. The beaches of Durban exhibited the most microplastics concentration followed by the Sodwana & Richards Bays, Ballito and Mtunzini. Black coloured particles dominated other colours. The long shore coastal Agulhas current aiding the degradation of primary microplastics from continent and sea contributes to increased microplastic concentration in Durban. SEM images highlighted weathering/degradation by exhibiting cracks, grooves, sharp edges, deep fissures, and layered degradation. EDS results identified elements C, Si, Al, Fe, Ca, Mg, Na, K, S, Ti, Cu and Zn which were added as additives during plastic production. FTIR spectral matches identified PP (62%), PES (31.2%), PC (29.8%), NY (18.92%), RY (17.2%), PAN (11.21%) and PS (28.9%) and PET (36.1%) (Vetrimurugan *et al.,* 2020).

Gerolin *et al.,* (2020) assessed the microplastics concentrations in sediment samples collected from Solimões, Negro and Amazon rivers. Microplastics concentrations (size: 0.063–5 mm) ranged from 417 to 8178 particles/kg of dried sediment, and microplastics (size: 0.063–1 mm) from 0 to 5725 particles/kg of dried sediment. The maximum microplastics concentrations were observed in samples collected at a depth of 5–7 m while minimum concentration was observed in samples collected at a depth of 34 m. Microplastics concentrations vary due to various reasons such as hydraulic characteristics, erosive-depositional behaviour, and their proximity to the sampling sites.

Bacteria in plastic degradation

Chandra *et al.* (2020) reported polyethylenes, like HDPE, MDPE, LDPE, LLDPE and poly (butylene terephthalate), nylons, polypropylene, polystyrene, polyvinylchloride, polyurethane, polyethylene terephthalate in water and identified several species of bacteria which undertaken these plastic degradation due to its extracellular hydrolytic enzymes such as lipase, CMCase, xylanase, chitinase, keratinase and protease. *Staphylococcus sp.* (P1A), *Pseudomonas sp*. (P1B), and *Bacillus sp.* (P1C)) degrade polythenes, of these, *Bacillus sp.* showed greater degradation (42.5%).

Agustien *et al.* (2016) screened 11 polyethylene degrading-bacteria from soil, of these BTS-5 showed highest potential in degrading polyethylene (11.7% w/w) and BTS-9, BTS-12 exposed lowest potential (0.9% w/w). *Bacillus cereus* and *Bacillus gottheilii* were used to study the biodegradation of microplastics and validated by assessment of the morphological and structural changes through scanning electron microscopy and Fourier transform infrared spectroscopy analyses.

Bakht *et al.,* 2020 studied the novel microorganisms for polyethylene degradation. Totally forty waste samples were collected from different landfills and dumpsites of which, eight samples were found to degrade polythene strips in liquid medium. Of these samples showed that two strains of microbes had high potential for polythene degradation. Biochemical tests and ribotyping were performed for characterization of isolated bacteria. Resultantly, two novel bacterial strains were identified named; *Bacillus wudalianchiensis UMT* (2A) and *Pseudomonas aeruginosa_UMT* (6). Analysis of these microbes further revealed that *Bacillus wudalianchiensis_UMT and Pseudomonas aeruginosa_UMT* have capability to degrade 6.6% and 4.8% polyethylene respectively and the results disclosed that these bacteria have great

potential to degrade polythene in less time as compare to natural degradation process and can contribute to reduce pollution from our environment.

Hou *et al.,* 2022 determined the PE degradation efficiency of two *Pseudomonas*, identified by 16S rDNA analysis, and elucidated their potential mechanisms through whole genome sequencing. PE mulch lost 5.95 ± 0.03% and 3.62 ± 0.32% of its mass after incubated with *P. knackmussii* N1-2 and *P. aeruginosa* RD1-3 strains respectively during 8-week incubation period. Moreover, considerable pits and wrinkles were observed on PE. The hydrophobicity of PE films also decreased, and new oxygenic functional groups were detected on PE mulch by Fourier Transform Infrared Spectrometry (FTIR).

Singh *et al.,* 2016 studied the isolation, identification, screening and degradation of pretreated polythene by microorganisms obtained from soil. A total of 15 bacteria were recovered from different areas. Further Screening of polythene degrading microorganism was done by zone of clearance method out of 15 bacteria only 3 showed the positive results and identified to be *Staphylococcus sp* (P1A), *Pseudomonas sp.* (P1B), and *Bacillus sp.* (P1C). A total of three isolates P1A, P1B, PIC and one Consortium PID (P1A+P1B+P1C) were used for degradation of 10 and 40 micron polythene. *Bacillus sp*. (PIC) showed 42.5% followed by *Staphylococcus sp.* (P1A) 20% *Pseudomonas sp.* (P1B) 7.5 % and consortium (PID) 5% degradation by weight loss in 40 days in 40 micron polythene. This study concluded that Bacillus sp may act as solution for the problem caused by polythene in nature.

Gong *et al.,* 2018 studied the micro-size polyethylene terephthalate (PET) particles degradation. A combinatorial processing based on whole-cell biocatalysts was constructed for biodegradation of PET. Compared with enzymes, the products can be used by strain growth and do not accumulated in culture solution. Thus, feedback inhibition of products can be avoided.

When PET was treated with the alkaline strain under high pH conditions, the product concentration was higher and the size of PET particles decreased dramatically than that of the biocatalyst under neutral conditions. This shows that the method of combined processing of alkali and organisms is more efficient for biodegradation of PET.

Chen *et al.,* 2020 investigated the hyperthermophilic composting (*h*TC) technology in full-scale (200 t) for *in situ* biodegradation of sludge-based MPs. After 45 days of *h*TC treatment, 43.7% of the MPs was removed from the sewage sludge, which is the highest value ever reported for MPs biodegradation. The underlying mechanisms of MPs removal were investigated in lab-scale polystyrene-microplastics (PS-MPs) biodegradation experiments. The *h*TC inoculum degraded 7.3% of the PS-MPs at 70 °C in 56 days, which was about 6.6 times higher than that of the conventional thermophilic composting (*c*TC) inoculum at 40 °C. Analyses of the molecular weight and physicochemical properties of the PS-MPs residuals indicated that hyperthermophilic bacteria in *h*TC accelerated PS-MPs biodegradation through excellent bio-oxidation performance. High-throughput sequencing suggested that *Thermus*, *Bacillus*, and *Geobacillus* were the dominant bacteria responsible for the highly efficient biodegradation during *h*TC. These results reveal the critical role of hyperthermophilic bacteria in MPs biodegradation during *h*TC, highlighting a promising strategy for sludge-based MPs removal from the real environment.

Auta *et al.,* 2017 studied eight bacterial strains isolated from mangrove sediment in Peninsular Malaysia to mitigate the environmental impact of microplastics and develop a clean-up option. The bacterial isolates were screened for their potential to degrade UV-treated microplastics from polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS). Only two isolates, namely, *Bacillus cereus* and *Bacillus gottheilii*, grew on

a synthetic medium containing different microplastic polymers as the sole carbon source. A shake flask experiment was carried out to further evaluate the biodegradability potential of the isolates. Degradation was monitored by recording the weight loss of microplastics and the growth pattern of the isolates in the mineral medium. The biodegradation extent was validated by assessment of the morphological and structural changes through scanning electron microscopy and Fourier transform infrared spectroscopy analyses. The calculated weight loss percentages of the microplastic particles by *B. cereus* after 40 days were 1.6%, 6.6%, and 7.4% for PE, PET, and PS, respectively. *B. gottheilii* recorded weight loss percentages of 6.2%, 3.0%, 3.6%, and 5.8% for PE, PET, PP, and PS, respectively.

Niu *et al.,* 2021 investigated the vertical distribution of microplastics (with the size < 5 mm) and the bacterial community assemblages colonizing microplastics in urban river sediments at a depth from 0 to 50 cm. The results showed that both microplastics and associated microbial communities presented vertical profiles in river sediments. The mean concentration of microplastics increased from the shallow layers to the deep layers of sediments, and smaller microplastic particles were dominant in deeper layers. A greater degradation of microplastics in deeper layers was confirmed by contact angle measurements, scanning electron microscopy and Fourier transform infrared spectroscopy-attenuated total reflectance analyses. Unlike the surrounding sediments, the whole bacterial communities on microplastics exhibited higher frequency of positive correlations in the bacterial co-occurrence network, which indicated a less stability of bacterial communities on microplastics. The indicative plastic-degrading bacteria with an average abundance of 4.33% was found in the surrounding sediments, while on the microplastics 21.37% was found. This study provides new insight into the vertical distribution

and the potential microbial degrading characteristics of microplastics in urban river sediments, which expanded our understanding of the fate of microplastics in aquatic environments.

Sharma *et al.,* 2014 studied the in vitro biodegradation of polyethylene and PVC strips using the microorganisms isolated from the soil over a period of 1 month of incubation. The microbial species associated with the degrading capibilities were identified as one Gram (+)ve and one Gram (-)ve bacteria. The efficiencies of these two bacteria in the degradation of plastics were compared and one with the higher degrading capacity is identified to be *Bacillus cereus* strain.

Fungi in plastic degradation

Alshehrei 2017 studied ten fungal strains were isolated from Red Sea water; Jeddah, Saudi Arabia. These isolates were screened to examine their ability in biodegradation of Low density polyethylene, selected fungi which related to *Aspergillus* and *Penicillium* showed ability to degrade polyethylene films and powder. *Penicillium sp.* showed the highest percentage in reduction of polyethylene weight with (43.4%). Detection of morphological changes by SEM demonstrated that fungal growth was observed clearly on the treated film. Mycelia and conidia of *Aspergillus sp* and *Penicillium sp* have seen physically associated with the surface.

Pramila and Ramesh (2011) studied the biodegradation of low density polyethylene by fungi isolated from marine water used a SEM analysis and reported *Aspergillus spp*. showed the higher efficiency of degradation.

Paco *et al.,* 2017 studied the fungus *Zalerion maritimum* to different times of exposition to polyethylene (PE) pellets, in a minimum growth medium, was evaluated, based on the quantified mass differences in both the fungus and the microplastic pellets used. Additionally,

molecular changes were assessed through attenuated total reflectance Fourier transform Infrared Spectroscopy (FTIR-ATR) and Nuclear Magnetic Resonance (NMR). Results showed that, under the tested conditions, *Z. maritimum* is capable of utilizing PE, resulting in the decrease, in both mass and size, of the pellets. These results indicate that this naturally occurring fungus may actively contribute to the biodegradation of microplastics, requiring minimum nutrients.

Luz *et al.,* 2019 investigated the degradation of oxo-biodegradable plastic bags and green polyethylene by Pleurotus ostreatus. The degradation was possible by three reasons: (a) presence of pro-oxidant ions or plant polymer, (b) low specificity of the lignocellulolytic enzymes, and (c) the presence of endomycotic nitrogen-fixing microorganisms. The plastic bags' degradation by abiotic and microbial process using the exposure to sunlight and the use of a white-rot fungus were also studied. The degradation of plastic bags was more effective when the abiotic and biotic degradations were combined.

Sangale *et al.,* 2019 discovered the elite polythene deteriorating fungi (isolated from the rhizosphere soil of *Avicennia marina*). From 12 different eco-geographical locations along the West Coast of India, total 109 fungal isolates were recorded. The polythene deteriorating fungi were screened at varied pH (3.5, 7 and 9.5) based on changes in weight and tensile strength of the treated polythene at ambient temperature with continuous shaking for 60 days. BAYF5 isolate (pH 7) results in maximum reduction in weight (58.51 ± 8.14) whereas PNPF15 (pH 3.5) recorded highest reduction in tensile strength (94.44 ± 2.40). The highest percent weight gain (28.41 ± 6.99) with MANGF13 at pH 9.5. To test the reproducibility of the results, the elite polythene degrading fungal isolates based on weight loss and reduction in tensile strength were only used for repetition experiment and the results based on the reduction in tensile strength were found only reproducible. Polythene biodegradation was further confirmed using Scanning

Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The most efficient polythene deteriorating fungal isolates were identified as *Aspergillus terreus* strain MANGF1/WL and *Aspergillus sydowii* strain PNPF15/TS using both morphological keys and molecular tools.

Gao *et al.,* 2021 isolated a fungus (named *Alternaria* sp. FB1) that possessing a prominent capability of colonizing, degrading and utilizing PE. The molecular weight of PE film decreased 95% after the fungal treatment. GC-MS clarified a four-carbon product (named Diglycolamine) accounted for 93.28% of all degradation products after the treatment by strain FB1. They defined potential enzymes that involved in the degradation of PE through a transcriptomic method. The degradation capabilities of two representative enzymes including a laccase and a peroxidase were verified. Lastly, a complete biodegradation process of PE is proposed.

Khruengsai *et al.,* 2021 studied the biodegradation of 30 fungi from Thailand, screened in mineral salt medium agar containing low-density polyethylene (LDPE) films. Diaporthe italiana, Thyrostroma jaczewskii, Collectotrichum fructicola, and Stagonosporopsis citrulli were found to grow significantly by culturing with LDPE film as the only sole carbon source compared to those obtained from Aspergillus niger. These fungi were further cultured in mineral salt medium broth containing LDPE film as the sole carbon source for 90 days. The biodegradation ability of these fungi was evaluated from the amount of CO_2 and enzyme production. Different amounts of CO_2 were released from D. italiana, T. jaczewskii, C. fructicola, S. citrulli, and A. niger culturing with LDPE film, ranging from 0.45 to 1.45, 0.36 to 1.22, 0.45 to 1.45, 0.33 to 1.26, and 0.37 to 1.27 g/L, respectively. These fungi were able to secrete a large amount of laccase enzyme compared to manganese peroxidase, and lignin

peroxidase enzymes detected under the same conditions. The degradation of LDPE films by culturing with these fungi was further determined. LDPE films cultured with D. italiana, T. jaczewskii, C. fructicola, S. citrulli, and A. niger showed weight loss of 43.90%, 46.34%, 48.78%, 45.12%, and 28.78%, respectively. The tensile strength of LDPE films cultured with D. italiana, T. jaczewskii, C. fructicola, S. citrulli, and A. niger also reduced significantly by 1.56, 1.78, 0.43, 1.86, and 3.34 MPa, respectively. The results from Fourier transform infrared spectroscopy (FTIR) reveal an increasing carbonyl index in LDPE films culturing with these fungi, especially C. fructicola. Analysis of LDPE films using scanning electron microscopy (SEM) confirmed the biodegradation by the presence of morphological changes such as cracks, scions, and holes on the surface of the film. The volatile organic compounds (VOCs) emitted from LDPE films cultured with these fungi were analyzed by gas chromatography-mass spectrometry (GC-MS). VOCs such as 1,3-dimethoxy-benzene, 1,3-dimethoxy-5-(1-methylethyl)-benzene, and 1,1-dimethoxy-decane were detected among these fungi. Overall, these fungi have the ability to break down and consume the LDPE film. The fungus C. fructicola is a promising resource for the biodegradation of LDPE.

Enzymes for plastic degradation

Ahmad *et al.,* 2019 investigated the structures of two genes from *Roseateles depolymerans* strain TB-87 encoding the esterases Est-H and Est-L, which can degrade aliphatic-aromatic copolyesters, were annotated. Two open reading frames (ORFs) consisting of 1083 bp and 870 bp nucleotides, corresponding to est-H and est-L, encoding enzymes of 290 and 289 amino acids, respectively, were predicted. In addition, another ORF consisting of 735 bp encoding a chaperone-like protein (Est-Ch) of 244 amino acids was identified in the intergenic region of est-H and est-L. The presence of a promoter region upstream of est-H and the absence

of a terminator region downstream of the ORF and vice versa for est-Ch, suggests that est-H and est-Ch are polycistronically expressed. A homology search for Est-H and Est-L revealed homology with plastic degrading enzymes, such as esterases and cutinases, while Est-Ch showed homology with a bacterial lipase chaperone. As consensus lipase sequences (-Gly-His-Ser-Met-Gly-) were observed in these enzymes, Est- H and Est-L were hypothesized to be hydrolases with serine (Ser) in their active center. Three dimensional structures of Est-H and Est-L without their putative signal sequences were constructed using Est119 from Thermobifida alba strain AHK119 as the template; the structures and positions of the catalytic triad (Ser, Asp, His) active centers were similar to those of Est119. A mutant strain in which the annotated esterase-encoding genes were disrupted using a homologous recombination method lost the ability to form a clear zone on poly(butylene succinate-co-adipate (PBSA) emulsion-overlaid nutrient agar plates.

Maeda *et al.,* 2005 used biodegradable plastics as fermentation substrates for the filamentous fungus Aspergillus oryzae. This fungus could grow under culture conditions that contained emulsified poly-(butylene succinate) (PBS) and emulsified poly-(butylene succinate-co-adipate) (PBSA) as the sole carbon source, and could digest PBS and PBSA, as indicated by clearing of the culture supernatant. We purified the PBS-degrading enzyme from the culture supernatant, and its molecular mass was determined as 21.6 kDa. The enzyme was identified as cutinase based on internal amino acid sequences. Specific activities against PBS, PBSA and poly-(lactic acid) (PLA) were determined as 0.42 U/mg, 11 U/mg and 0.067 U/mg, respectively.

Suzuki *et al.,* 2014 studied Paraphoma-related fungal strain B47-9 secreted a biodegradable plastic (BP)-degrading enzyme which amounted to 68 % (w/w) of the total secreted proteins in a culture medium containing emulsified poly(butylene succinate-co-adipate)

(PBSA) as sole carbon source. The gene for this enzyme was found to be composed of an open reading frame consisting of 681 nucleotides encoding 227 amino acids and two introns. Southern blot analysis showed that this gene exists as a single copy. The deduced amino acid sequence suggested that this enzyme belongs to the cutinase (E.C.3.1.1.74) family; thus, it was named Paraphomarelated fungus cutinase-like enzyme (PCLE). It degraded various types of BP films, such as poly(butylene succinate), PBSA, poly(butylene adipate-co-terephthalate), poly(ε-caprolactone), and poly(DL-lactic acid). It has a molecular mass of 19.7 kDa, and an optimum pH and temperature for degradation of emulsified PBSA of 7.2 and 45 °C, respectively. Ca2+ ion at a concentration of about 1.0 mM markedly enhanced the degradation of emulsified PBSA.

Juarez *et al.,* 2021 studied the molecular docking simulation for catalytic enzyme degradation of PE using individual enzymes: laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP) and unspecific peroxygenase (UnP). PE-binding energy, PE-binding affinity and dimensions of PE-binding sites in the enzyme cavity were calculated in each case. Four hypothetical PE biodegradation pathways were proposed using individual enzymes, and one pathway was proposed using a synergic enzyme combination. The results shown that in nature, enzymes act in a synergic manner, using their specific features to undertake an extraordinarily effective sequential catalytic process for organopollutants degradation. In this process, Lac (oxidase) is crucial to provide hydrogen peroxide to the medium to ensure pollutant breakdown. UnP is a versatile enzyme that offers a promising practical application for the degradation of PE and other pollutants due to its cavity features.

Wei *et al.,* 2022 studied the enzymatic hydrolysis of a biodegradable polyester (poly(ε-caprolactone)) by Amano Lipase PS in an aqueous (buffer) environment yielded rapidly an excessive number of microplastic particles; merely 0.1 g of poly(ε-caprolactone) film was

demonstrated to yield millions of particles. Microplastic particles formed had irregular shapes with an average size of around 10 μm, with only a few reaching 60 μm. The formation of microplastic particles resulted from the uneven hydrolysis/erosion rate across the polymer film surface, which led to a rough and undulating surface with ridge, branch, and rod-shaped micro-protruding structures. The consequent detachment and fragmentation of these micro-sized protruding structures resulted in the release of microplastics to the surroundings. Together with microplastics, hydrolysis products such as acidic monomers and oligomers were also released during the enzymatic hydrolysis process, causing a pH decrease in the surrounding liquid. The results suggest that the risk of microplastic pollution from biodegradable plastics is notable despite their biodegradation.

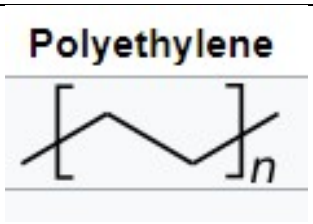
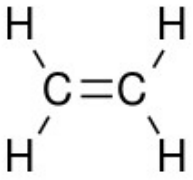
Zrimec *et al.,* 2021 constructed hidden Markov models from experimentally verified enzymes and mined ocean and soil metagenomes to assess the global potential of microorganisms to degrade plastics. By controlling for false positives using gut microbiome data, compiled a catalogue of over 30,000 nonredundant enzyme homologues with the potential to degrade 10 different plastic types. While differences between the ocean and soil microbiomes likely reflect the base compositions of these environments, and find that ocean enzyme abundance increases with depth as a response to plastic pollution and not merely taxonomic composition. By obtaining further pollution measurements, they observed that the abundance of the uncovered enzymes in both ocean and soil habitats significantly correlates with marine and country-specific plastic pollution trends.

Mohanan *et al.,* 2020 studied the petroleum-derived (petro-)polymers such as polyethylene (PE), polyethylene terephthalate (PET), polyurethane (PU), polystyrene (PS), polypropylene (PP), and polyvinyl chloride (PVC) are extremely recalcitrant to natural

biodegradation pathways. Some microorganisms with the ability to degrade petro-polymers under *in vitro* conditions have been isolated and characterized. In some cases, the enzymes expressed by these microbes have been cloned and sequenced. The rate of polymer biodegradation depends on several factors including chemical structures, molecular weights, and degrees of crystallinity. Polymers are large molecules having both regular crystals (crystalline region) and irregular groups (amorphous region), where the latter provides polymers with flexibility. Highly crystalline polymers like polyethylene (95%), are rigid with a low capacity to resist impacts. PET-based plastics possess a high degree of crystallinity (30–50%), which is one of the principal reasons for their low rate of microbial degradation, which is projected to take more than 50 years for complete degraded in the natural environment, and hundreds of years if discarded into the oceans, due to their lower temperature and oxygen availability. The enzymatic degradation occurs in two stages: adsorption of enzymes on the polymer surface, followed by hydro-peroxidation/hydrolysis of the bonds. Microbial and enzymatic degradation of waste petro-plastics is a promising strategy for depolymerization of waste petro-plastics into polymer monomers for recycling, or to covert waste plastics into higher value bioproducts, such as biodegradable polymers via mineralization.

The discovery of bacterial enzymes that specifically breakdown PET by palm *et al.,* 2019, is a possible approach. PET is converted to mono-(2-hydroxyethyl) terephthalate by *Ideonella sakaiensis* PETase, a structurally well- characterized consensus/- hydrolase fold enzyme (MHET). MHETase, hydrolyzes MHET to form the PET educts terephthalate and ethylene glycol. They published crystal structures of MHETase bound to a nonhydrolyzable MHET homologue and active ligand- free MHETase. MHETase has a typical α/β-hydrolase domain and a lid domain that confers substrate selectivity, similar to feruloyl esterases.

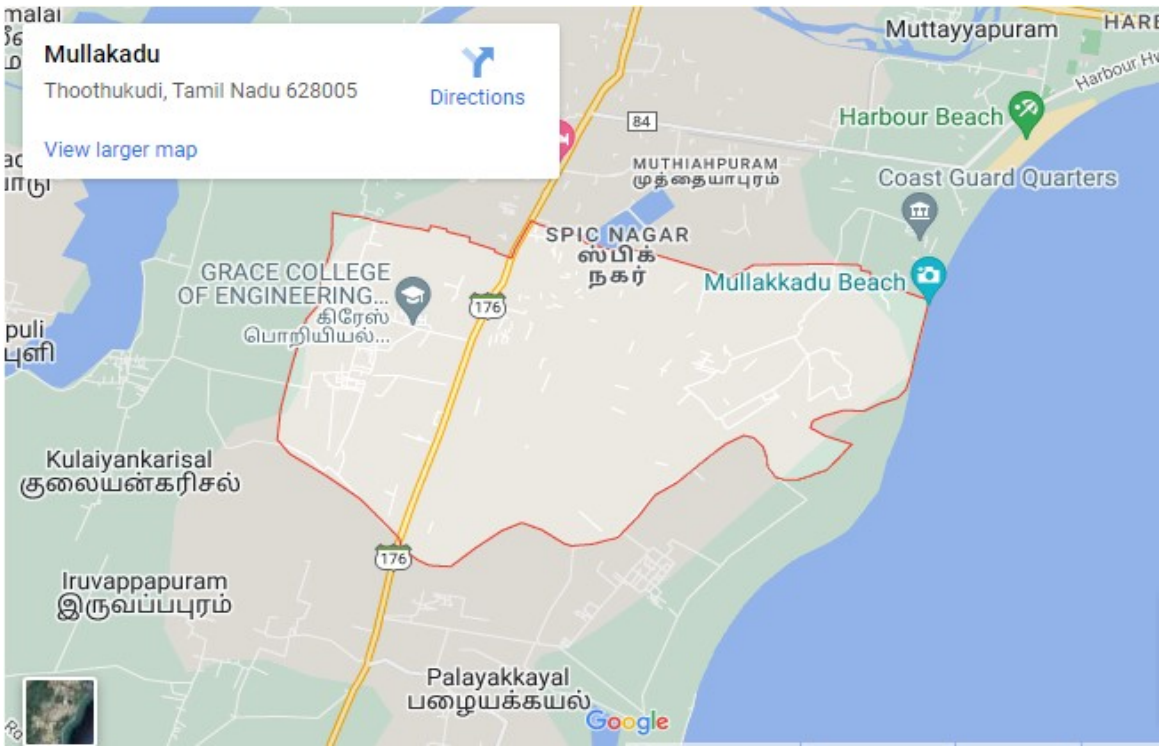
POLYTHENE

Traditional name	Polythene or Polyethylene (PE)
IUPAC name	Poly(methylene)
Source based name	Polyethene
Chemical formula	$(C_2H_4)_n$
Structure	
Monomer (Ethylene)	
CAS number	9002-88-4
Density	0.88-0.96 g/cm
Melting point	115-135^{o}C

Adopted from Jones et al. (2008)

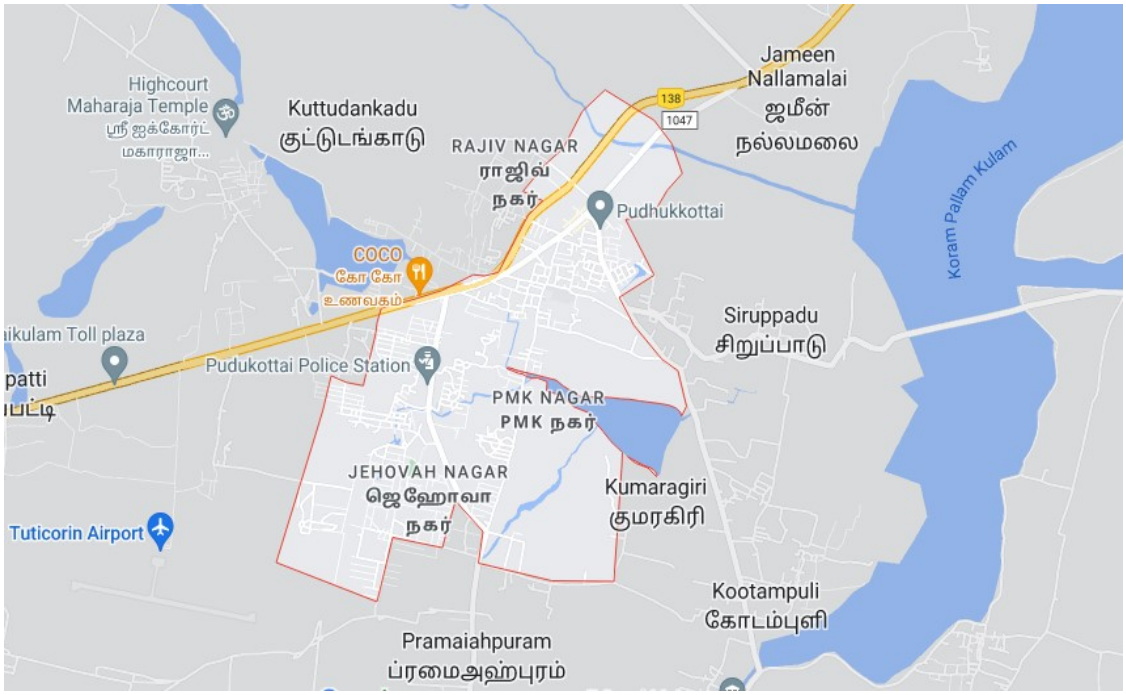
STUDY AREA

MULLAKKADU



Mullakkadu is a small Village in Thoothukkudi Block in Tuticorin District of Tamil Nadu State, India. It comes under Mullakkadu Panchayath. It is located 10 KM towards South from District head quarters Thoothukudi. 8 KM from Thoothukudi Rural. 625 KM from State capital Chennai. Mullakkadu Pin code is 628005. It is near to Bay of Bengal. There is a chance of humidity in the weather. (http://www.onefivenine.com/india/villages/Tuticorin/Thoothukkudi/Mullakkadu). The village Mullakkadu surrounded with many industries. The industrial wastes are dumped in the soil and during rainy season it has been flooded over the water channel of Mullakkadu.

PUDHUKKOTTAI



Pudhukkottai is a small Village in Thoothukkudi Block in Tuticorin District of Tamil Nadu State, India. It comes under Kumaragiri Panchayath. It is located 13 KM towards west from District head quarters Thoothukudi. 11 KM from Thoothukudi Rural. 627 KM from State capital Chennai. Pudukottai Pin code is 628103. It is near to Bay of Bengal. There is a chance of humidity in the weather. (http://www.onefivenine.com/india/villages/Tuticorin/Thoothukkudi/Pudukottai). The village Pudhukkottai surrounded by many industries and these industrial wastes are dumped in the soil and during rainy season it have been flooded over the water bodies of Pudhukkottai.

MATERIALS AND METHODS

Sample collection

The samples were collected from Mullakkadu village and Pudhukkottai village of Thoothukudi District, Tamil Nadu State, India. Both freshwater and soil samples were used for this present study. The freshwater and soil samples collected from the study area using glass bottles and stoppered properly to prohibit the environmental contamination.

Isolation of plastic degrading microbes

The collected freshwater and soil samples were serially diluted from 10^{-1} to 10^{-5} to isolate a pure colonies of microorganisms (bacteria and fungi) which are capable of degrading plastics.

Isolation of fungal colonies

Pour plate method was followed for the isolation of fungal colonies. The suspensions up to 10^{-6} were transferred to Petriplates along with Potato Dextrose Agar and incubated at 28°C for 7 days. After the incubation period the fungal colonies were counted, isolated and placed on new agar plates and the cultured plates were stored under refrigerated condition.

Degradation of micro and nanoplastics using fungi

Polythene disposable gloves (15µM thickness) were used as a microplastic source. The half strength Potato Dextrose broth medium was prepared and 100 mL of medium was poured into a 250 mL conical flask and 100 mg of microplastics were put inside the conical flask. The pure fungal culture was also inoculated and incubated for 30 days at 28°C.

Detection of plastics biodegradation

After the incubation period, the microplastic sheets was disinfected thoroughly with 70% ethanol for 30 minutes and then washed with distilled water. The collected microplastic residues were kept inside a Hot Air Oven for 2 hours at 100^{oC} for drying purpose. The weight reduction of the plastics were calculated using following formulae (Saminathan *et al.,* 2014):

$$Weight\ Loss = Initial\ weight\ of\ plastics - Final\ Weight\ of\ plastics$$

$$Percentage\ of\ weight\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Isolation of bacteria

The pour plate method was followed for the isolation of bacterial culture.1 ml of samples from each serial dilution was poured to Petriplate and add 30 mL of Nutrient agar. The agar plates were incubated at $37^{o}C$ for 48 hours. After the incubation period the bacterial colonies was counted using Colony Counter and the bacterial colonies were isolated and grown further by streak plate method (Gupta *et al.,* 2010) and stored under refrigerated condition.

Screening of plastic degrading bacteria

The isolated bacterial strains will be inoculated on mineral medium with plastic polymers and will be incubated at room temperature. The clear zone will formed by the bacteria, if there's no clear zone but bacteria can grow in the medium, then it will be continued for biodegradation test of plastics (Kambe *et al*., 1995).

Degradation of micro and nanoplastics using bacteria

The half strength Nutrient broth medium was prepared and 50 mL of medium was poured into a 100 mL conical flask and 30 mg of microplastics were put inside the conical flask. The pure bacterial culture was also inoculated and incubated for 30 days at 37°C using incubator .

Detection of plastics biodegradation

After the incubation period, the microplastic sheets was disinfected thoroughly with 70% ethanol for 30 minutes and then washed with distilled water. The collected microplastic residues were kept inside a Hot Air Oven for 2 hours at 100^{oC} for drying purpose. The weight reduction of the plastics were calculated using following formulae (Saminathan *et al.,* 2014):

$$Weight\ Loss = Initial\ weight\ of\ plastics - Final\ Weight\ of\ plastics$$

$$Percentage\ of\ weight\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100$$

Scanning electron microscopy (SEM) analysis

The microbes treated plastic sheets will be subjected to analyze qualitatively using SEM. The samples will be allowed to ionize using the ion sputter on a metal stub for 20 min. After gold coating, the samples will be kept under vacuum to view microscopically from 50 X to 15000 X magnification using SEM.

Species identification

Genomic DNA Extraction

The selected bacterial and fungal culture was used to inoculate Luria Bertani (LB) broth and mycological broth respectively, and will incubate overnight at 37 ºC. The culture (1.5 ml) will be spun at 7000 rpm for 3 min. The pellet is resuspended in 400 µl of Sucrose TE. Lysozyme will be added to a final concentration of 8 mg/ml and the solution will be incubated for 1h at 37 ºC. To the tube, 100 µl of 0.5M EDTA (pH 8.0), 60 µl of 10 % SDS and 3 µl of proteinase K from

20 mg/ml stock will be added and incubated at 55°C overnight. Extracted with equal volume of phenol: chloroform (1:1), centrifuged (10000 rpm; 10 min) and the supernatant will be transferred to a sterile tube. The supernatant will be extracted twice with phenol: chloroform and once with chloroform: isoamylalcohol (24:1) and ethanol precipitated. RNase treatment will be done to avoid RNA contamination in the DNA sample. The DNA pellet will be resuspended in sterile distilled water and stored at 4 °C for immediate use and at – 20°C for long-term storage.

PCR amplification

16S rRNA genes of the bacterial isolates was amplified with genomic DNA isolates as template and 16s Forward Primer: 5'-AGAGTRTGATCMTYGCTWAC-3' and 16s Reverse Primer: 5'-CGYTAMCTTWTTACGRCT-3' in the composition and amplification cycle. Each reaction mixture contained 1 µl of template DNA, 400 ng of two primers, dNTPs (2.5mM each) 4 µl, 10X Taq DNA Polymerase Assay Buffer 10 µl and 3 units (1µl) of Enzyme Master Mix (Bioron). The PCR program consists of an initial denaturation step at 94°C for 5 min, followed by 30 cycles of DNA denaturation at 94°C for 30 sec, primer annealing at 55°C for 30 sec, and primer extension at 72°C for 2 min was carried out in Thermal Cycler. After 35 cycles, a final extension at 72°C for 20 min was added. PCR amplification targeting bacterial 16S rRNA gene will be performed using the Prokaryotes 16s rRNA specific primer (Teske *et al.,* 2002).

Cloning and Sequencing

The PCR product will be sequenced and will be analyzed and the annotated sequence will be submitted in GenBank.

FT-IR analysis of microplastic sample

The microplastic sample was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on Thermoscientific Nicot iS5 iDl transmission, between 4000-400 cm^{-1} (Kareru et al., 2008).

RESULTS

Isolation of microorganisms

The microorganisms (bacteria and fungi) isolated from the serially diluted freshwater and soil samples ranging from 10^{-1} to 10^{-5}. Pour plate method was used for the isolation of both bacteria and fungi and the colonies of each plate was counted using colony counter. The results of isolated bacterial and fungal colonies were presented in **Table 1 & 2** and **Plate 1&2.** Totally 60 bacterial strains were isolated from the sample. For bacterial culture, Pudhukkottai soil sample (JS II) showed highest number of colonies (93) at 10^{-1} concentration while Mullakkadu soil sample (SM I) showed lowest number of colonies (15) at 10^{-5} concentration. For fungal culture, totally 18 fungal strains were isolated from different study area. Of these Pudhukkottai soil sample (JS II) showed highest number of colonies (19) at 10^{-1} concentration while Mullakkadu water (MS II) and Pudhukkottai water (P I) showed lowest number of colonies (2) at 10^{-5} concentration. The control plate does not contain any bacterial or fungal colonies.

FTIR spectroscopy analysis of microplastic sample

The FTIR spectroscopy analysis was carried out to predict the functional groups present in the polyethylene sheets. The results of FTIR spectral studies were presented in **Table 3** and **Figure 1.**

From the spectral data, presence of C-1, C-Br, C-Cl, C-H, C-F, O-H, CH_2, NO_2, C=O and N-H were identified. These bonding are responsible for the presence of alkyl halide, phenol, aldehyde, carboxylic acid, amine salt and alcohol in the polyethylene sheets.

Table 1. Bacterial colonies observed after serial dilution of different samples

Sample	Sample ID	No. of Colonies Observed					
		Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Mullakkadu water	MS I	0	88	73	69	54	40
	MS II	0	92	87	72	67	54
Pudukottai water	P I	0	38	29	25	23	18
	P II	0	83	54	41	35	35
Mullakkadu soil	SM I	0	53	42	36	25	15
	SM II	0	65	60	56	51	44
Pudukottai soil	JS I	0	48	37	28	26	20
	JS II	0	93	67	52	49	43

Table 2. Fungal colonies observed after serial dilution of different samples

Sample	Sample ID	No. of Colonies Observed					
		Control	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Mullakkadu water	MS I	0	16	14	9	5	3
	MS II	0	14	11	9	4	2
Pudukottai water	P I	0	14	9	6	4	2
	P II	0	13	8	6	4	3
Mullakkadu soil	SM I	0	18	13	8	5	4
	SM II	0	17	12	7	4	3
Pudukottai soil	JS I	0	18	13	6	5	3
	JS II	0	19	12	7	4	3

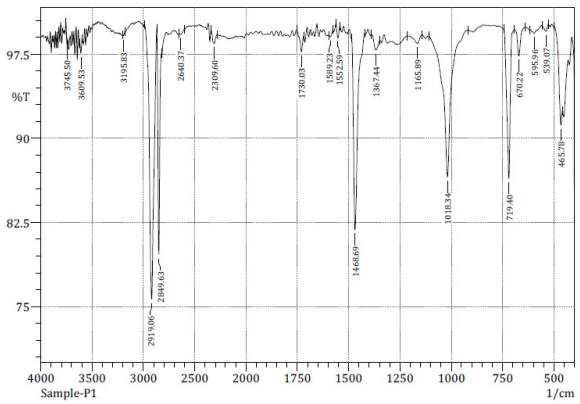


Figure 1. FT-IR analysis of Polyethylene sheets

Table 3. FT-IR analysis of polyethylene gloves

Peak Value	Functional group	Intensity
465.78	C- l	Strong
539.07	C – Br Alkyl halides	Strong
595.96	C- Br Alkyl halides	Strong
670.22	C- Cl	Strong
719.4	C- H bend	Medium
1018.34	C- F	Strong
1165.89	C- F	Strong
1367.44	O –H bending, phenol	Medium
1468.69	CH_2 bend	Medium
1552. 59	NO_2 Stretch	Strong
1589.23	NO_2 Stretch	Strong
1730.03	C = O Stretch, aldehyde	Strong
2309.6	Unknown	Unknown
2640.37	O- H Stretch, carboxylic acid	Strong, broad
2849.63	N –H stretch, amine salt	Strong, broad
2919.06	N- H stretch, amine salt	Strong, broad
3195.83	O- H stretch, carboxylic acid	Strong, broad
3609.53	O-H stretch, alcohol	Medium , sharp
3745.5	Unknown	Unknown

Table 4. Degradation of polyethylene sheets using various bacterial strains

S. No.	Bacterial stains isolated from different samples	Weight of microplastics		Percentage of polyethylene degradation
		Initial Weight (in mgs)	Final Weight (in mgs)	
1.	MS-A	30	26	13.33
2.	MS-B	30	29	3.33
3.	MS-C	30	27.5	8.33
4.	MS-D	30	27	10
5.	MS-E	30	28	6.67
6.	MS-F	30	30	0
7.	MS-G	30	27	10
8.	MS-H	30	29	3.33
9.	MS-I	30	30	0
10.	MS-J	30	30	0
11.	MS-K	30	29	3.33
12.	MS-L	30	30	0
13.	MS-M	30	26.5	11.67
14.	MS-N	30	27	10
15.	MS-O	30	30	0
16.	P-A	30	30	0
17.	P-B	30	30	0
18.	P-C	30	30	0
19.	P-D	30	30	0
20.	P-E	30	30	0
21.	P-F	30	27	10
22.	P-G	30	30	0
23.	P-H	30	30	0
24.	P-I	30	30	0
25.	P-J	30	30	0
26.	P-K	30	30	0
27.	P-L	30	30	0
28.	P-M	30	30	0
29.	P-N	30	30	0
30.	P-O	30	30	0
31.	SM-A	30	30	0
32.	SM-B	30	27	10
33.	SM-C	30	30	0

34.	SM-D	30	30	0
35.	SM-E	30	28	6.67
36.	SM-F	30	30	0
37.	SM-G	30	27	10
38.	SM-H	30	30	0
39.	SM-I	30	30	0
40.	SM-J	30	30	0
41.	SM-K	30	30	0
42.	SM-L	30	30	0
43.	SM-M	30	27	10
44.	SM-N	30	30	0
45.	SM-O	30	30	0
46.	JS- A	30	30	0
47.	JS-B	30	30	0
48.	JS-C	30	28	6.67
49.	JS-D	30	30	0
50.	JS-E	30	30	0
51.	JS-F	30	30	0
52.	JS-G	30	30	0
53.	JS-H	30	30	0
54.	JS-I	30	29	3.33
55.	JS-J	30	30	0
56.	JS-K	30	28	6.67
57.	JS-L	30	30	0
58.	JS-M	30	30	0
59.	JS-N	30	30	0
60.	JS-O	30	30	0

Table 5. Degradation of polyethylene sheets using various fungal strains

S. No.	Fungal stains isolated from different samples	Weight of Polyethylene		Percentage of polyethylene degradation
		Initial Weight (in mgs)	Final Weight (in mgs)	
1.	A	100	81	19
2.	B	100	75	25
3.	C	100	10	90
4.	D	100	12	88
5.	E	100	75	25
6.	F	100	26	74
7.	G	100	11	89
8.	H	100	11	89
9.	I	100	16	84
10.	J	100	10	90
11.	K	100	62	38
12.	L	100	11	89
13.	M	100	84	16
14.	N	100	70	30
15.	O	100	31	69
16.	P	100	11	89
17.	Q	100	10	90
18.	R	100	13	87

Bacterial degradation of polyethylene sheets

The results observed after one month of incubation of the experiment set-up are reported in **Table 4 & 5** and **Plate 5, 6 and 8**. During the incubation period, the bacterial sample along with the plastic sheets were kept at 37^{o}C with shaking at certain interval. The half strength media was provided to the culture, in order to increase the microplastic degradation. At the end of the incubation period it was inferred that the bacterial isolates possessed the capability to degrade the plastic strips which was shown by the weight difference of the plastic strips compared to the control ones. Totally 60 bacterial strains were isolated from the collected soil and freshwater samples and studied its ability to degrade micro and nanoplastics. Of these 11 species shown good results in degradation (i.e.) above 40%, 41 species shown moderate level of degradation (i.e.) fall between 11% and 39% while 8 species shown very low level of degradation (i.e.) below 10%.

Fungal degradation of polyethylene sheets

The results observed after one month of incubation of the experiment set-up are reported in **Table 5** and **Plate 7.** During the incubation period, the fungal sample along with the plastic sheets were kept at 28^{o}C with shaking at certain interval. The half strength media were provided to a culture, in order to increase the microplastic degradation. At the end of the incubation period it was inferred that the fungal isolates possessed the capability to degrade the plastic strips which was shown by the weight difference of the plastic strips compared to the control ones. Totally 18 fungal strains were isolated from the collected soil and freshwater samples and studied its ability to degrade micro and nanoplastics. Of these 9 species shown good results in degradation i.e.

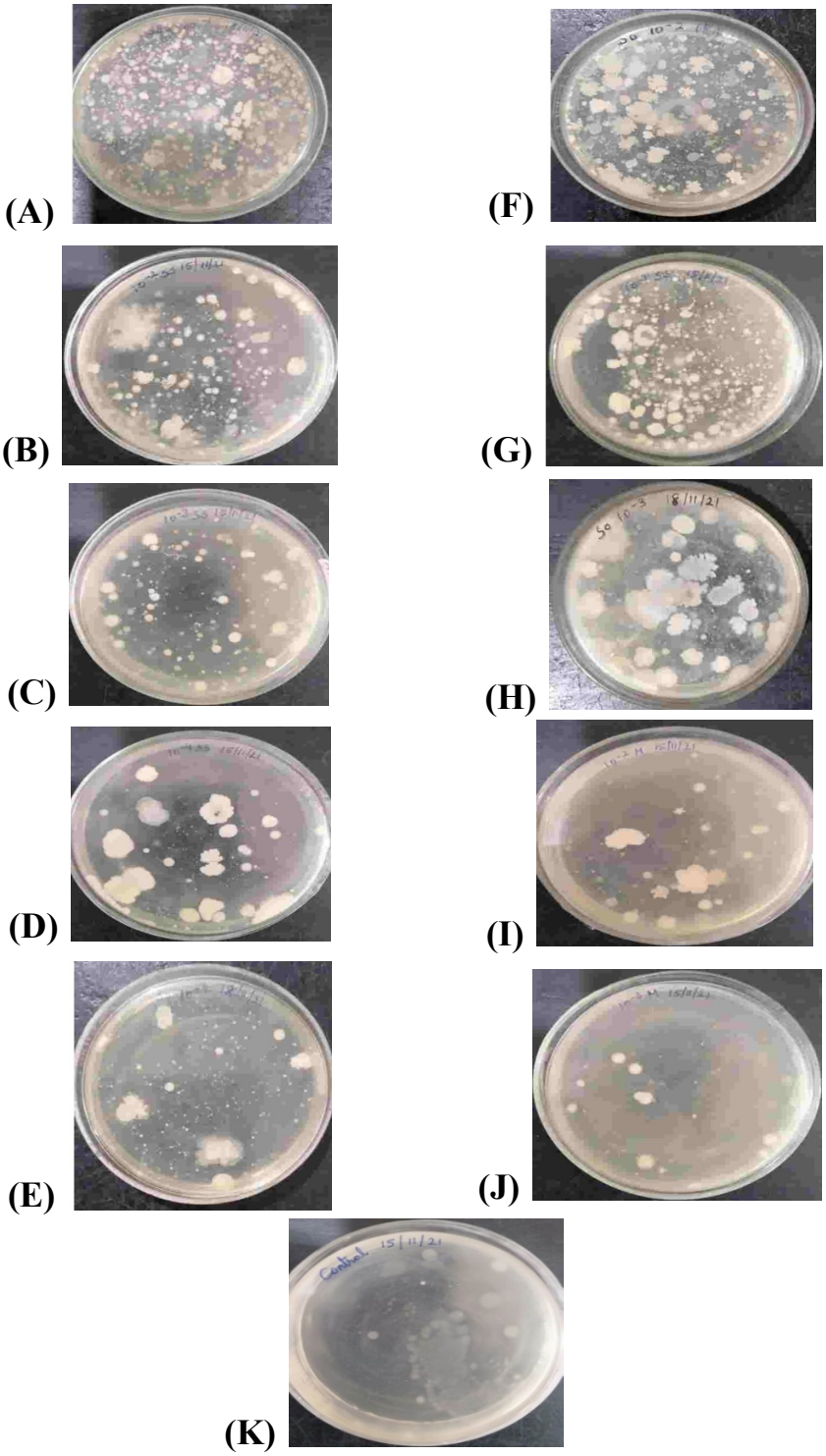


Plate 1. Bacterial colonies observed after serial dilution of soil samples collected from Mullakkadu (A, B, C, D, E) and Pudhukkottai (F, G, H, I, J). K. Control

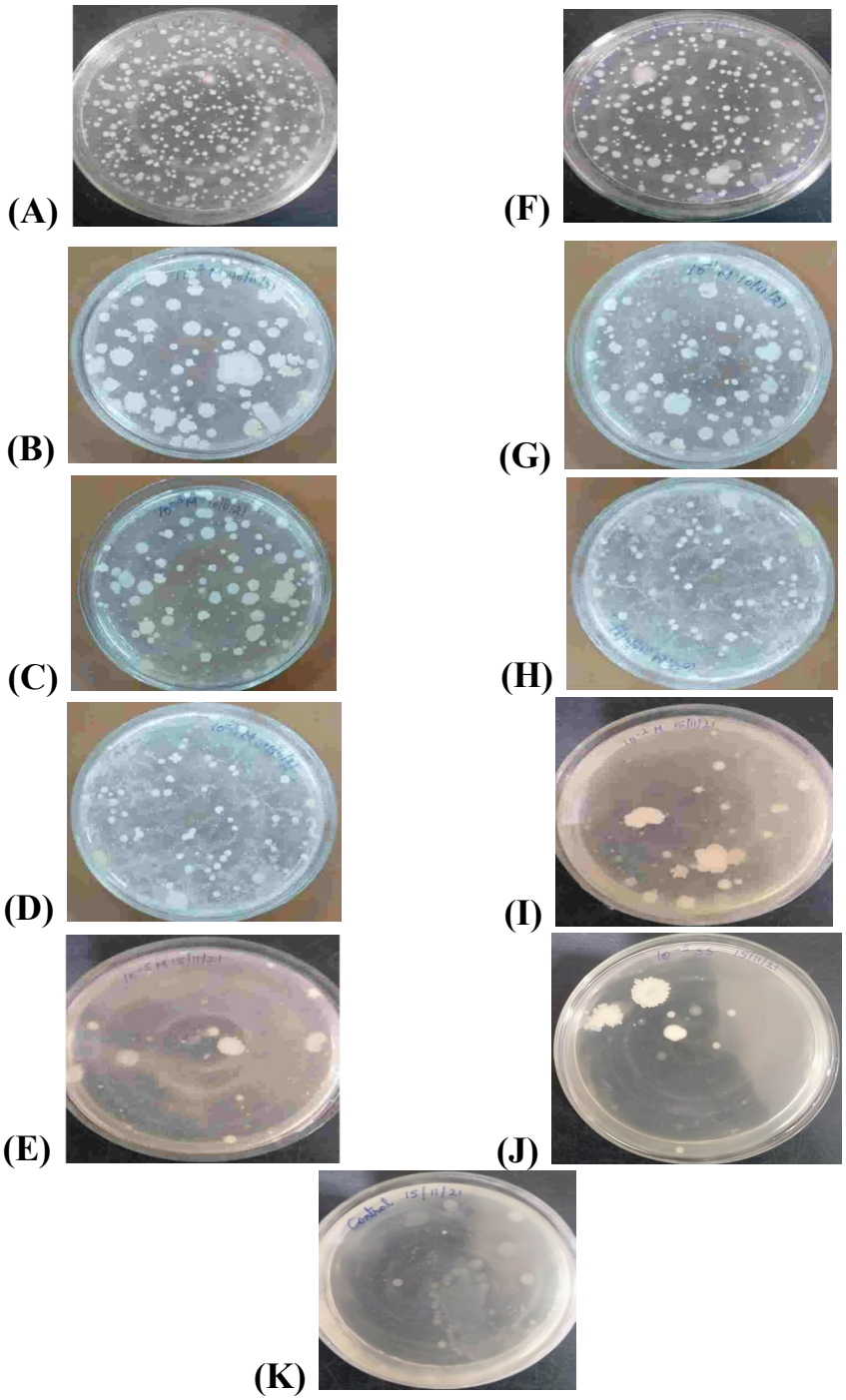
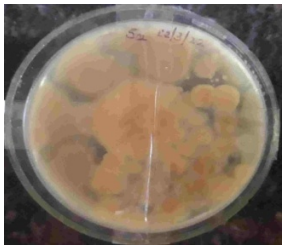
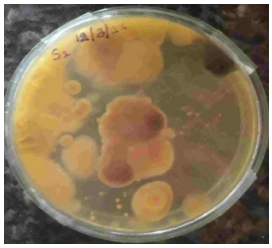


Plate 2. Bacterial colonies observed after serial dilution of freshwater samples collected from Mullakkadu (A, B, C, D, E) and Pudhukkottai (F, G, H, I, J). K. Control

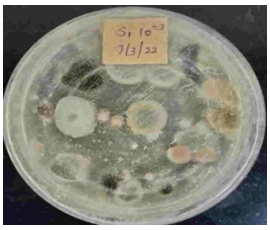
(A)



(B)



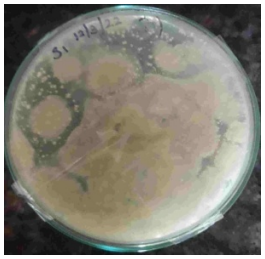
(C)



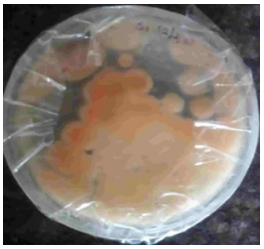
(D)

(E)

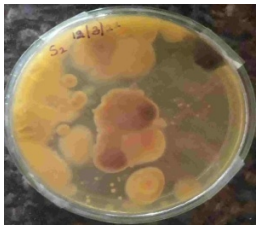
(F)



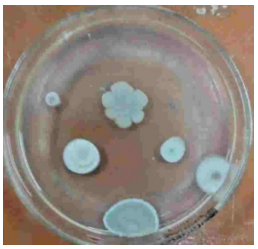
(G)



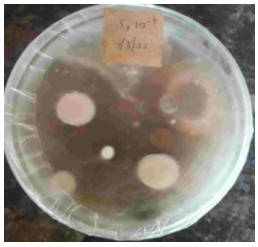
(H)



(I)



(J)



(K)

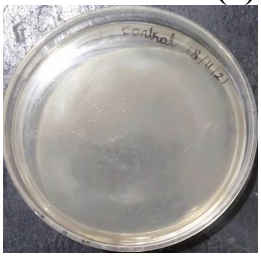
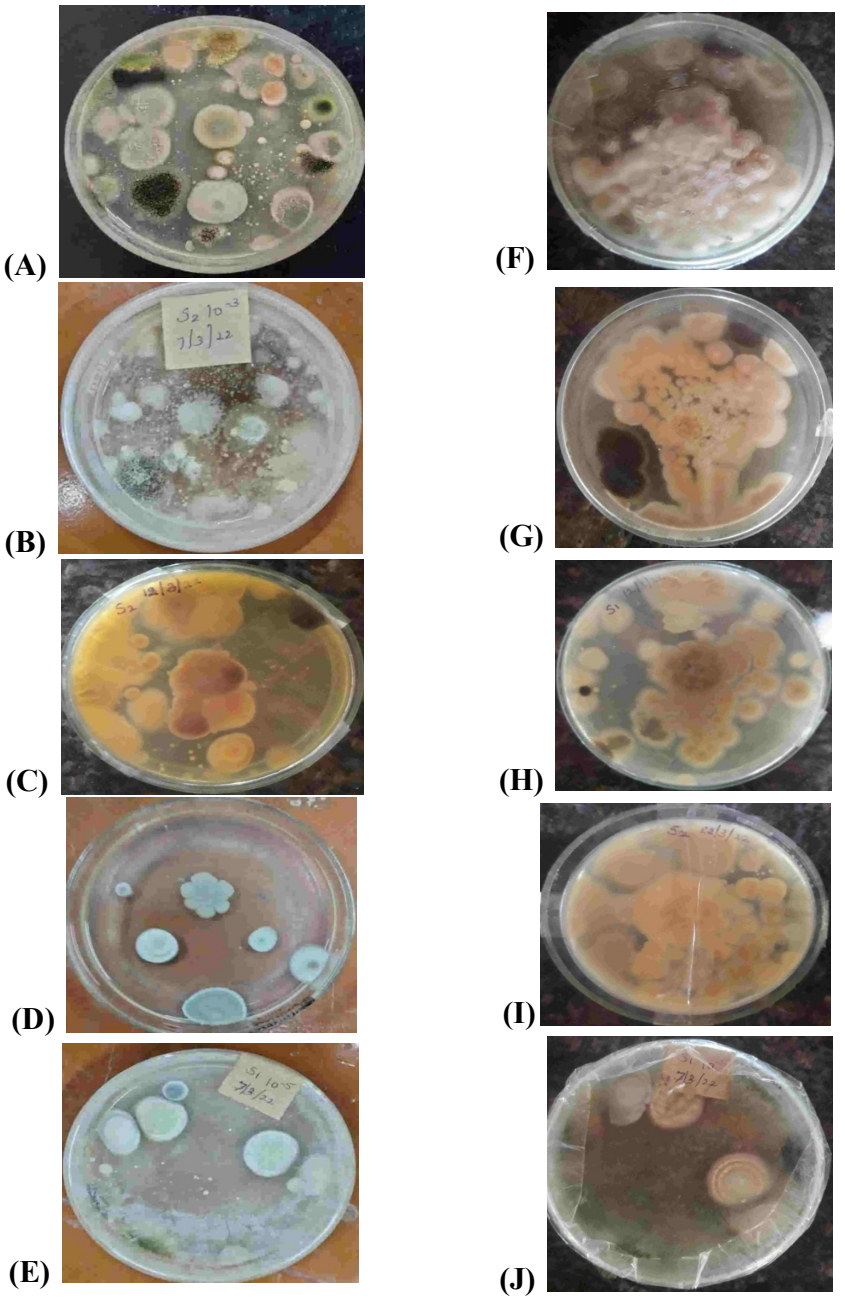


Plate 3. Fungal colonies observed after serial dilution of soil samples collected from Mullakkadu (A, B, C, D, E) and Pudhukkottai (F, G, H, I, J). K. Control



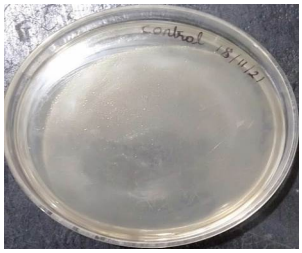
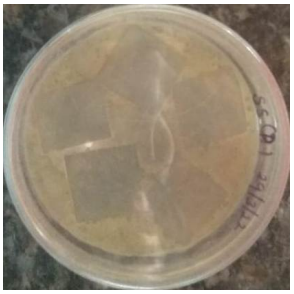
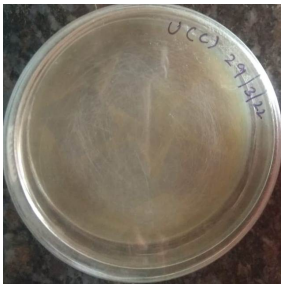
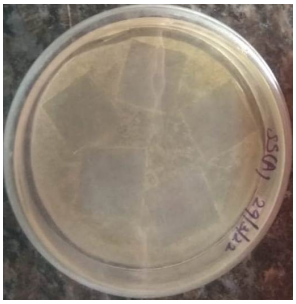
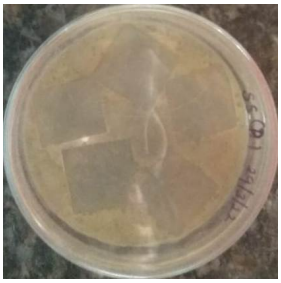
(K) 

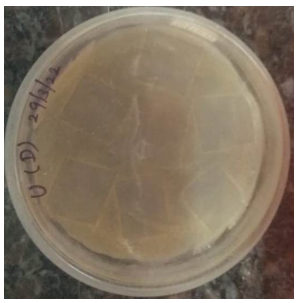
Plate 4. Fungal colonies observed after serial dilution of freshwater samples collected from Mullakkadu (A, B, C, D, E) and Pudhukkottai (F, G, H, I, J). K. Control











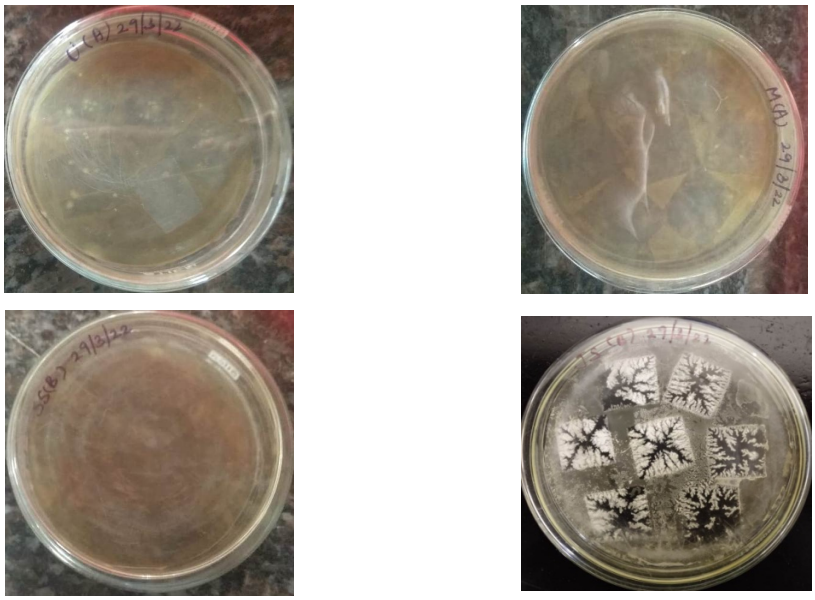
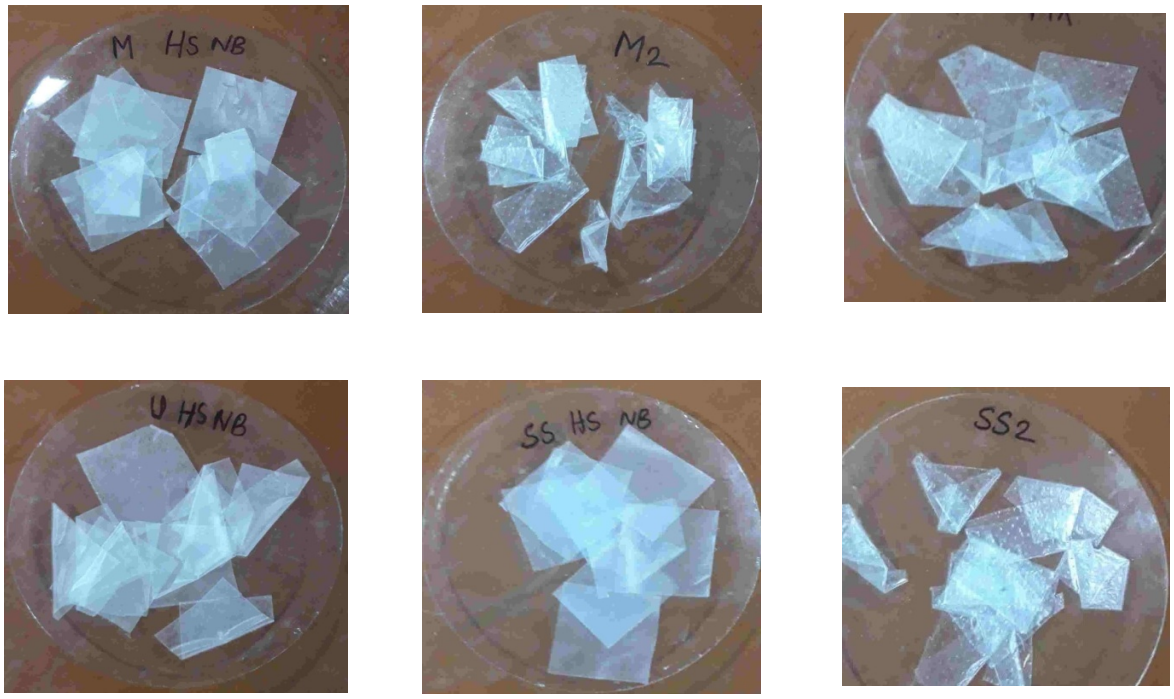


Plate 5. Test for polyethylene degradability using isolated bacterial strains



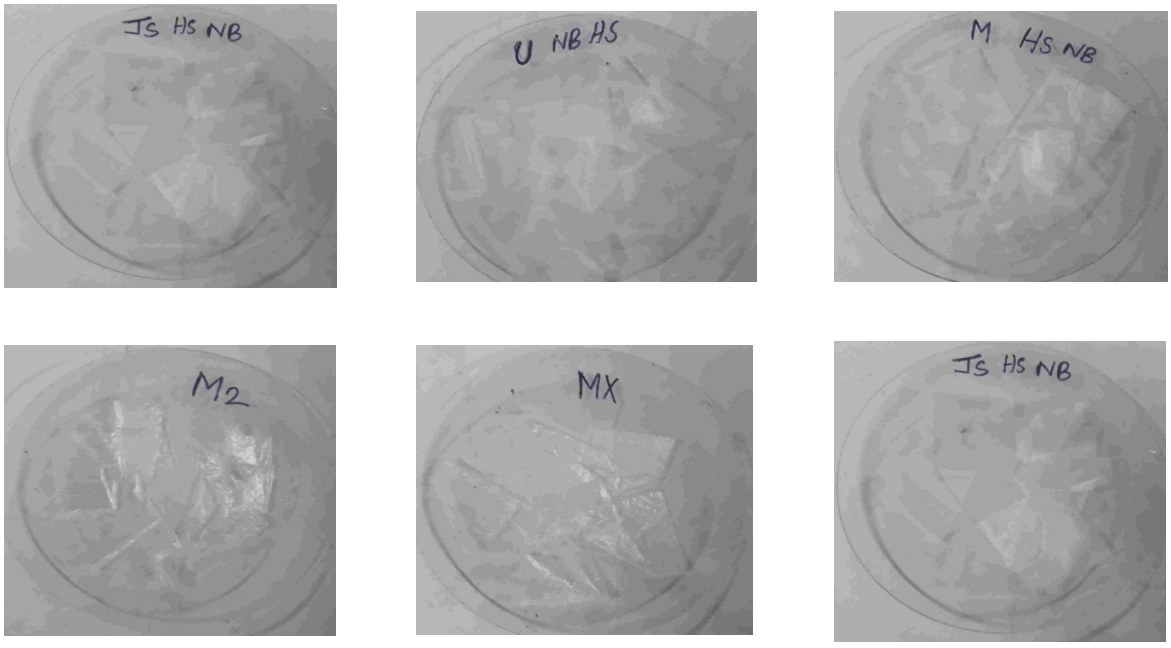


Plate 6. Weight loss of polyethylene sheets after degradability test with isolated bacterial strains

Plate 7. Weight loss of polyethylene sheets after degradability test with isolated fungal strains

A

B

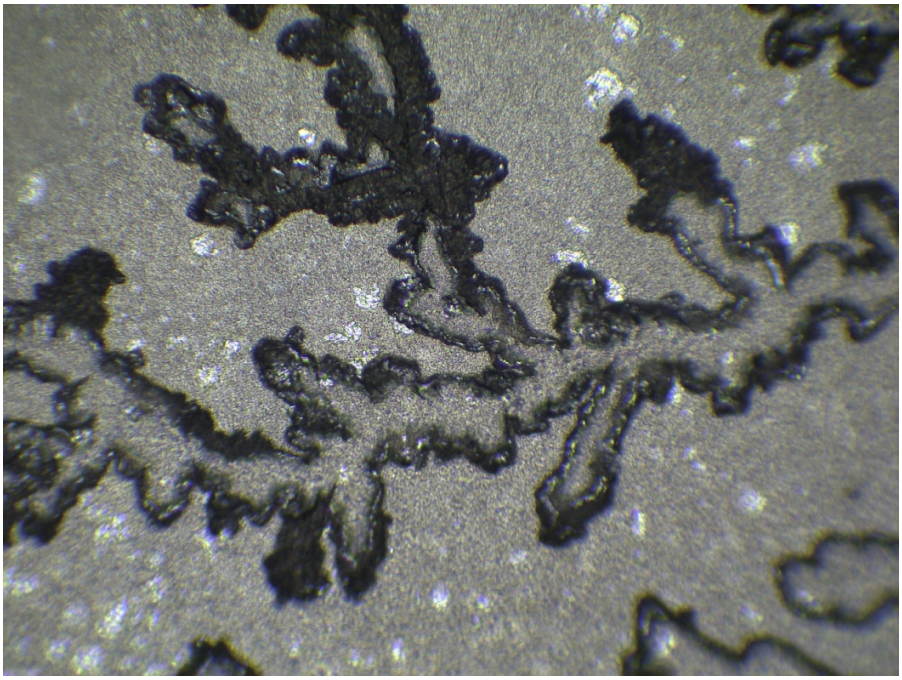
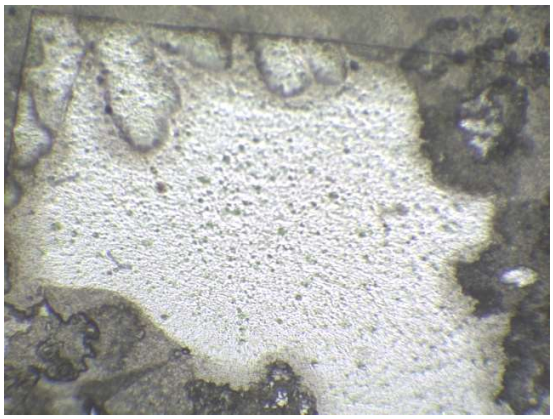
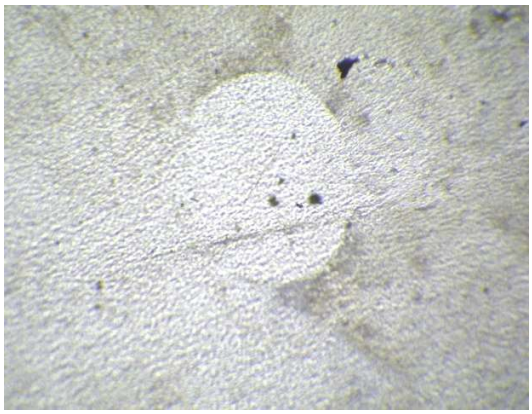
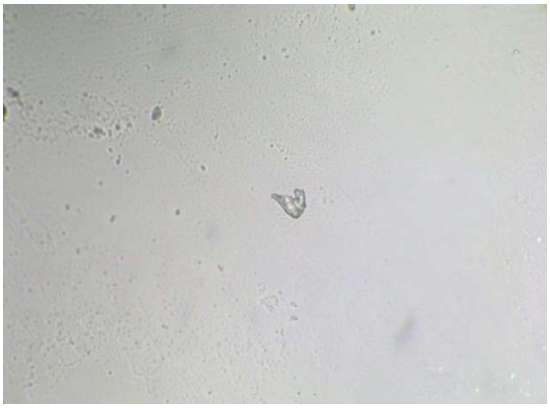


Plate 8. (A) A bacterial biofilm developed on the polyethylene sheets during incubation period of degradation test; (B) Bacterial biofilm under light microscope with 10X magnification

(A) 

(B) 

(C) 

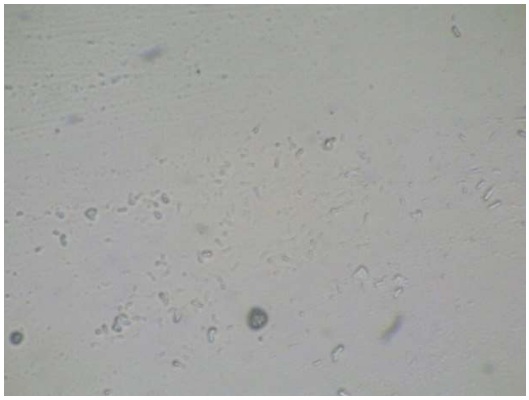
(D) 

Plate 9. Polyethylene sheets treated with bacteria shown under light microscope with 4X (A), 10X (B), 40X (C) and 100X (D) magnification

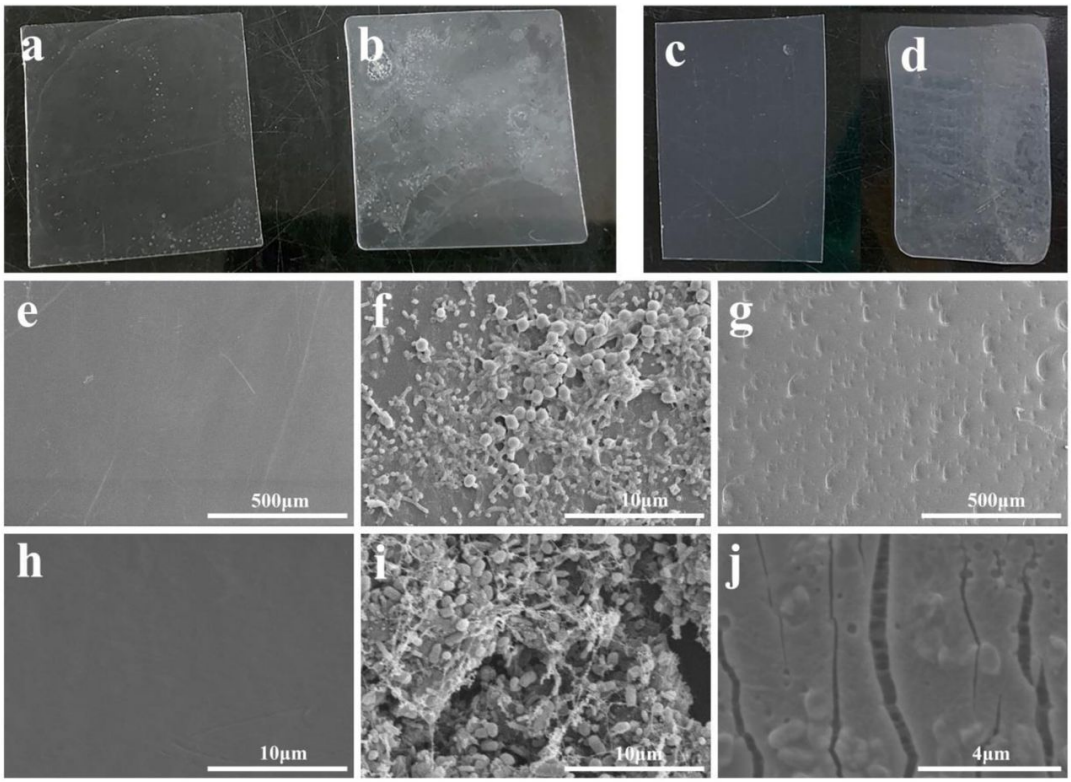


Plate 10. Observation of the colonization and degradation effects of a *Bacillus sp.* (JS-B) on PE sheets. **a & c**, Morphology of PE film without treatment. **b & d**, Morphology of PE sheet treated by the bacteria. **e & h**, SEM observation of PE sheet without treatment. **g, f, i & j**, SEM observation of the colonization of the bacterial community on the PE sheet after 30 days incubation. The type of PE sheet used for this assay is 15 μ in thickness.

above 80%, 5 species shown moderate level of degradation i.e. fall between 26% and 79% while 4 species shown low level of degradation i.e. below 25%.

Both bacterial and fungal degraded polyethylene sheets exhibits a wells, wavy appearance, and somewhat fade colour due to the action of microorganisms, which was observed under light microscope using 4X, 10X, 40X and 100X magnification. The result observed under light microscope was shown in **Plate 8 & 9.**

SEM analysis

The scanning electron microscopic images clearly reveals the presence of erosions, abrasions, grooves and ridges on the surface of the bacteria treated PE sheet. Such kind of deteriorative actions were not seen in the bacteria untreated control PE sheet (**Plate 10**). Based on the high-resolution SEM imaging, it was very clear that the erosions, abrasions, grooves and ridges were formed by the colonization and degradation of PE by bacteria.

DISCUSSION

DISCUSSION

The present investigation deals with the isolation, identification and plastic degrading ability of different bacterial and fungal stains from different samples collected from Mullakkadu and Pudukkottai of Thoothukudi District. Different kinds of changes were observed in isolated microbial strains during this investigation. The main area of this research is to find out the micro and nanoplastics degrading microorganisms. In this present investigation, the one use gloves made up of 15µ polyethylene polymers was used as a microplastic source. This polyethylene polymers are converted into tiny particles named as micro and nanoplastics during its incarnation due to climatic conditions and environmental factors (Scalenghe, 2018; Law and Thompson, 2014). It is clear that the polymers to some extent can be degraded in the appropriate environment in right concentration. Here, we proved that both bacterial and fungal strains have the ability to degrade the polyethylene sheets at some extent. The biofilm was formed on the polyethylene sheets during the incubation period. The degradation was happened when the biofilm starts to form on the surface of the plastic sheets. The microbes utilize these insoluble substrates by enzymatic activities was proved using chitinase and glucanase assay reported by Sharma et al. (2014). Development of such biofilms on the surface of synthetic waste can prove to be a very efficient method for degradation of these polymers *in vitro*. The results obtained from the present study showed that the isolated strains were survived on the plastic sheets and utilize it as their energy source due to lack of culture medium. The *in vitro* condition itself gave the better results; if it will be taken into the large scale application it may prove its environmental profitability.

Fourier transform infrared spectroscopy is a physicochemical analytical technique which provides a clear picture of the composition chemicals. FT-IR spectroscopy of polyethylene

sheets was helped to find out the functional groups present in plastic gloves i.e. polyethylene sheets. FTIR is employed to elucidate the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of respective chemical functional groups (Bobby *et al.,* 2012).

SUMMARYAND CONCLUSION

SUMMARY AND CONCLUSION

The microbial strains from various study area were successfully isolated with a potential to degrade synthetic polymer such as polyethylene sheets. Totally 60 bacterial strains and 18 fungal strains were isolated from different study area of these all species have the ability to degrade micro and nanoplastics but with different potentiality. The overall results revealed that the fungal strains possessed very good micro and nano plastic degradation than bacterial strains that has been proved with the weight loss of plastic sheets. The FT-IR analysis of polyethylene sheets revealed that the presence of C-1, C-Br, C-Cl, C-H, C-F, O-H, CH_2, NO_2, C=O and N-H bonds these are responsible for the presence of alkyl halide, phenol, aldehyde, carboxylic acid, amine salt and alcohol.

Current scenario exhibited the Earth suffocation due to Covid-19 pandemic for past 3 years. The use and throw PPE kits, face masks, gloves, etc. created a huge plastic pollution to the environment during the pandemic period. The degradation of such kinds of plastics using microorganism especially bacteria and fungi gives a hope to revive the nature as like past decades.

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PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF ACALYPHA INDICA AND PHYSALIS MAXIMA

A short term project work submitted to

ST.MARY`S COLLEGE (Autonomous), THOOTHUKUDI

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MAY - 2022

CERTIFICATE

This is to certify that this project work entitled **"PHYTOCHEMICAL STUDIES AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF ACALYPHA INDICA AND PHYSALIS MAXIMA"** is submitted to **St. Mary's college (Autonomous), Thoothukudi** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the award of the degree of **Master of science in Botany**, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2021 – 2022 by **MARIA REENA MAXILDA. N** **(Reg No. 20APBO06)**

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INTRODUCTION

In today's world, there are still a lot of people who do not have adequate access to basic needs or basic necessities such as food, water, education, health services, clean environment and other resources. That is the major concern being addressed by many governments at all levels instead of the rapidly growing population on one hand and a deteriorating environment on the other hand. Over the past 20 years, there has been a lot of interest in the investigation of plant materials as sources of new antibacterial agents. The medicinal plants are useful for healing as well as curing of many human diseases because of the phytochemical constituents present in them.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg, and Newman, 2001). There are over 4,00,000 species of plants in the world (Pitman and Jargensen, 2002) out of which only a small fraction of about 35,000-70,000 species of plants have been screened for their medicinal use (Veeresham, 2012). India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species (Jain, 1994). Many plants have been sources of medicines since ancient times. According to World Health Organization, 80% of the population of the world depends on traditional medical practitioners for their medicinal needs. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field.

Phytochemicals are the chemicals that present naturally in plants, Now a days, these phytochemicals become more popular due to their countless medicinal uses,

phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as "man friendly medicines''.

Medicinal plants are richest bioresource of drugs in traditional system of medicine and it also responsible for different colours, flavours and smell of plants. They also functions as medicaments. These medicinal values of plants active substance that produce a definite physiological action on the human body (Karunyadevi *et al.,* 2009). Fruits and vegetables values have been greatly known for their numerous healthful properties. Among the various evidence revealing that medicinal and culinary herbs have some endemic species, a diet rich in fruits and vegetables and phytochemicals which decrease the risk of cardiovascular diseases and some forms of cancer of particular interest (Javanmardi *et al* ., 2003)

Plants have not only nutritional value but also in the eyes of the local people, they have medicinal and ritual or magical values. Traditional medical plants have important contribution in the health care system of local communities as the main source of medicine for the majority of the rural population. These medical systems are heavily dependent on various plant species and plant based products.

Since time immemorial, plants have been indispensable source of both preventive and curative traditional medicine preparations for human beings and livestocks. These are more than 35,000 plant species being used in various human cultures around for medicinal purposes. According to WHO, ever 80% of the world's population relies upon traditional plant based - systems of medicines to provide them

primary healthcare. Fransworth and soejarto also echoed, same with their estimation that 70-80% of people worldwide rely chiefly on traditional, largely herbal medicine to meet their primary healthcare needs. It is further indicated that herbal medicine is still the mainstay of about 75-80% of the world population mainly in the developing countries for primary healthcare.

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs in the recent years. There has been an increasing awareness about the importance of medicinal plants. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality.

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For a long period of time,

plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased.

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache *et al*., 2001). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini *et al.,* 2003). Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011).

Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Abiramasundari *et al*., 2011).

Antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as ‘forgotten plants’. Taking into account the increasing

demand for natural ingredients that might be used as food additives, components of functional foods, preventing plant diseases and nutraceuticals as well as for other applications. It is reasonable to revise the 'forgotten plants' by assessing their applicability and benefits using modern scientific analysis methods (Rahman *et al*., 2011). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Gislene *et al*., 2000).

Within the last few years, infections have increased to a great extent and antibiotics resistance effects have become an ever-increasing therapeutic problem. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Therefore, it is very important to carry out screening of these plants in order to find out their use in folk medicine and to reveal the active principle by isolation and characterisation of their constituents. Systematic screening of the plants may result in the discovery of new novel bioactive compounds like alkaloids, flavonoids, tannins and phenolic compounds.

The emergence and spread of multi-drug resistant (MDR) enteric bacterial pathogens have substantially threatened the current anti-bacterial therapy. MDR enteric bacterial infections often lead to increased mortality, longer length of stays in hospitals, and higher cost of treatment and care the therapeutic alternatives for these pathogens are extremely limited and physicians are forced to use expensive or

previously discarded drugs, such as colistin that are associated with significant side effect to the patients health.

Therefore it is necessary to search other potential alternatives that can be effective in the treatment of these problematic bacterial infections. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions.

In the evolutionary study of phytochemicals, it was believed that there was little free oxygen in the atmosphere when plants first evolved the direct consequence of this is that as plants metabolize, oxygen concerntration in the world increased. This polluted the environment and to deal with in the plants began to synthesize antioxidants molecules to protect it from highly reactive species that are cytotoxic to the plant cells. Moreover, the damaging effects of microbes on the cell structures of plants especially the important biomolecules has left with no options than to synthesize more bioactive compounds to protect it.

Herbal medicines are gaining priorities in treating various health ailments of diverse origins in man. Before the inventions of the modern synthetic medicines, man's dependence was totally on plants. Traditional systems of plant based products have existed with the changes in culture, traditions and mode of life of man; except for a short period when the inventions of the modern synthetic medicines came into existence. Plant based antimicrobials have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Continued exploration of plant derived antimicrobials is needed today (Hussain *et al.,* 2004).

The pathogenic microorganisms can be controlled with the antibiotics presently available; however the need of new antibiotics has increased due to current problems of resistance associated with them (Prabhat *et al*., 2005). The drug resistant bacteria and fungal pathogens have complicated the treatment of infectious diseases. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms this has necessitated a search for new antimicrobial substances from other sources including plants. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harbone and Baxter, 1995). The substances that can either inhibit the growth of pathogen or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena, 1999). It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogen.

Phytochemistry is the study of the chemicals produced by plants particularly the secondary metabolites, synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure and environmental conditions. The proper understanding of phytochemical is essential for drug discovery and for the developement of novel therapeutic agents against major diseases. The study of phytochemicals has been instrumental in the discovery of new plant natural products which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, nutraceuticals and dietary supplement industries.

In general, bacteria have the genetic ability to transmit and acquire resistance against the drugs used as therapeutic agents. One way to prevent antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents (Shah, 2005). According to Zaheer *et al.* (2010) antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Researchers are increasingly turning their attention to the medicinal plants and it is estimated that, plant materials are present in, or have provided the models for 25-50% Western drugs (Khan *et al.,* 2009). Many commercially proven drugs used in modern medicine was initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable in the treatment of various illness (Manju *et al.,* 2011). Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may likely to differ (Fabricant and Fansworth, 2001). There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Costa *et al.*, 2008).

The cell envelope of bacteria is the first barrier antibacterials need to cross in order to reach their targets. In Gram positive bacteria, the cell envelope is composed of a cytoplasmic membrane and a thick, rigid cell wall. The cell wall is composed of cross linked layers of peptidoglycans associated with anionic polymers, called

teichoic acids, and surface proteins. The function of the cell wall is primarily to confer shape and mechanical strength to bacteria. Gram negative bacteria have an extra outer membrane (OM) composed of a special lipid bilayer: the outer leaflet of the OM is composed of glycolipids, mainly lipopolysaccharides (LPS), instead of phospholipids. LPS molecules bind each other tightly, especially if divalent cations (Ca^{2+} and Mg^{2+}) are present to screen the negative charge of phosphate groups present on the molecules. The acyl chains are largely saturated, which facilitates tight packing. These structural features make the OM very impermeable, specially to hydrophobic molecules. Furthermore, outer membrane proteins (OMPs) can be associated to the OM and function as channels for the diffusion of small molecules. The peptidoglycan cell wall in Gram negatives is relatively thin without teichoic acids. For any antibacterial action, four main steps are required: (i) uptake of the antibacterial from solution, (ii) penetration to the target site, (iii) accumulation in target, and (iv) effective interaction with target. The uptake of an antibacterial across the OM in Gram negatives is often determined by its overall physical character (size, charge, hydrophobicity or amphiphilicity) rather than by specific chemical structure.

Crossing can occur by (i) channel-mediated diffusion through porins (i.e. water-filled channels with low substrate selectivity), (ii) by lipid-mediated diffusion though OM bilayer (although occurrence of this type of influx is expected to be very slow in healthy cells), (iii) by carrier-mediated diffusion via substrate-specific transporters, or (iv) by selfpromoted uptake (as pore-forming cationic peptides). Passage across the cell wall in both Gram positives and negatives is not restricted, as this open network conveys little barrier to most antibacterials (up to 30-57 kDa). For antibacterials with targets inside the cytosol, passage across the cytoplasmic

membrane in both Gram positive and negative bacteria is usually mediated by active, carrier-mediated transport systems. Bacteria can be intrinsically resistant to antibacterials, i.e., have the ability to resist the antibacterial action as a result of inherent structural and functional characteristics. Main causes of intrinsic resistance are (i) absence of a susceptible target (ii) prevention of access to targets or (iii) active efflux of the antibacterial preventing the effective accumulation in the target. In addition to intrinsic resistance, bacteria can acquire or develop resistance to antimicrobials by (i) genetic or post-translational modification of the target, (ii) chemical inactivation, or (iii) modification of the cell envelope to reduce influx or increase the efflux. Gram negative bacteria are known to have a higher intrinsic resistance towards antimicrobials than Gram positive bacteria. This is primarily due to the limited permeability conferred by LPS, especially for hydrophobic molecules, and size restrictions of porins (generally MW < 600-700). In addition, double-membrane-spanning intrinsic efflux pumps keep an effective excretion of antibacterials across the double membrane of Gram negatives. Efflux pumps are transport proteins with broad substrate specificity involved in the expulsion of toxic substances. Studies indicate that recognition and uptake of substrates by the efflux pumps in Gram negatives can take place directly from the periplasm, from the outer leaflet of the inner membrane or from the cytoplasm.

Historically, natural extracts have been used as safe and effective remedies for infections in traditional medicine. Because nature can more effectively provide stereochemical and functional group diversity than synthetic chemistry, natural sources, like marine organisms, plants and uncultured bacteria, are extensive sources of new antibacterials. The majority of the antibacterials introduced to the market

during the discovery era and since the 1980s has been derived from natural compounds. Within natural products, plant extracts have displayed high effectiveness, low toxicity and low resistance development, probably due to their multiple mechanisms of action derived from the plethora of structurally diverse compounds produced by secondary plant metabolism. Some of the major antimicrobial compounds produced by plants are phenolic compounds (e.g. phenolic acids, flavonoids, isoflavonoids, tannins), quinones, coumarins, alkaloids, lectins, polypeptides and terpenoids.

Natural products have played an essential role in the discovery and development of therapeutic agents since ancient times. Most antimicrobials were originally isolated by screening soil-derived actinomycetes during the golden era of antimicrobial discovery (1930s-1960s). Later came the golden era of medicinal chemistry, where optimization of existing molecular scaffolds created successive generations of important antibiotic classes. This approach was successful in dealing with the succeeding waves of resistant bacterial pathogens. In the 1990s, target-based high-throughput screening of synthetic compound libraries was performed. However, these chemical libraries proved to be inefficient in generating lead compounds that could effectively penetrate bacterial cells. The synthetic chemicals often lacked the extensive functional group chemistry and chirality that natural products display. This resulted in a significant discovery void. Misuse of antibiotics, lack of discovery and innovation has led to the so-called resistance era faced today. Resistance to common bacteria has reached alarming levels in many parts of the world, due to the almost

inevitable selection of antimicrobial-resistant bacteria that arise after widespread use of a new antibiotic, both for veterinary and human use. The emergence of multidrug-resistant pathogens with a variety of resistance mechanisms is characteristic of the resistance era. Despite the boom of genomic sciences, target-based high-throughput screening and advances in rational drug design, interest in natural product revitalized as a result of a pressing need for new antimicrobial scaffolds.

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983 and 1994 (Cragg *et al.,* 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria.

According to World Health Organization (Santos *et al.,* 1995) medicinal plants would be the best source to obtain a variety of drugs. Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from Ayurveda the traditional system of medicine in India. Ayurvedic system of medicine has its long history of therapeutic potential. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been

conducted worldwide to prove antimicrobial activities from medicinal plants (Alonso-Paz *et al.,* 1995).

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen *et al.,* 1987) as well as tannin (Saxena *et al.,* 1994). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004).

Plants and herbal extracts are well known for their ability to have therapeutic potential and have been exploited worldwide to cure many severe ailments. They are the basis of Ayurvedic medicine, Unani form of medication and Siddha. From time immemorial, lot of work has been done in this field and still more and more efforts are required to exploit these precious resources for the benefit of human kind. The recorded use of plants in the treatment of ailments dates back to antiquity. Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases.

Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and protection against predators and infections. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines (Abubakar *et al.,* 2010). Plants have provided a source of inspiration for novel drug compounds, as plant- derived medicines have made large contributions to human health and well-being. Their role is two-fold in the development of new drugs: they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or; a phytomedicine to be used for the treatment of disease (Ciocan and Bara, 2007).

Herbal medicines already form the basis of therapeutic use in developing countries but recent years have also seen an increase in the use of herbal medications in the developed world as well. Some studies focusing on the investigation of traditional African (Abubakar *et al.,* 2010), Caribbean (Abubakar *et al.,* 2010 and Indian (Abubakar *et al.,* 2010) medicinal plants have resulted in the identification of new sources of therapeutic agents. The uses of plant extracts, as well as other alternative forms of medical treatments, have enjoyed great popularity in the late 1990s (Cowan, 1999). It is estimated that today, plant materials are present in, or have provided the models for 50% of Western drugs. Many commercially proven drugs used in modern medicine were initially used in crude forms in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are

relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Ciocan and Bara, 2007).

Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite the progress made in the understanding of microorganisms and their control, incidents of epidemics due to drug resistant microorganisms and the emergence of hitherto unknown disease causing microbes, pose enormous public health concerns (Iwu *et al.,* 1999). Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Another driving factor for the renewed interest in 3 plant antimicrobials in the past 20 years has been the rapid rate of (plant) species extinction (Cowan, 1999).

Antimicrobial multiple drug resistance towards commonly used commercial drugs has resulted in an increase in the search for antimicrobial agents from natural sources. Plant derived antimicrobial agents are a largely untapped resource with enormous medical potential and much more investigation is needed in this area (Abubakar *et al.,* 2010). Plants have provided a good source of anti-infective agents; emetine, quinine and berberine remain highly effective instruments in the fight against microbial infections.

Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections. Plants containing protoberberines and related alkaloids, picralima type indole

alkaloids and garcinia biflavonones have been found to be active against a wide variety of microbes (Iwu *et al.,* 1999). Therefore clinical microbiologists have good reasons to be interested in the topic of antimicrobial plant extracts, since it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians and several are already being tested in human beings. In addition, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics (Ciocan and Bara, 2007). Extracts were screened for their antibacterial potency as antibacterial potentiators and resistance modifying agents. Phytochemical characterization of extracts was undertaken by the well described biochemical methods. There are many plants which have not been fully studied and utilized for their therapeutic potential, especially as antimicrobial agents. Therefore efforts were made to tap known medicinal plants and also unexploited plants for their potential activity to combat infectious diseases. The potential of *Acalypha indica* and *Physalis maxima* plant extracts for antibacterial activity against important human pathogenic bacteria was evaluated.

REVIEW OF LITERATURE

2.1. PLANT DERIVED MEDICINES: ORIGINS AND HISTORICAL PERSPECTIVE

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman, 2001). The human use of plants as traditional medicine date back to middle Paleolithic age, approximately 60,000 years ago as per some fossil records. The plants were used as foods, spices, flavors, insect deterrents, ornamentals, fumigants and cosmetics. At present, natural products represent over 50% of all drugs in clinical use, in which natural products derived from higher plants represent 25% of the total. The World Health Organization (WHO) estimated that over 80% of the people in developing countries rely on traditional remedies and about 855 traditional medicines used crude plant extracts. This means that about 3.5 to 4 billion of the global population depend on plant resources for drugs (Maridass and Britto, 2008).

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. The maximum therapeutic and minimum side effects of medicinal herbs have been demonstrated or verified in numerous scientific investigations. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Maridass and Britto, 2008).

2.2. MEDICINAL PLANTS AND PLANT DERIVED DRUGS: MARKET DEMANDS AND CURRENT SCENARIO

Almost 95% of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates. In the recent years there has been greater expansion of indigenous drug industry in India. Consequently the demand for the new material (medicinal plants) has enormously increased. As per latest estimate, in our country, there are about eight thousand licensed pharmacies of ISM (Institute for Supply Management) engaged in the manufacture of bulk drugs. The total annual requirement of the raw materials of these pharmacies was estimated to be thousands of quintals. The annual demand of the global market is $32 million of medicinal plants fromdeveloping countries. The herbal drug production in our country has been estimated to beRs. 4000 crores in the year 2000. Out of 15,000-20,000 medicinal plants, our rural communities use 7,000-7,500 medicinal plants. About 130 pure compounds, which are extracted from 100 species of higher plants of Indian origin, are used throughout the world. Hence, India can play a major role for supplying the raw herbs, standardized extracted materials and pure compounds isolated from natural resources (Maridass and Britto, 2008).

Natural products have provided many effective drugs which include a wide range of older drugs such as quinine and morphine and newer drugs such as paclitaxel (Taxol), artemisinin, etoposide, mevastatin and camptothecin). Further evidence of the importance of natural products is provided by the fact that almost half of the world's 25 bestselling pharmaceuticals in 1991 were either natural

products or their derivatives (Maridass and Britto, 2008).

The number of higher plant species on our planet is estimated around 250,000 (lower level at 215,000 and an upper level as high as 500,000). Of these, only 6% have been screened for biological activity and only 15% have been pharmacologically screened. Moreover, plant extracts contain up to several thousands of secondary metabolites. The major types of compounds identified in Indian medicinal herbs include alkaloids, saponins, flavonoids, anthroquinones, terpenoids, coumarins, lignans, polysaccharides, polypeptides and proteins. Efficient detection and rapid characterization of these components based on molecular characterizations offer better understanding of the pharmacological applications of these herbal medicines (Maridass and Britto, 2008).

2.3.1. PLANT DERIVED DRUGS AND TRADITIONAL SYSTEMS OF MEDICINE

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs is beyond the reach of three quarters of the third world's population,although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the

financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future (Joy *et al.*, 2001).

Among ancient civilizations, India has been known to be rich repository of medicinal plants. About 8,000 herbal remedies have been codified in Ayurveda. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and/or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structures from about 90 species of plants. Some of the useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemisinin and ephedrine among others (Joy *et al.*, 2001).

In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important. Where the active molecule cannot be synthesized economically, the product must be obtained from the cultivation of plant material. About 121 major plant drugs have been identified for which no synthetic ones are currently available. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance (Joy *et al.*, 2001).

2.3.2. WOUND HEALING AGENTS FROM PLANTS

Wound healing occupies an important field of research in modern biomedical sciences. Wound healing involves cellular, physiological, biochemical and molecular processes which result ultimately in connective tissue repair and the formation of a fibrous scar. Healing of wound is an important part of the reparative process. Hence, efforts aimed at achieving a perfect wound healing has inspired many researchers in trying various therapeutic options which were thought to aid or accelerate the wound healing process. Durodola (1977) demonstrated the effectiveness of crude extract of *Ageratum conyzoides* in inhibiting the growth of *Staphylococcus aureus*, a major wound pathogen in *in-vitro* cultures of the organism. Much work has recently been done on the wound healing effect of several medicinal plants (Maridass and Britto, 2008).

2.3.3. ANTIDIARRHOEAL AGENTS FROM PLANTS

Diarrhoea is a major health problem especially for children under the age of 5 years and up to 17% of all death in the indoor pediatric patients is related to diarrhoea. Worldwide incidence of diarrhoeal death account for more than 5-8 million each year in infants and small children less than 5 years, especially in developing countries.

According to WHO estimate for the year 1998, there were about 7.1 million deaths associated to diarrhoea. A range of medicinal plants with antidiarrhoeal properties has been widely used by the traditional healers; however, the effectiveness of many of these antidiarrhoeal traditional medicines has not been scientifically evaluated (Maridass and Britto, 2008).

2.3.4. PLANT DERIVED ANTIBACTERIAL CHEMOTHERAPEUTIC AGENTS

Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases, ranked 5th in 1981, had become the 3rd leading cause of death in 1992, with an increase of 58%. More than hundreds of plants worldwide are used in traditional medicine as treatments for bacterial infections. Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public. In addition the pharmaceutical industry continues to examine their potential as sources of novel growth factor, immunomodulatory and antimicrobial activity (Maridass and Britto, 2008).

2.4. ANTIMICROBIAL COMPOUNDS FROM PLANTS: AN OVERVIEW

For centuries man made use of medicinal plants even though he was unable tofind a rational explanation for their effects. It was not until the 19th century and the rapid development of organic chemistry and pharmacology, that man determined which active principles of group of principles are responsible for a given therapeutic effect. Thebeneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Therefore, random screening of plants for active chemicals is as important as the screening of ethnobotanically targeted species (Ciocan and Bara, 2007).

2.4.1. Phenolics and Polyphenols, Simple phenols and phenolic acids

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group ofphenyl propane-derived compounds which are in the highest oxidation state. The herbs tarragon and thyme contain caffeic acid, which is effective against viruses, bacteria, and fungi. Catechol and pyrogallol are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two OH groups, and pyrogallol has three. The site (s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity. In addition, some authors have found that more highly oxidized phenols show more inhibition of the microbes (Ciocan and Bara, 2007).

2.4.2. Quinones

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins, often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface- exposed adhesins, cell wall polypeptides and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism. Kazmi *et al.* (1994) described ananthraquinone from *Cassia italica*, which was bacteriostatic for

Bacillus anthracis, *Corynebacterium pseudodiphthericum* and *Pseudomonas aeruginosa* and bactericidal for *Pseudomonas pseudomalliae*. Hypericin, an anthraquinone from St.John's wort (*Hypericum perforatum*), which has received much attention lately as an antidepressant, has been earlier reported to have antimicrobial properties (Ciocan and Bara, 2007).

2.4.3. Flavones, flavonoids and flavonols

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described for quinones. More lipophilic flavonoids may also disrupt microbial membranes (Ciocan and Bara, 2007).

Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas. It was noticed that teas exerted antimicrobial activity and that they contain a mixture of catechin compounds. These compounds inhibited *in vitro Vibrio cholerae, Streptococcus mutans, Shigella* and other bacteria. Flavonoid compounds exhibit inhibitory effects against multiple viruses. Numerous studies have documented the effectiveness of flavonoids such as swertifrancheside, glycyrrhizin (fromliquorice) and chrysin against HIV (Ciocan and Bara, 2007).

2.4.4. Tannins

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3000 and they are found in almost every plant part: bark, wood, leaves, fruits, and roots. They are divided into two groups, condensed and hydrolyzable tannins. Hydrolyzable tannins are based on gallic acid, usually as multiple esters with D-glucose; while the more numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers. Tannins may be formed by condensations of flavan derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units (Ciocan and Bara, 2007).

Stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, has been assigned to tannins. Their mode of antimicrobial action may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. According to some studies, tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Ciocan and Bara, 2007).

2.4.5. Coumarins

Coumarins are phenolic substances made of fused benzene and a-pyrone rings. They are responsible for the characteristic odor of hay. As of 1996, at least 1,300 coumarins had been identified. Several coumarins have antimicrobial properties. Thornes in 1954 sought an agent to treat vaginal candidiasis and found coumarins *in vitro* to inhibit *Candida albicans*. Phytoalexins,which are hydroxylated

derivatives of coumarins, are produced in carrots in response to fungal infection and are presumed to have antifungal activity. Antimicrobial activity of coumarins has also been documented in woodruff (*Galium odoratum*) extracts. Data about specific antibiotic properties of coumarins are scarce, though many reports give reason to believe their utility as antimicrobial phytochemicals (Ciocan and Bara, 2007).

2.4.6. Terpenoids and Essential Oils

Terpenoids are synthesized from acetate units and share their origins with fatty acids. They differ from fatty acids in that they contain extensive branching and are cyclized. Examples of common terpenoids are menthol and camphor (monoterpenes) and farnesol and artemisinin (sesquiterpenoids). Terpenes or terpenoids are active against bacteria, fungi, viruses, and protozoa. In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria. The ethanol-soluble fraction of purple prairie clover yielded a terpenoid called petalostemumol, which showed excellent activity against *Bacillus subtilis* and *Staphylococcus aureus*, though lesser activity against Gram negative bacteria and *Candida albicans*. On the other hand, two diterpenes had broad-spectrum activity and worked equally well against *Staphylococcus aureus, Vibrio cholerae, Pseudomonas aeruginosa* and *Candida* spp. (Ciocan and Bara, 2007).

2.4.7. Lectins and Polypeptides

Peptides inhibitory to microorganisms were first reported in 1942. Recent interest has been focused mostly on studying anti-HIV peptides and lectins, but the inhibition of bacteria and fungi by these macromolecules, such as that from the herbaceous *Amaranthus*, has long been known. Thionins are peptides commonly found in barley and wheat. They are toxic to yeasts and Gram negative & Gram

positive bacteria. Fabatin, a newly identified peptide from fava beans, appears to be structurally related to g-thionins from grains and inhibits *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus hirae* but not *Candida* or *Saccharomyces* (Ciocan and Bara, 2007).

2.4.8. Alkaloids

Alkaloids rank among the most efficient and therapeutically significant plant substances. They are chemically very diverse group of organic nitrogen compounds. The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is C10H16, and they occur as diterpenes, triterpenes, and tetraterpenes (C20, C30, and C40), as well as hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain additional elements, usually oxygen, they are termed terpenoids (Ciocan and Bara, 2007).

Generally they are extremely toxic though they do have a marked therapeutic effect in minute quantities. For this reason plants containing alkaloids were not often used in folk medicine, and if used, then only for topical application. Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents all over the world for their analgesic, antispasmodic and bactericidal effects (Ciocan and Bara, 2007).

2.4.9. Other Antimicrobial Compounds From Plants

Many phytochemicals not mentioned above have also been found to exert antimicrobial properties. There are reports of antimicrobial properties associated with polyamines (in particular spermidine), isothiocyanates, thiosulfinates,

glycosides, and glucosides. Polyacetylenes deserve special mention. Acetylene compounds and flavonoids from plants traditionally used for treatment of malaria fever and liver disorders have also been associated with antimalarial activity. In the early 1990s, researchers found that the monosaccharide fructose present in cranberry and blueberry juices competitively inhibited the adsorption of pathogenic *Escherichia coli* to urinary tract epithelial cells, acting as an analogue for mannose. Clinical studies have borne out the protective effects of cranberry juice. Many researchers are now seeking a second active compound from cranberry juice which contributes to the antimicrobial propertiesof this juice (Ciocan and Bara, 2007).

2.5.1. THE CHALLENGE AND THREAT OF ANTIMICROBIAL RESISTANCE

The development of resistance in microrganisms is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones. Under continued selection pressure, the selected resistant organisms multiply and spread to other geographic locations as well as to other microbes by transfer of resistance genes. Selection of resistant strains occurs so rapid for some bacteria that clinical usefulness of the antibiotics is lost over a period of time. The emergence and spread of microbes resistant to cheap and effective first-line drugs has become a common occurrence. The problem is even more evident in bacterial infections such as diarrhoeal, respiratory tract, meningitis, sexually transmitted infections and tuberculosis, which contribute most to the global infectious disease burden. Resistance to penicillin in *Staphylococcus aureus* first appeared in 1942, immediately following its clinical use. By the late 1960s, more

than 80% of both community and hospital acquired staphylococcal isolates were resistant to penicillin (Sibanda and Okoh, 2007).

At present most clinical isolates of *Staphylococcus aureus* are multi-drug resistant (resistant to three or more of agents such as ciprofloxacin, erythromycin, clindamycin, gentamicin, trimethoprim/sulphamethoxazole, linezolid and vancomycin). Global resistance rates in *Streptococcus* isolates are as high as 80% for erythromycin and 50% for penicillins. Recently, strains of *Mycobacterium tuberculosis* that are resistant to virtually all classes of drugs currently available for the treatment of TB (isoniazid, rifampicin, fluoroquinolones, aminoglycosides like amikacin, kanamycin and capreomycin) have been identified, earning a new classification termed, extremely drug resistant tuberculosis (XDR-TB). When infections become resistant to first-line antimicrobials, treatment has to be switched to second or third-line drugs, which are mostly expensive. In many poor countries, the high cost of such replacement drugs is prohibitive, with the result that some diseases can no longer be treated in areas where resistance to first-line drugs is widespread. Hence, there is need to develop alternative approaches in addition to the search for new antimicrobial compounds. Such approaches might include strategies that target resistance mechanisms coupled with antibiotics (Sibanda and Okoh, 2007).

2.5.2. MECHANISMS OF ANTIMICROBIAL RESISTANCE IN PATHOGENICBACTERIA

Resistance to antimicrobials arises as a result of three main strategies: enzymatic inactivation of the drug, modification of target sites and extrusion by efflux. While chemical modifications could be significant in antibiotic resistance,

exclusion from the cell of unaltered antibiotic represents the primary strategy in denying the antibiotic, access to its targets (Sibanda and Okoh, 2007).

2.5.2.1. Alteration of target site

Chemical modifications in the antibiotic target may result in reduced affinity of the antibiotic to its binding site. This is a mechanism employed by a number of pathogenic bacteria in evading the effect of antibiotics. Modifications are usually mediated by constitutive and inducible enzymes. Resistance to macrolides, lincosamide and streptogramin B antibiotics (MLSB resistance) in pathogenic *Streptococcus* species isa result of methylation of the N^6 amino group of an adenine residue in 23S rRNA. This is presumed to cause conformational changes in the ribosome leading to reduced binding affinity of these antibiotics to their binding sites in the 50S ribosomal subunit. β-lactam antibiotics function by binding to and inhibiting the biosynthetic activity of penicillin binding proteins (PBPs), thereby blocking cell wall synthesis. In *Staphylococcus aureus* and *Streptococcus pneumoniae*, resistance to β-lactams can be a result of mutations leading to the production of PBP2a and PBP2b respectively. The two proteins have a reduced affinity for β-lactams and yet they take over the functions of normal PBPs in the presence of inhibitory levels of β-lactams. This mechanism of resistance is also responsible for β-lactam resistance in non-β-lactamase producing *Haemophilus influenzae*(Sibanda and Okoh, 2007).

2.5.2.2. Enzymatic inactivation

The production of hydrolytic enzymes and group transferases is a strategy employed by a number of pathogens in evading the effect of antibiotics. Genes that code for antibiotic degrading enzymes are often carried on plasmids and other

mobile genetic elements. The resistance to β-lactam antibiotics by both gram negative and gram positive bacteria has long been attributed to β-lactamases. These enzymes confer significantantibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four- membered β-lactam ring. Resistance to aminoglycosides in Gram negative bacteria is most often mediated by a variety of enzymes that modify the antibiotic molecule by acetylation, adenylation or phosphorylation (Sibanda and Okoh, 2007).

2.5.2.3. Antibiotic efflux

It is now widely recognized that constitutive expression of efflux pump proteins encoded by house-keeping genes that are widespread in bacterial genomes are largely responsible for the phenomenon of intrinsic antibiotic resistance. Several studies have shown that active efflux can be a mechanism of resistance for almost all antibiotics. The majority of the efflux systems in bacteria are non-drug-specific proteins that can recognize and pump out a broad range of chemically and structurally unrelated compounds from bacteria in an energy-dependent manner, without drug alteration or degradation. The consequence of this drug extrusion is that, it leads to a reduced intracellular concentration of the antimicrobial such that the bacterium can survive under conditions of elevated antimicrobial concentration. The MIC of the drug against such organisms will be higher than predicted (Sibanda and Okoh, 2007).

Multi-drug resistance efflux pumps are ubiquitous proteins present in both Gram positive and Gram negative bacteria as either chromosomally encoded or plasmid encoded. Although, such proteins are present constitutively in bacteria, the continued presence of the substrate induces over-expression. This increased

transcription is responsible for the acquired resistance. In Gram negative bacteria, the effect of the efflux pumps in combination with the reduced drug uptake due to the double membrane barrier is responsible for the high inherent and acquired antibiotic resistance. Efflux transporters constitute about 6 to 18% of all transporters found in any given bacterial cell. Currently, much attention is being paid towards understanding the operating mechanisms of these pumps which has potential applications in the design of transport inhibitors that could be used in combination with antibiotics in development of clinically useful drugs (Sibanda and Okoh, 2007).

The MDR pumps of pathogenic bacteria belong to five families of transporters: the major facilitator super family (MFS), the adenosine triphosphate (ATP)-binding cassette (ABC) super family, the small multi-drug resistance (SMR) family and the resistance-nodulation-cell division (RND) super family and the multi-drug and toxic compound extrusion (MATE) family. The NorA protein of *Staphylococcus aureus* is the best studied chromosomally encoded pump in pathogenic Gram positive bacteria. It is present in *Staphylococcus epidermidis* but appears to be absent in *Enterococcus faecalis* or in Gram negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*. Overexpression of the NorA gene in *Staphylococcus aureus* confers resistance to chloramphenicol and fluoroquinolone antimicrobials (Sibanda and Okoh, 2007).

2.5.3. RESISTANCE MODIFYING AGENTS IN COMBINATION WITH ANTIBIOTICS TO OVERCOME RESISTANCE

The selection pressure exerted by the continued presence of antimicrobial agents facilitates the emergence and dissemination of antibiotic resistance genes.

Over generations, the genotypic makeup of bacterial populations is altered. The clinical implications of this are that many infections become untreatable leading to serious morbidity and mortality. Although the introduction of new compounds into clinical use has helped to curtail the spread of resistant pathogens, resistance to such new drugs, has developed in some cases. For instance, resistance to the lipopeptide, daptomycin among clinical isolates of *Enterococcus faecium* has already been detected, even though the drugwas first licensed in 2003 (Sibanda and Okoh, 2007).

It has been observed that antibiotic combinations can have synergistic benefits and interactions between existing antibiotics. Several therapeutic regimens are based on synergistic interactions between antibiotics with different target sites. As new antimicrobial compounds are discovered, there is need to assess their potentials in combination therapies with older antibiotics that have been rendered ineffective by the development of resistant strains. The use of agents that do not kill pathogenic bacteria but modify them to produce a phenotype susceptible to the antibiotic could be an alternative approach to the treatment of infectious diseases. Such agents could render the pathogen susceptible to a previously ineffective antibiotic. Since the modifying agent applies little or no direct selective pressure, this concept could slow down or prevent the emergence of resistant genotypes. The inhibition of resistance expression approach was used in the production of Augmentin, a combination of amoxicillin and clavulanic acid, where clavulanic acid is an inhibitor of class-A β-lactamases. This combination has been used clinically since the late 1970s. A similar approach can be used for target-modifying enzymes and for efflux systems (Sibanda and Okoh, 2007).

A number of *in vitro* studies have reported the use of plant extracts in

combination with antibiotics, showing significant reduction in the minimum inhibitory concentrations of the antibiotics against some resistant strains. The curative effect of plant extracts in this combination study has been referred to as resistance modifying/modulating activity. This ability of plant extracts to potentiate antibiotics has not been well explained. Speculation is that inhibition of drug efflux and alternative mechanisms of action could be responsible for the synergistic interactions between plant extracts and antibiotics (Sibanda and Okoh, 2007).

2.5.3.1. Efflux pump inhibition in combination with antibiotics as a strategy for overcoming resistance

The discovery and development of clinically useful efflux pump inhibitors (EPIs) represents a significant advance in the development of therapeutic regimens for the treatment of MDR related conditions. This approach termed the EPI strategy is based on blocking the activity of the efflux pumps, thereby resulting in the accumulation of the antibiotic inside the bacterial cell and consequently increasing its access to its target sites. This will lead to increased susceptibility of the bacterium, thus implying that the therapeutic effect of the drug is achieved with low concentrations. Combining broad spectrum efflux pump inhibitors with current drugs can recover clinically relevant activity of those compounds and thus may provide new dimensions to the ever increasing need for development of new antimicrobial agents. In addition, this approach will lead to the preservation and improvement of the usefulness of old and cheap antibacterial agents. Finally this could reduce the appearance and spread of resistant mutants (Sibanda and Okoh, 2007).

2.5.3.2. Multiple targets and mutual interference strategies

A combination of antimicrobials with different target sites and mechanisms of action can be beneficial in reducing resistance development since the chances that a pathogen could simultaneously develop resistance against more than one drug is low. Other combinations may involve antibiotics and other compounds that are not antimicrobial but can enhance the activity of the antibiotics. Combinations between antibiotics and known or new antimicrobial compounds might uncover some beneficial potential useful in curbing antimicrobial resistance. Some drug formulations in current use are already based on the concept of dual targets or mutual interference. For example, the combination of trimethoprim and sulphamethoxazole (cotrimoxazole) involves a mutual interference of two sequential steps in the bacterial folate biosynthesis pathway. Sulphamethoxazole competitively inhibits bacterial dihydropteroate synthetase, an enzyme involved in the first step towards folic acid synthesis. Trimethoprim inhibits the enzyme dihydrofolate reductase, involved in the next step in the folic acid pathway. The synergy between epigallocatechin gallate (EGCG) in tea catechins and oxacillin was attributed to the combined action of EGCG and oxacillin on the biosynthesis of the cell wall, thus bypassing the resistance mechanism resulting from the reduced affinity of penicillin binding proteins (PBPs) to oxacillin (Sibanda and Okoh, 2007).

2.5.3.3. Beta-lactamase inhibitors as antibacterial potentiators

One of the important mechanisms of β-lactam resistance in bacteria is production of β-lactamase and therapeutic control of the β-lactamase producing bacteria is a major clinical problem for more than 50 years (Yang *et al.*, 2009). The use of β-lactamase inhibitors in combination with β-lactam antibiotics is currently the most successful strategy to combat this specific resistance mechanism. A number

of β-lactamase inhibitors such as clavulanic acid, sulbactam, tazobactam and succinic acids have been isolated from natural products or synthesized for the improvement of medicines (Yang *et al.*, 2009). These inhibitors have been used to augment the activity of β-lactam antibiotics against the β-lactamase producing bacteria (Sibanda and Okoh, 2007).

Though, the development of drug combinations containing the β-lactamase inhibitors has given clinicians a novel approach to control these resistant organisms, the current marketed inhibitors are not active against all β-lactamases. In the early 1990s, β- lactamases that were resistant to clavulanic acid were discovered (Yang *et al.*, 2009). From the late 1990s, multi-drug resistant Enterobacteriaceae (mostly *Escherichia coli*) that produce extended spectrum β-lactamases have emerged within the community settings as an important cause of urinary tract and bloodstream infections (Darwish and Aburjai, 2010). So there is a requirement for new β-lactamase inhibitors to be combined with β-lactam antibiotics to fight these resistant bacteria (Yang *et al.*, 2009).

Some plant extracts, combined with antibiotics, have exhibited synergistic activityagainst the standard bacterial strains as well as drug resistant bacteria (Yang *et al.*, 2009) and studies, though few, in regard to the β-lactamase inhibitory components from plant extracts, especially from Traditional Chinese Medicines (TCM), have also been reported (Yang *et al.*, 2009).

2.5.4. PLANTS AS SOURCES OF NEW ANTIMICROBIALS AND RESISTANCEMODIFYING AGENTS

Plants have traditionally provided a source of hope for novel drug

compounds, as plant herbal mixtures have made large contributions to human health and well-being. Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Literature is flooded with compounds that have been isolated from a variety of medicinal plants. Despite this abundant literature on the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been exploited for clinical use as antibiotics (Sibanda and Okoh, 2007).

The observation that plant derived compounds are generally weak compared to bacterial or fungal produced antibiotics and that these compounds often show considerable activity against Gram positive bacteria than Gram negative species has been made by many researchers. It was hypothesized that plants produce compounds that can be effective antimicrobials if they find their way into the cell of the pathogen; especially across the double membrane barrier of gram negative bacteria. Production of efflux pump inhibitors by the plant would be one way to ensure delivery of the antimicrobial compound (Sibanda and Okoh, 2007).

The bases for understanding the action of plant antimicrobials, vast majority of such compounds are agents with weak or narrow-spectrum activities that act in synergy with intrinsically produced efflux pump inhibitors. There is reason therefore to believe that, plants could be a source of compounds that can increase the sensitivity of bacterial cells to antibiotics. Such compounds could be useful particularly against antibiotic resistant strains of pathogenic bacteria. The rich chemical diversity in plants promises to be a potential source of antibiotic resistance

modifying compounds and has yet to be adequately explored (Sibanda and Okoh, 2007).

2.5.4.1.Efflux pump inhibitors from plant sources

The antihypertensive plant alkaloid reserpine was first isolated from the roots of *Rauwolfia vomitoria* Afz. Its EPI activity was originally demonstrated against the Bmr efflux pump, which mediates tetracycline efflux in *Bacillus subtilis*. Reserpine also potentiated the activity of tetracycline (a 4-fold reduction in MIC) in two clinical isolates of MRSA, which possessed the Tet (K) efflux protein. Reserpine also reversed NorA- conferred MDR, and Kaatz and Seo (1995) showed that it enhanced the activity of norfloxacin against *S. aureus*. NorA is one of the major MDR transporters in *Staphylococcus aureus* and causes a significant decrease in susceptibility towards fluoroquinolones. In another study, the MICs of the fluoroquinolones ciprofloxacin, moxifloxacin and sparfloxacin were lowered by as much as 4-fold in *Staphylococcus aureus* isolates in the presence of reserpine. Fluoroquinolone resistance in *Streptococcus pneumoniae* has also been negated in the presence of reserpine (Stavri *et al.,* 2006).

2.5.4.2. Resistance modifying activities of plant crude extracts: the basis for isolation of potentially useful compounds

The isolation of resistance modifying compounds from plants is to be realistic, screening for such activities in crude extracts is the first step in identifying leads for isolation of such compounds. Among Euphorbiaceae family *Acalypha indica* L. is an angiospermic plant, distributed in approximately 60 countries, and is grown widely within the tropical Africa, South Africa, and South East Asia. It grows here and there in Bangladesh like waste lands, road side's crevices in walls, rocky hillsides, forest

edges and river banks. It prefers moist and shaded places (Nautiyal *et al.,* 2015). The World health organization has estimated that 80% population of the developing countries is unable to afford the pharmaceutical drugs and depend on traditional herbal medicine to sustain their primary health care needs (Sankarnarayanan *et al.,* 2010).

Acalypha indica is used medicinally in several countries and are a source of the many potent and powerful drugs (Selvamani *et al.,* 2013). It is being employed for a protracted time as remedies for human diseases, because they contain component of therapeutic value (Nostro *et al.,* 2000). Every part of this plant has been used as traditional medicine for long time.

Phytochemical are non-nutritive plants chemicals that have either defensive or disease protective properties. They are non-essential nutrients and mainly produced by plant to supply them protection. Here non-essential nutrients, meaning that they are not required by the physique for sustaining life (Dillard and German, 2000). Agrawal (2017) investigated the phytochemical properties of *Acalypha indica* and the leaf extracts were subjected to a quantitative chemical analysis of phytochemical compounds. Another experimented with dried roots, stems, and leaves to see which had the most moisture, ash, and water activity.

One such underutilized source is the *Physali*s genus in the Solanaceae family, which also contains more commonly known food crops such as tomato, pepper, and potato. The *Physalis* genus has gained more interest as a potential source for new food crops because it contains many edible species; however, they are underutilized within the current food production system yielding their nutritional benefits widely

unrealized. Overall, the majority of *Physalis* species grow wild, with only few species cultivated as food crops or ornamentals.

Among those cultivated are tomatillo, golden berry and ground cherry. These tomato relatives have been cultivated for both dietary and medicinal purposes. Over time, interest in these edible *Physalis* has grown because of their chemical compounds and culinary properties. Golden berry is rich in nutrients and other bioactive compounds; notably vitamins A, B, C, antioxidants known to benefit human health. In folk medicine, other parts of the plant have been used for their believed medicinal properties in treatment of diseases such as cancer and hepatitis. Specifically, members of the Malayali tribes in the Kolli Hills of India use *P. peruviana* whole plant extract to treat skin diseases, and the Tribes of Thiashola, Manjoor, and Western Ghats use the leaves and seeds to treat jaundice and glaucoma.

The fresh fruits of *P. peruviana* are used as strengthen the immune system, lowering blood sugar, destroys intestinal parasites and healing of skin diseases. The aim of the present study was to evaluate the antimicrobial activity of *P. peruviana*, Exotic fruits play an important role in nutrition and are an excellent base for dietetic products due to their beneficial bioactive compounds and low energetic value. Highly valued for its unique flavour, texture and colour, recent research has shown *Physalis peruviana* L. fruit to be rich in many beneficial compounds. For instance, the *Physalis* fruit is rich in pro-vitamin A, ascorbic acid, and in some vitamins of the B complex (thiamine, niacin, and vitamin B12). Additionally, the fruit is rich in crude protein, phosphorous and iron, although calcium content is low. Some of the health benefits of *Physalis* include: blood purification, reduction of the albumin in the kidneys,

reconstruction and strengthening of the optic nerve, alleviation of throat infections, elimination of intestinal parasites, and treatment of prostate problems.

In Indonesia, *Physalis peruviana* grows naturally in bushes in field and even on the edge of the forest. *Physalis* is a genus of the plants belonging to the family Solanaceae. *Physalis* is known to be almost similar to several species such as blueberry pitaya (Rotringuez *et al.,* 2014). The *Physalis peruviana* fruit is nutritionally rich with high content of vitamins, minerals and antiodidants (Pereda *et al.,* 2018). The cultivation of *Physalis peruviana* is not too difficult, as this plant has good adaptive capabilities in hot as well as cool climates (Oliverira *et al.,*).

Solanaceae are charecterized by the predominance of with steroids being *Physalis* the most important (Tomassini *et al.,* 2000). Recent studies with extracts of *Physalis* leaves have demonstrated important biological activities, as antifungal, antitumoral and insect repellent due to bioactive compounds as whitanolides, phenols and ethanolics (Franco *et al.,* 2007). *Physalis peruviana* fruit has a long history of ethnomedial purposes worldwide, as an antimycobacterial, antilukemic antipyretic and diuretic agent and it was used in the treatment of cancer, hepatitis, asthma, malaria, dermatitis, rheumatism fevers and many other conditions. The fruit contains vitamins, minerals, polysaccharides, protein, polyphenols, polysterols and many other functional nutrients. The phytochemical composition of cape gooseberry fruit outlines its antimicrobial, antiviral, antioxidant, anti-inflammatory, immunomodulatory, hepatoprotective and other properties making it valuable for the neutraceutical and pharmaceutical industries. *Physalis peruviana* is a medicinal plants widely used in folk medicine for treating diseases such as malaria, asthma, dermatitis ,diuretic, antiseptic, antifungal, cataract-cleaning, antiparasitic properties (Kasali *et al.*, 2013).

2.5.4.3. Plant compounds with resistance modifying activities

Withanolides extracted from tomatillo, 17-epi-philadelphicalactone, withaphysacarpin, philadelphicalactone C, and ixocarpalactones A have all exhibited cytotoxicity against human renal carcinoma, kidney carcinoma, and melanoma cancer cell lines. Withanolides represent a large group of steroids found in the *Physalis* genus. This group has received considerable attention based on the potential health benefits associated with their biological activity as it relates to properties that include anti-inflammatory, anti-microbial, antitumor, antifeedant, and immunosuppressive.

An additional type of steroids, known as physalins. Preliminary findings by Physalins are also present in the leaves of *P. peruviana*. In recent decades, due to the large amount of research on phytochemistry and pharmacognosy, natural products from plant sources have gained particular importance in the treatment of different kinds of diseases (Newman and Cragg, 2016).

Physalis species is considered as an important therapeutic plant that is popular worldwide. *Physalis* species that lacks scientific scrutiny is *P. peruviana*, commonly known as Cape gooseberry, and its fruit belongs to the Solanaceae family. Recent research has shown that *P. peruviana* is a promising source of phytochemicals. Different parts of the plant show antioxidant properties (Wu *et al.*, 2004). Chemical studies on *P. peruviana*, showed the presence of withanolides, steroids, alkaloids, and glycosides (Wu *et al.*, 2006). Many chemical compounds viz. 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di- and tri-glycosides are reported to be present in *P. peruviana* (Keith *et al.*, 1992).

The qualitative analyses of the extracts from the root and leaf of *Acalypha indica* exhibited the presence of phytochemical constituents like alkaloids, flavonoids,

phenolic compound, saponins and sterol (Chitravadivu *et al.,* 2009). *Acalypha indica* leaf and flower showed the antioxidant activity due to presence of flavonoids, kaempherol, glycosides, mauritianin, clitoria, nictiflorin (Dinesh Kumar *et al.,* 2012). The whole plant contains various saponins and alkaloids. Acalyphamide a new amide is isolated from root. Acalyphin, a cyanogenic glycoside is isolated from leaves and twigs (Mahato *et al.,* 1983). GC-MS analysis of MeOH root extract of *Acalypha indica* reported some novel medicinal chemicals like imipramine, proadifen, phenytain, dimethylnitromethane, methoxyphenamine (Sinha *et al.,* 2012).

2.6. EXPERIMENTAL APPROACHES TOWARDS PHYTOCHEMICAL EXTRACTION, ANALYSIS AND ANTIMICROBIAL SCREENING

There are multiple factors that may affect the outcome of susceptibility tests. Methods that are standardized are more likely to be reproducible than unstandardized methods. Standardization is vital for intra and interlaboratory reproducibility since results may be considerably influenced by the method used. Standard criteria for evaluation of plant antimicrobial activity are lacking to some extent and sometimes results greatly differ between authors. Hence, on some occasions, it becomes difficult to compare the obtained results, when dealing with plant extracts, with published results because a number of variables influence the results, like the environmental and climatic conditions under which the plant grew, choice of plant extracts, choice of extraction method, antimicrobial test method and test microorganisms (Ncube *et al.,* 2008).

2.6.1. PREPARATION OF PLANT EXTRACTS

Extraction methods, which are used pharmaceutically, involve separation of

active portions of plant tissues from the inactive/inert components by using selective solvents along with appropriate extraction technology. During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity. The basic parameters influencing the quality of an extract are: a) the plant part used as starting material, b) the solvent used for extraction and c) the extraction technology. Effect of plant material depends on the nature of the plant material; its origin; degree of processing; moisture content and particle size, while variations in extraction method include type of extraction; time of extraction and temperature. The nature of solvent as well as solvent concentration and polarity also affect the quantity and secondary metabolite composition of an extract (Ncube *et al.,* 2008).

2.6.1.1. Plant material

Fresh or dried plant material can be used as a source for secondary plant components. However, most scientists working on the chemistry of secondary plant components have tended to use dried plant material for numerous reasons. Differences in water content may affect solubility of subsequent separation by liquid-liquid extraction and the secondary metabolic plant components should be relatively stable, especially if to be used as an antimicrobial agent. Moreover, many plants are used in the dry form (or as an aqueous extract) by traditional healers. Plants are usually air dried but some researchers dry the plants in the oven at about 40°C for 72 hrs. In addition, plants will have different constituents depending on the climatic conditions in which they are growing. The choice of plant material used in the extract preparation is generally guided by the traditional use of the plant and the ease of handling of the different plant parts (Ncube *et al.,* 2008).

2.6.1.2. Choice of solvent

Successful determination of biologically active compounds from plant material depends largely on the type of solvent used in the extraction procedure. Properties of a good solvent in extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract and preservative action. In addition, it should not cause the extract to complex or dissociate. The choice of solvent is very well influenced by what is intended with the extract. Since the end product will contain traces of residual solvent, the solvent should be non-toxic and should not interferewith the bioassay. The choice will also depend on targeted compounds (Ncube *et al.,* 2008).

In a study to determine the optimal conditions for extraction of tannins and other phenolics, aqueous acetone was found better at extracting total phenolics than aqueous methanol. In another study where twenty different solvents were evaluated, chloroform was found to be the best solvent for the extraction of non-polar, biologically active compounds from the roots of *Angelica archangelica*. Traditional healers use primarily water but plant extracts from organic solvents give more consistent antimicrobial activity compared to water extracts. Polyphenolic compounds such as flavonols and most other bioactive compounds are in general soluble in polar solvents such as methanol. Most antimicrobial active components are not water soluble and thus organic solvent extracts have been found to be more potent. Water-soluble compounds, like polysaccharides and polypeptides are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents. Water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics are only important as

antioxidant compounds. Methanol, ethanol and ethyl acetate are the most commonly used solvents for investigations of antimicrobial activity in plants. Dichloromethane has also been used by several researchers. Some authors use a combination of these solvents to obtain the best solvent systems for extraction. Acetone, though not a very commonly used solvent, has been used by a number of authors (Ncube *et al.*, 2008).

In a study by Masoko and Eloff (2006), where the antifungal activity of *Combretum* species was investigated, from the extractants used, which included hexane, dichloromethane, acetone and methanol, it was discovered that acetone and methanol extracted more compounds from the leaves than the other solvents. Both acetone and methanol were found to extract saponins which have antimicrobial activity. Eloff (1998a)examined a variety of extractants for their ability to solubilise antimicrobials from plants, rate of extraction, ease of removal and toxicity in bioassay, among other things and acetone was found to be the best extractant. It gave the lowest minimum inhibitory concentration against the Gram positive bacteria and the largest number of different components and inhibitors, but different results may be obtained with other plants and generalization cannot be made on the usefulness of acetone as a better extractant (Ncube *et al.*, 2008).

2.6.1.3.The extraction methods

Variations in extraction methods are generally found in the length of the extraction period, solvent, pH, temperature, particle size and the solvent-to-sample ratio. The longer the contact between solvent and the plant material, the better is extraction. The period of extraction can be shortened by grinding the plant material finer as this will increase the surface area for extraction, thereby increasing the rate

of extraction. Shaking the plant material-solvent mixture will also increase the rate of extraction. In the study by Eloff (1998b), 5 mins extractions of very fine particles of diameter 10 μm gave higher quantities than 24 hrs in a shaking machine with less finely ground material (Ncube *et al.*,2008).

In one study, sequential extraction with various solvents at room temperature was compared with extraction in a water bath at 37°C for 30 mins with distilled water adjusted to pH 2.0 with hydrochloric acid and then neutralized with sodium hydroxide, before extraction with diethyl ether. It was concluded that the latter method had higher activity which was attributed to the acidified aqueous environment promoting easy extraction. The solvent-to-sample ratio affects the quantity and quality of constituents obtained. In a study to identify the optimal conditions for extracting sugars from non- defatted soybean, a solvent ratio of 5:1 at 25°C or 50°C for 15 mins was found to give the best yield of sugar. In some studies, solvent to sample ratios of 10 mL: 1 g solvent to dry weight ratio has been ideally used (Ncube *et al.*, 2008).

The method that has been widely used by researchers investigating antimicrobial activity is homogenization in solvent. Dried plant material is ground in a blender, put in solvent and shaken vigorously for 5 mins or left for 24 hrs after which the extract is filtered and fresh solvent is added to the residue for another 24 hrs. Some authors reported shaking unhomogenized dry leaves in solvent for about 5 mins, followed by filteration and concentration under reduced pressure to obtain an extract. This actually gave a higher yield and bioactivity than using the same method but with homogenized (macerated) extract. Meyer and Dilika (1996) used these different methods on the same plant and found that the homogenized

dichloromethane extract generally had higher activity than the shaken extract of the same solvent. Another common method is serial exhaustive extraction which involves successive extraction with solvents of increasing polarity, from a non-polar (hexane) to a more polar solvent (methanol). This ensures that a wide polarity range of compounds could be extracted and is ideal when one has to screen the plant for a variety of compounds (Ncube *et al.,* 2008).

Some methods are employed when a particular class of compounds is targeted. For example, extraction of essential oils is done by steam distillation, volatile solvent extraction or supercritical fluid extraction (SFE). Maceration, maceration with sonication, soxhlet extraction and SFE with hexane or carbon dioxide was compared for the extraction of low-polarity compounds from *Mikania glomerata*. SFE-hexane proved to be the most effective for extraction. These newer methods, including microwave assisted methods, are proving to be more efficient than the conventional methods (Ncube *et al.,* 2008).

Soxhlet extraction of dried plant material using organic solvents is another commonly employed extraction method. Here, the sample is continually exposed to fresh solvent, which improves the efficiency of the method. It works well for compounds that can withstand the temperature of the boiling solvent, but cannot be used for thermolabile compounds because prolonged heating may lead to degradation of compounds. Other common extraction methods include a) maceration (for fluid extract) where whole or coarsely powdered plant material is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved; b) percolation; and c) infusion which is prepared by immersing the plant material for some time in cold or hot water (Ncube *et al.,* 2008).

2.6.2. PHYTOCHEMICAL SCREENING: QUALITATIVE ANALYSIS

The term qualitative phytochemical analysis refers to the procedures involved in establishing and proving the identity of the phytochemical constituents present in the crude plant extract. The pharmacological actions of crude drugs are determined by the nature of their constituents. Thus the plant species may be considered as a biosynthetic repository not only for the chemical compounds and constituents but also for a multitude of phytochemicals and substances including alkaloids, terpenoids, flavonoids, and glycosides etc, which exert definite physiological effects. These phytochemicals and substances are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used (Sofowora, 1993). Qualitative phytochemical screening is mainly based on the standard procedures described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

2.6.3. ANTIMICROBIAL SCREENING (ANTIMICROBIAL SUSCEPTIBILITYTESTING): SCOPE AND METHODS

The Antimicrobial Susceptibility Test (AST) is used to determine the efficacy of potential antimicrobials from biological extracts against a number of diverse microbial species. Though AST methods are used to screen plant extracts for antimicrobial activity, they are largely used to determine the usefulness of an antimicrobial in combating infections by determining its Minimum Inhibitory Concentration (MIC). *In vitro* susceptibility tests are particularly important in clinical research if an organism is suspected to belong to a species that has shown resistance to commonly used antimicrobial agents. They are also vital in

epidemiological studies of susceptibility and in comparisons of new and existing antimicrobial agents (EUCAST, 2003; Ncube *et al.,* 2008).

Successful discovery of novel natural antimicrobials has necessitated the development of new bioassay techniques which are sensitive enough to detect small amounts of biologically active chemicals. Standardized *in vitro* tests are essential for screening plant extracts or compounds and more studies should be conducted for MIC determination of natural products in order to get results that are comparable to those of currently used antibiotics. Evaluation of the performance of a susceptibility test should include criteria such as ease of use, reproducibility, test sensitivity and specificity. Though, current standard methods, approved by various bodies like the National Committee for Clinical Laboratory Standards (NCCLS) [now known as Clinical and Laboratory Standards Institute (CLSI)], British Society for Antimicrobial Chemotherapy (BSAC) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST), exist for guidelines of antimicrobial susceptibility testing of conventional drugs. However, these might not be exactly applicable to plant extracts and modifications have to be made (Ncube *et al.,* 2008).

AST standard tests can be divided into diffusion and dilution methods. Common diffusion tests include agar well diffusion, agar disk diffusion and bioautography, while dilution methods include agar dilution and broth micro/macrodilution. The broth and agar based methods are the conventional reference methods for AST. There are other commercial custom-prepared methods like the agar screen plate, Epsilometer test and the Vitek system which could be used in place of the standard reference methods, eventhough these are not common in

routine AST and for testing activity of plant extracts. The major problem in the diffusion and dilution based AST is one of availabilityof the active principles which depend on the solubility of the test compound (Ncube *et al.,* 2008).

2.6.3.1. Agar disk diffusion assay

Agar diffusion techniques have been widely used to assay plant extracts for antimicrobial activity, though there are limitations with the technique. Disk diffusion is suitable for identification of leads but not effective for quantification of bioactivity. These diffusion techniques generally do not distinguish bactericidal and bacteriostatic effects. The MIC cannot be determined and these are classically used for preliminary screening; as qualitative tests, since the amount of extract in the disk is not quantitatively determined. Some researchers however have reported MIC values obtained by the agar diffusion method, though high activity in the disk diffusion assay does not necessarily correlate to low MIC values in the microtitre plate method. The agar disk diffusion method can mainly be used for AST of pure substances because when it is applied to mixtures containing constituents exhibiting different diffusion rates, results may be unreliable (Ncube *et al.,* 2008).

In this method, 6 mm paper disks, saturated with filter sterilized plant extract at the desired concentration, are placed onto the surface of a suitable solid agar medium. Mueller Hinton is usually the medium of choice, though sometimes tryptone soy agar or nutrient agar has been used. The media is preinoculated with the test organism at inoculum size of 1 x 10^8 CFU/mL of bacteria as reported by some authors. There have been some variations noted on the impregnation of disks with the antimicrobial substances. Sometimes it is impregnated before placing on the agar plate, while sometimes the disk is placed on the plate and then impregnated.

Mbata *et al.* (2006) soaked the paper disks in the leaf extract for about 2 hrs, while Basri and Fan (2005) left the disks to dry overnight under a laminar flow cabinet. Some authors refrigerate the plates for 1-2 hrs at 4°C to allow pre-diffusion of the extracts from the disk into the seeded agar before incubation. The plates are then incubated at 37°C for 18-24 hrs for bacteria and 48 hrs for fungi. Zones of inhibition are then measured from the circumference of the disks to the circumference of the inhibition zone or recorded as the difference in diameter between the disks and the growth free zones around the disks (Ncube *et al.*, 2008).

2.6.3.2. Agar well diffusion assay

The principle of the agar well diffusion is the same as that of the agar disk diffusion. A standardized inoculum culture is spread evenly on the surface of gelled agar plates. Wells of between 6 and 8 mm are aseptically punched on the agar using a sterile cork borer allowing at least 30 mm between adjacent wells and the Petri dish. Fixed volumes of the plant extracts are then introduced into the wells and the plates areincubated at 37°C for 18-24 hrs for bacteria and 48 hrs for fungi (Ncube *et al.*, 2008).

2.6.3.3. Agar dilution assay

The agar dilution assay is more versatile than the broth dilution and do not presentproblems encountered with the latter, that is, sample solution, contamination and determination of MIC breakpoints. In this method a stock solution of the extract is prepared in its extracting solvent, usually filter-sterilized and then incorporated in molten agar, cooled to 50°C in a water bath, to obtain different concentrations of the extract in the agar. Usually Mueller Hinton agar is used though some authors have used nutrient agar. Inoculum preparation also differs between authors and some

have used overnight culture dilutions of 1:100 or 1:10 in broth (Ncube *et al.*, 2008).

CLSI (2006b) recommends an inoculum density of about 10^7 CFU/mL and using replicator pins, micropipette or standard loop to transfer about 1-2 μL (10^4 CFU) of the inoculum. Some have reported leaving the plates overnight, before streaking, to allow the solvent to evaporate. The organisms are streaked or inoculated in radial patterns on the agar plates and incubated at 37°C for 24 to 48 hrs. The MIC is defined as the lowest concentration of the extract inhibiting the visible growth of each microrganism on theagar plate (Ncube *et al.*, 2008).

Awal *et al*. (2004) has demonstrated toxicity of leaf and seed extracts of *Cassia alata* by using brine shrimp cytotoxicity assay. In another study by Mongelli *et al*. (2003), cytotoxic evaluation of components of *Bolax gummifera* was done through this assay. Chowdhury *et al*. (2004) described the cytotoxic potential of extracts and purified components of *Stachytarpheta urticaefolia* using this assay.

2.7. SAFETY CONCERNS AND RELEVANCE OF *IN VIVO* TOXICITY ASSESSMENT OF PHYTOCHEMICALS AND HERBAL EXTRACTS

The use of herbal medicines and natural remedies by the customary practitioners for treatment of diseases remains the main stay of health care system, gaining increasing popularity, particularly among the rural public in the developing countries. Their growingattractiveness could be credited to their advantages of being effective and affordable sources of medical care (Ogbonnia *et al*., 2010), they being an alternative way to compensate for some apparent deficiencies in conventional pharmacotherapy (Patrick- Iwuanyanwu *et al*., 2012).

Though there is growing disillusion with modern medicine coupled with the misconception that herbal products being natural may be free of adverse and toxic

effects associated with conventional allopathic medicines (Ogbonnia *et al*., 2010), the popularity and accessibility of the traditional remedies has generated concerns regarding the safety and efficacy of these herbal preparations. Herbal remedies are considered safer and less damaging to the human body than synthetic drugs. However, the lack of standardization has been a major concern regarding use of herbal medicines. Although herbal preparations may be considered safe, some are known to be toxic at high doses and othersmay show adverse effects after prolonged use (Patrick-Iwuanyanwu *et al*., 2012).

They could be contaminated with microbial and foreign materials like aflatoxins, heavy metals and pesticide residues. The presence of any of the likely contaminants is a potential health risk to a vast population that depends on herbal medicines for their healthcare needs. Increased morbidity and mortality associated with the use of herbs or traditional medicines has raised worldwide attention in the last few years. Upon exposure,the clinical toxicity may vary from mild to severe and even life threatening making the safety and toxicity evaluations of these preparations vital (Ogbonnia *et al*., 2010).

Acute toxicity testing measures the adverse effects that occur within a short time of administration of single dose of a chemical substance, phytochemical, herbal product or a drug candidate. Such studies are performed mainly in rodents and provide information on the health hazards likely to arise from short-term exposure of these substances. They are typically an initial step in the assessment of the toxic characteristics of a substance for both health and environmental effects (Rispin *et al*., 2002).

Acute toxicity studies can be used to identify doses connected with target

organ toxicity and lethality and may be referred to humans. They serve as the foundation for hazard classification and labeling of chemical substances and can provide information for comparison of toxicity and dose-response among them. Acute toxicity data may also give information about the mode of toxic action of a substance, which can assist in the analysis and treatment of toxic reactions. They are used to regulate biological products and can serve to set up dosing levels for repeated dose studies. Acute oral toxicity testing in the rodents is also used to establish the level of lethality to non-rodents and other terrestrial mammals (Rispin *et al*., 2002). Several acute toxicity studies of plant extracts and phytochemicals are reported in literature. For example, acute toxicity studies (oral and intraperitoneal) of extracts and powders of *Syzygium aromaticum* (clove) and *Lawsonia inermis* (henna) in mice has been reported by Agbaje *et al.* (2009), Parle and Khanna (2011) and Chakraborty *et al.* (2011).

The acute oral toxicity testing in rodents is primarily based on 'Up-and-Down' procedure described by Dixon (1991) and is performed according to CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) recommended 'Organization for Economic and Cultural Development (OECD) 425 guidelines' (1998; 2001).

2.8. *IN VIVO* EFFICACY TESTING OF PHYTOCHEMICALS AND PLANTEXTRACTS

Two prime examples of animal studies from literature are the descriptions of the effects of oolong tea polyphenols on dental caries in rats and cholera in mice. Another bacterial infection model, *Pseudomonas aeruginosa* lung infection of athymic rats, was used to test subcutaneous administrations of ginseng. Treated rats

had decreased lung pathology and bacterial load, though enhanced humoral immunity was partially responsible (Cowan, 1999).

In a study by Saini *et al.* (2009), the *in vivo* antibacterial efficacies of *Syzygium aromaticum* (clove) and *Ocimum sanctum* (tulsi) were demonstrated in mice against *Klebsiella pneumoniae* in a respiratory tract infection model. Another antibacterial *in vivo* study is of *Lawsonia inermis* (henna) herb against *Mycobacterium tuberculosis* H37Rv inexperimental models of guinea pigs and mice tuberculosis (Sharma, 1990).

Many of the *in vivo* studies to date have examined the antiprotozoal effectiveness of plant extracts. Two recent studies with rats used experimental *Entamoeba* infections to test the effectiveness of a crude herbal extract and an ethanolic extract of *Piper longum* fruits, containing the alkaloid piperine. Perrett *et al.* (1995), after applying the chloroform extract of the legume *Milletia thonningii* topically to the skin of mice, found that it prevented the establishment of subsequent *Schistosoma mansoni* infection (Cowan, 1999).

OBJECTIVES

The broad objective of the research was to screen and evaluate medicinal plants, plant products and extracts for their antimicrobial activity leading to a safe and alternative anti-infective agent with therapeutic potential.

The specific objectives were to screen and evaluate medicinal plants, plant products and extracts:

1. As antimicrobial agents

2. As potentiators of antimicrobial agents

MATERIALS AND METHODS

4.1. Collection and identification of plant materials

The fresh plant materials of *Acalypha indica* Roxb. and *Physalis maxima* Hook. are collected from Thoothukudi. The collected materials were identified using standard monographs (Gamble, 1935).

4.2. Histochemical studies

Methods in plant histochemistry was carried out in freshly collected material.

4.2.1. Calcium oxalate (silver hydrogen peroxide method) (Krishnamurthy, 1988)

Fresh or formalin fixed and paraffin embedded tissue

- Section the material, deparaffinise and bring down to water.
- Treat with 2N acetic acid for 15 mins to remove phosphate and carbonate.
- Treat with 1% silver nitrate in 15% hydrogen peroxide (equal parts of 30% hydrogen peroxide and 2% silver nitrate) for 15 mins at 22^0c.
- Wash in distilled water.
- Counter stain with 2% safranin for 1to 3 mins.
- Dehydrate clear and mount.

Calcium oxalate deposits and crystals stains black while the background is red.

4.2.2. Starch

Starch is the most important carbohydrate reserve of the plant. It is intracellular and occurs as granules in membrane bound cytoplasmic organelles the plastids (chloroplasts and amyloplasts). It is a homopolysaccharides and on hydrolysis yields only D- glucose. However, it

is composed of two basic molecules, the amylose and the amylopectin (Ca 80% of the total starch). The proportion of the above two polymers in starch often varies between different species, starch differs from cellulose another homopolysaccharide, having the linkage between glucose units α1-4 and not β1-4 having some random branching in the chain.

4.2.2.1. Iodine-potassium iodide reaction (Krishnamurthy, 1988)

- Section the material, deparaffinise and bring down to water.
- Mount sections in iodine -potassium iodide solution

Starch will appear blue to black in a few minutes newly formed starch may appear red to blue.

4.2.3. Lignin (potassium iodide-iodine–sulphuric acid method) (Krishnamurthy, 1988)

- Fresh tissue preferable
- Section the material
- Stain the section potassium iodide solution (Lugol's iodine)
- Transfer the section to60%-70% sulphuric acid solution.

Lignins become yellow or orange or brown.

4.2.4. Proteins

Proteins are high molecular weight biopolymers they are composed of α-amino carboxylic acid commonly called amino acids. Amide linkage between amino acid residues called peptide links. Generally it is around 50 residues with a molecular weight of about 6000, there are 20 amino acids which are represented in majority of proteins. Three dimensional type of protein is complicated and it is defined by its primary, secondary, tertiary and sometimes quaternary structure.

4.2.4.1. Total protein (Coomassie Brilliant blueR 250 CBB methods) (Krishnamurthy, 1988)

- Fresh tissue or tissue fixed in 2.5 % glutaraldehyde and embedded paraffin.
- Section the material deparaffinise and bring down to water.
- Stain in 0.02% coomassie brilliant blue R 250 in Clarke solution (PH2.0).
- Rinse in clarke's solution.
- Destain in fresh clarke's solution for 20 minutes
- Dehydrate in 98% and absolute ethanol, 5 min each and mount.

Proteins stain blue.

4.2.5. Tannins (Krishnamurthy, 1988)

- Section the material
- Treat in Lugol's iodine solution (iodine 4g potassium iodide is dissolved in 100 mL water)
- Add Ammonium hydroxide (10 mL of ammonium hydroxide is dissolved in 90 mL of distilled water).

Tannin stains bright red colouration of the plant tissue.

4.3. Phytochemical analysis

4.3.1. Extraction of compounds

The fresh plant materials of *Acalypha indica* Roxb. and *Physalis maxima* Hook. are collected from Thoothukudi. The collected samples were cut into small fragments and shade dried until the material has become uniform. The dried plant material was finely powdered using blender, and sieved to get particles of uniform size. The final uniform powder was used for extraction of active constituents of the plant materials.

The leaves of *A. indica* and fruits of *Physalis maxima* was collected from Thoothukudi. About 25 g of dry powder was packed in Soxhlet apparatus for extraction of respective soluble bioactive molecules from the plant materials by the use of different solvent (n-butyl alcohol, isopropyl alcohol, ethanol and water). Fractions containing volatile solvents, were concentrated with the help of evaporator. The concentrated extract was loaded to sterilized collecting tube and refrigerated for further studies.

Preliminary phytochemical screening of plant was done following the standard procedures adapted by the various workers.

Test for tannins - Ferric chloride test (Ciulei and Istodor, 1995)

To 1 mL of the extract, 2 mL of 5% $FeCl_3$ was added. Dark blue or green -black indicates the presence of tannins.

Test for saponins - Frothing test (Harbrone, 1998)

The crude extract is mixed with 5 mL of distilled water and shaken vigorously, resulting in the formation of stable foam which is a positive indication for saponins.

Test for Flavonoids (Savithramma *et al.*, 2011; Selvaraj *et al.*, 2014)

For identification of flavonoids, 2 mL of plant extract, 1 mL of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Coumarins (Harbrone, 1998)

For identification of coumarins, 1 mL of plant extract, 1 mL of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for Terpenoids (Harbrone, 1998)

For identification of terpenoids, 0.5 mL of the plant extract, 2mL of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

Test for Quinines

One mL of the peel extract was added to 1 mL conc. sulphuric acid. Formation of red color indicates the presence of quinones.

Test for Alkaloids - Wagner's test (Clarke, 1975)

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL water) and observed for the formation of reddish brown colour precipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

Test for Sterols (Egwaikhide and Gimba, 2007)

Extract (1 mL) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour. No dark pink or red colour precipitate, absence of sterols.

Test for Carbohydrate - Fehling's test (Harbrone, 1998)

5 mL of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Test for Glycosides (Clarke, 1975)

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaoH solution was added. Formation of yellow colour indicates the presence of glycosides.

Test for Protein - Ninhydrin test (Harbrone, 1998)

0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for phenol (Harbrone, 1998)

To 1 mL of the extract, 2 mL of distilled water was added and followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

4.4. Antibacterial activity

4.4.1. Test microorganisms

The standard strains of *Bacillus subtilis, Staphylococcus aureus, Escherichia col*i and *Vibrio cholerae* were used as test organisms Cultures of bacteria were grown on nutrient broth (Hi Media, Mumbai) at 37^{o}C for 12–14 h and were maintained and preserved on nutrient agar slants (Hi Media, Mumbai) at 4^{o}C prior to use.

4.4.2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the crude extract was determined according to the method described by the Clinical and Laboratory Standards Institute (CLSI, 2012a), with some modifications. Two fold serial dilutions of the extract and antibiotics were made with Mueller Hinton Broth (MHB) to give concentrations ranging from 2000 to 8000 µg/mL for crude extract and 5 to 1000 µg/mL for antibiotics. Hundred microliters of test bacterial suspension were inoculated in each tube to give a final concentration of 1×10^{5} CFU/mL. The tubes were incubated for

24 h at 37ºC. The control tube did not have any antibiotics or crude extract, but contained the test bacteria and the solvent used to dissolve the antibiotics and extract. The growth was observed both visually and by measuring OD at 600 nm. The lowest concentration of the crude extract showing no visible growth was recorded as the MIC. Triplicate set of tubes were maintained for each concentration of the test sample. Ampicillin were used as positive control.

4.4.3. Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration was determined according to the method of Smith-Palmer *et al.* (1998). About 100 µL from the tubes not showing bacterial growth in the MIC test were serially diluted and plated on nutrient agar. The plates were incubated at 37ºC for 24 h. Minimum bactericidal concentration is defined as the concentration at which bacteria failed to grow on nutrient agar inoculated with 100 µL test bacterial suspensions.

4.4.4. Antibacterial assay by disc diffusion technique

The antibacterial activity of the extract was determined by the disc diffusion method (CLSI, 2012b) against human pathogenic bacteria. The test cultures maintained in nutrient agar slant at 4°C were sub-cultured in nutrient broth to obtain the working cultures approximately containing 1×10^6 CFU/mL. The MIC concentration of the crude extract was incorporated in a 6 mm sterile disc. Mueller Hinton (MH) agar plates were swabbed with each bacterial strain and the test discs were placed along with the control discs. Ampicillin discs (5 µg/disc) were used as positive control. Plates were incubated overnight at 37°C for 24 h. Clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents (extract and antibiotics) was determined by measuring the zone of inhibition measured in mm and expressed as diameter in millimeter (mm).

4.5. FTIR Characterization:

FTIR with transmittance mode was used to characterize the presence of specific chemical groups in the tested catheter samples. FTIR measurements were carried out on a Nicolet 6700 spectrometer (Thermo Fisher Scientific Inc., Warsaw, Poland) equipped with a deuterated triglycine sulfate detector (DTGS/KBr) and a versatile Attenuated Total Reflectance (ATR) sampling accessory with a diamond crystal plate. Spectra were recorded in the spectral range of 4000–600 cm^{-1} at 4 cm^{-1} spectral resolution, 2 sample gain, and 32 sample/background scans using OMNIC 8.1 computer software (Thermo Fisher Scientific Inc.).

4.6. GC-MS analysis:

The volatile compounds were analyzed by gas chromatograph mass spectrometer (GC/MS) by a Thermo Scientific trace GC Ultra Couple with single quadruple MS and a fused silica capillary column TG-5MS (30 m × 0.251 mm, 0.1 mm film thickness).The oven temperature was maintained initially at 40 °C for 3 minutes and then programmed from 40 to 280 °C with rate of 4 °C/min. Helium was used as carrier gas, at 1 ml/min flow rate. The determination of all the identified compounds was made using a percent relative peak area. A tentative identification of the components was made in function with the relative retention time and the mass spectra with those of The National Institute of Standard and Technology, NIST Willy library data of the GC/MS system (Usaizan *et al.,* 2014).

RESULTS

5.1. DESCRIPTION OF THE PLANT

Botanical Name: *Acalypha indica* L.

Synonym: *Acalypha chinensis* Benth.

Family: Euphorbiaceae

Habit: Herb

Habitat: Rocky hillsides, shades of thickets, road sides, wastelands and open plains.

Distribution: Widely distributed in the East Asian countries such as India, Myanmar, Thai land, Malaysia, Vietnam and Indonesia

Plant Characteristics: *Acalypha indica* is an annual herb which is very common in southern India and considered as a major weed. The plant body contains several alkaloids. Leaves have long petiole, ovate or rhombic ovate, acute, cuneate at base, cranate-serrate, upto 3 inches long, 2 inches broad and glabrous. Spikes axillary and monoecious. Both male and female flowers are produced in the axillary spikes. Male flowers are ebracteate, minute, uppermost and few. Anthers vermiculiform. Male flowers are followed by a bunch of sterile flowers. Female flowers produced below, subtended by prominent bracts. These bracts are larger, leafy, dendate, alternate

on erect spikes. Ovary hispid and trilobed. There are three numbers of bifid styles are present. Capsules are three valved and covered with bract.

Flowering & Fruiting: June to December.

Uses: The leaves are used in the treatment of respiratory problems, rheumatoid arthritis, scabies and other skin diseases. They can also be used to treat bronchitis, pneumonia and asthma. The decoction made from the whole plant is used in the treatment of epilepsy, mouth ulcers and emmenagogue. The plant is used by many ethnic communities to treat and manage a number of diseases including Ganglions, diarrhea, leprosy, laxative, ring worms, intestinal worms, boils, swellings and venereal disease.

Botanical Name: *Physalis maxi*ma L.

Synonym: *Physalis pruinosa*

Family: Solanaceae

Habit: Herb

Habitat: Plains, Grasslands, moist places, river margins and margins of cultivation lands, rice fields and unused wastelands.

Distribution: Widely distributed in tropical Africa, Asia and Australia.

Plant Characteristics: *Physalis maxima* is a herbaceous plant which reaches a maximum height of about 2 feets. It is a perennial species and is well known for its medicinal properties. The

leaves and fruits are mostly harvested from wild conditions. Stem is angular, highly branched and green colored. Leaves alternate, petiolate, oblique, stipulate, more or less 10 cm long, margin toothed, apex acute and leathery. Flowers solitary, axillary, pedicellate and yellow colored. Calyx united, green hairy and persistent. Corolla five, yellow colored, gamopetalous and cup like. Stamens five, epipetalous and having greenish yellow colored anther lobes. Ovary round shaped, yellowish and the stigma is greenish yellow colored. Fruit is a small berry, globose, fleshy and overtopped by inflated calyx. Upon ripening the berry turns from green to yellow color and the outer shell turns from pale green to light brown color. Seeds many, compressed and can be propagated through seeds.

Flowering: November to February.

Uses: It is a traditional medicinal plant used by the local village people. It is generally used as a diuretic agent and is a preferable medicinal plant in the treatment of various urinary problems. The ripe fruits are sweet and edible. It is a commonly available medicinal plant in southern India

5.2. Histochemical analysis

Histochemical studies of *Acalypha indica* and *Physalis maxima* reveals the presence of calcium oxalate crystals in the cortical regions (Figure 1 e). Starch the most important carbohydrate reserve of the plant were recorded in *Acalypha indica* and *Physalis maxima* (Figure 1 a and b). Starch content was high in stem of *Acalypha indica*. Amide linkage between amino acid residues called peptide. Coomasie dye reacts with plant tissue that appears blue in phloem and epidermal tissues of *Acalypha indica* and *Physalis maxima*. Tannins content is reported in the histochemical studies of *Acalypha indica* and *Physalis maxima* as bright red colouration on

the vascular bundles of xylem and phloem (Figure 1 c and d). Tannins content was abundantly reported *Physalis* stem.

5.3. Phytochemical analysis

Phytochemical screening of the n-butanol, ispropyl alcohol, ethanol and aqueous extracts of *A. indica* leaves and *P. maxima* showed the presence of various medically active constituents (Table 1, Figure 2.1.1-4 and Figure 2.2.1-3). The qualitative analysis of the extracts from leaf sample of *Acalypha indica* and *Physalis maxima* revealed the presence of phytochemical constituents such tannins, saponin, alkaloids, flavonoids, coumarins, terpenoids, quinine, sterols, carbohydrate, glycoside, protein and phenolic compounds.

In the present study n-butanolic leaf extract of *Acalypha indica* indicates the presence of alkaloids, flavonoids, coumarins, quinine, carbohydrate, glycoside, protein and phenolic compounds. Isopropyl alcoholic leaf extract of *Acalypha indica* confirms the presence of alkaloids, saponins, flavonoids, coumarins, quinine, carbohydrate, glycoside and phenolic compounds. Ethanolic leaf extract of *Acalypha indica* indicates the presence of alkaloids, flavonoids, coumarins, terpenoids, sterols and glycosides. Aqueous leaf extract of *Acalypha indica* confirms the presence of tannins, alkaloids, flavonoids, coumarins, terpenoids, quinine, sterols, carbohydrate, glycosides and protein.

The current investigation reveals the presence of tannins, saponins, alkaloids, flavonoids, coumarins, terpenoids, quinine, sterols, carbohydrate, glycoside, and phenolic compounds in n-butanolic fruit extract of *Physalis maxima*. Saponins, alkaloids, flavonoids, coumarins, terpenoids, quinine, carbohydrate and glycosides were present in isopropyl alcoholic fruit extract of *Physalis maxima.* Ethanolic fruit extract of *Physalis maxima* were reported for the presence of saponins, alkaloids, flavonoids, coumarins, quinine, sterols, carbohydrate, glycoside, and

phenolic compounds. Tannins, flavonoids, coumarins, terpenoids, quinine, carbohydrate and glycosides were present in aqueous fruit extract of *Physalis maxima*.

5.4.1. Antimicrobial activity of crude extract

During this investigation, an attempt has been made to decipher the effect of these secondary metabolites towards its antibacterial activities.

5.4.1.1. Minimum Inhibitory Concentration (MIC)

MIC indicated that the tested crude ethanolic extract of *A. indica* inhibited *B. subtilis, S. aureus, E. coli* and *V. cholerae* at 4000 µg/mL (Table 2). All the tested bacterial strains showed an MIC within 4000 µg/mL in *A. indica*. In *P. maxima,* the MIC ranged within 2000 µg/mL against the tested bacterial strains *B. subtilis, S. aureus* and *V. cholerae. E. coli* the MIC was recorded at 4000 µg/mL. Ampicillin showed varying MIC against the tested bacterial strains. *B. subtilis, S. aureus* and *V. cholerae* were very sensitive to ampicillin with MIC of 1 µg/mL. *E. coli* was also sensitive to ampicillin at 2 µg/mL.

5.4.1.2. Minimum Bactericidal Concentration (MBC)

The crude ethanolic extract of *A. indica* showed an MBC of 8000 µg/mL against all the tested bacterial strains (Table 2). The MBC for *P. maxima* ranged between 4000-8000 µg/mL. *B. subtilis, S. aureus* and *V. cholerae* the MBC was 4000 µg/mL. Whereas for *E. coli* the MBC was 8000 µg/mL. The MBC values of ampicillin was 2 µg/mL against all the tested bacterial strains.

5.4.1.3. Antimicrobial activity by disc diffusion method

The antimicrobial activity of crude ethanolic extract of *A. indica* against the tested bacteria were depicted in Table 3. All the tested bacterial strains were sensitive to *A. indica* (Figure 3). The zone of inhibition varied between 12 to 16 mm. *S. aureus* was highly sensitive

with the maximum zone of inhibition 16 mm. the least activity was recorded against *V. cholerae. E. coli* was moderately inhibited. *P. maxima* fruit extract showed maximum antibacterial activity. Inhibiting all the tested bacterial strains. Maximum zone of inhibition was recorded against *V. cholerae* with the zone of inhibition 20 mm. Whereas *B. subtilis* and *E. coli* the inhibition zone was 18 mm and 16 mm for *S. aureus.* Ampicillin inhibited all the tested bacterial strains with the zone of inhibition ranging between 22 – 25 mm.

5.5. FTIR

The results revealed more number of peaks were detected, informing the complex structure of the compound. Table 4 shows the analysis of extract of *P. maxima*. The results conclude the occurrence of 15 peaks of which 3 major peaks, 3 medium peaks and three low peaks (Figure 3). Based on the analysis, the compound showed the presence of peak 678.9, indicating the group might be C-Br stretching alicyclic axial. The peak value 928.66 and 993.27 shows the presence of groups asymmetric stretching ring and C-H out of plane bending (aldehyde group). The peak values 1054.99, 1139.85, 1250.75, 1395.4 and 1632.63 confirms the presence of groups primary alcohol, asymmetric Dialkyl saturated cyclic, diaryl anti symmetric epoxides, phenol and associated primary amides. The group is imides cyclic in the peak 1727.14, C=O stretching vinyl or phenolic esters in 1784.03, O-H Stretching carboxylic acid in 2360.71, symphonic acid -O-H stretching in 2888.2, C-H stretching cyclobutane in 2981.74, Single bridge polyvalent alcohols in the peak 3448.49.

5.6. GCMS

GC-MS chromatogram of ethanol extract of *P. maxima* fruit are shown in the Figure 4. Major phyto components present in the leaf of *P. maxima* along with molecular formula and molecular weight were presented in Table 5. The GC-MS chromatogram of ethanol extract of *P.*

maxima fruit showed the presence of several active principle compounds. GCMS data confirmed the presence of 20 different compounds like 2-Methyl-7-phenylindole, at Propiophenone, 2'-(trimethylsilo..., 1,2-Bis(trimethylsilyl)benzene, Octasiloxane, 1,1,3,3,5,5,7,7,9,..., Propiophenone, 2'-(trimethylsilo..., 2-Ethylacridine, Tetrasiloxane, decamethyl-, 2-Methyl-7-phenylindole, Indolizine, 2-(4-methylphenyl)-, Tris(tert-butyldimethylsilyloxy)..., 9H-Fluorene-4-carboxylic acid, 9..., Cyclotrisiloxane, hexamethyl-, 2,4-Cyclohexadien-1-one, 3,5-bis..., Benzo[h]quinoline, 2,4-dimethyl-, 9H-Fluorene-4-carboxylic acid, 9..., 5-Methyl-2-trimethylsilyloxy-ace..., Methyltris(trimethylsiloxy)silane, Silicic acid, diethyl bis(trimet..., 2,4,6-Cycloheptatrien-1-one, 3,5..., Hexasiloxane, 1,1,3,3,5,5,7,7,9,... and 1H-Indole, 1-methyl-2-phenyl-.

Consequently, every identified phytochemical of the ethanolic extract of *Physalis maxima* fruit has its bioactivity and is significant for therapeutic values which can be used in the future herbal formulations in a safe and cost-effective way.

DISCUSSION

Plants have since ancient times provided mankind with various medicinal agents and natural products serving as source of many drugs (Balandrin *et al.,* 1993). Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. According to the World Health Organisation, medicinal plants would be the best source to obtain a variety of drugs.

Histochemical studies, reveals the presence of calcium oxalate crystals in the cortical regions of *Acalypha indica* stem than the *Physalis maxima*. Hydrogen peroxide converts oxalate into carbonate which reacts with silver nitrate in the presence of light to form black deposits starch is the most important carbohydrate reserve of the plant starch will appear blue to black in a few minutes because the accumulation of iodine in a few minutes because the accumulation of iodine in the centre of the helical starch molecule presence of proteins were recorded in *Acalypha indica* and *Physalis maxima*. Starch content was high in stem of *Acalypha indica*. Amide linkage between amino acid residues called peptide. Coomasie dye reacts with plant tissue that appears blue in phloem and epidermal tissues. Tannins content in plant materials react with Lugol's iodine and ammonium hydroxide. Bright red colouration on the vascular bundles of xylem and phloem. Tannins content was abundantly reported *Physalis* stem.

Results reveal that leaf extract of *A. indica* were rich sources of flavonoids, coumarins, alkaloids and glycosides. Tannins were present in less concentration. Sterols and saponin were absent in most of the solvent extracts. Among the solvents used aqueous extract were reported with maximum phytochemical constituents. *A. indica* used in folk and indigenous medicine for the treatment of eye infection, wounds, joint pain, arthritis and many other diseases (Doughari, 2006) the occurrence of phytochemicals and their medicinal role has not been adequately

studied. The results of the phytochemical screening of *A. indica* leaves by Oudhia (2003) showed that they are very rich in tannins, saponins, terpenoids, alkaloids and phlobatanins which are best known for their antimicrobial and antiviral properties. Britto *et al.,* (2011) have also identified & reported the presence of alkaloids, tannins, saponins, steroids, flavonoids, terpenoids and cardiac glycosides in ten medicinal plants belonging to different families.

Phytochemical analysis shows that most of the phytochemicals got dissolved in methanol solvent followed by hexane, ethyl acetate and petroleum ether. A particular phytochemical has its own affinity to a particular solvent. In the above result hexane has high affinity towards alkaloids and flavanoids and low affinity towards amino acids. Ethyl acetate has high affinity towards amino acids and good affinity towards alkaloids and saponins. Methanol has a good affinity towards alkaloids, phenols and flavanoids and low affinity towards saponins and amino acids. Petroleum ether has a good affinity towards alkaloids phenols and low affinity towards flavonoids (Mim-E-Tasmim *et al.*, 2021).

In the preliminary phytochemical testing of the *Acalypha indica* extracts, the presence of alkaloids, glycosides, phenols, tannins, saponins, and steroids were demonstrated according to (Kumar and Rani 2016). Phytochemical analysis on different parts of *Acalypha indica* by Mim-E-Tasmim *et al.* (2021) reveals that alkaloid, phenol,tannins, flavonoids, terpenoids, protein was absent in root, saponin was absent in root and flower, glycoside absent in root, stem, flower, similarly steroid was absent in root, stem, flower, of *Acalypha indica*.

Fruit extract of *Physalis maxima* using different solvents revealed the varying degree of phytochemical constituents. N-butanolic fruit extract of *Physalis maxima* revealed the presence of tannins, saponin, alkaloids, flavonoids, coumarins, terpenoids, quinine, sterols, carbohydrate, glycoside and phenolic compounds. In isopropyl alcoholic fruit extract of *P. maxima* saponin,

alkaloids, flavonoids, coumarins, terpenoids, quinine, carbohydrate, glycoside and protein were reported. Whereas in ethanolic fruit extract of *P. maxima* saponin, alkaloids, flavonoids, coumarins, quinine, sterols, carbohydrate, glycoside, protein and phenolic compounds. Aqueous fruit extract of *P. maxima* tannins, flavonoids, coumarins, terpenoids, quinine, carbohydrate, glycoside and protein. Aqueous extract of *P. maxima* showed less extraction of compounds than other solvents.

Flavonoids are present in all vascular plants and have been reported to exert multiple biological effects including anti-inflammatory, antiulcerogenic, antiallergic, antiviral and cancer activities (Harborne, 1973). Medicinally, tannins are used in antidiarrhoeal, haemostatic and antihaemorrhoidal preparations. Saponins are glycosides of steroids, steroid alkaloids found in plants, especially in the plant skins where they form a waxy protective coating. They are useful in lowering cholesterol, as antioxidants and anti-inflammatory agents. Terpenoids are large and diverse class of naturally occurring organic chemicals found in all classes of living organisms. The presence of terpenoids in the leaf extract of *A. indica* as indicated in this study is in agreement with the wide distribution and antibacterial properties of this compound reported by Nostro *et al.,* (2000) and Edeoga *et al.,* (2005) in other plants. Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia and are found as secondary metabolites in several plants like *Digitalis sp.., Convallaris, Euphorbia sp.,* (Edeoga *et al.,* 2005).

In the last decades, microbial infections have become a huge threat to human health, mainly due to the increase in microbial resistance (Li *et al.,* 2016). Emergence of microbial diseases implies serious loss in pharmaceutical industries. Usage of commercial antibiotics for human disease

treatment produce undesirable side effects. Therefore, research on antimicrobials and development are needed to improve the current therapeutic options against the microbial infections.

Hexane, chloroform, ethyl acetate and methanol extracts of *Acalypha indica* leaves showed significant zone of inhibition against Gram positive bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus faecalis* and one Gram-negative bacterium *Pseudomonas aeruginosa*. The gram positive bacteria are more susceptible than the gram negative bacteria. These different antibacterial activities could be due to the nature and concentration of antibacterial compounds plus its/their mode of action (Komathi *et al.,* 2013).

Leaves and flowers contain flavanoids (Ghani 1998). The 95% ethanol extract of this plant possessed antibacterial activity against *Staphylococcus aureus* and hot water extract against *Escherichia coli* (George and Pandalai, 1949). Arambewela *et al* (1999) reported that the essential oils of *K. galanga* root and rhizome showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Parvez *et al*., 2005 reported that the petroleum ether and methanol extract of *K. galanga* showed significant activity against Gram positive and Gram negative bacteria.

The antibacterial activity of plant extracts was not only due to one main active chemical but to the combined action of additional other compounds (Sunayana, 2003). The standard antibiotics used in the study inhibited all the tested bacteria at the lowest concentration due to the presence of single compound. Examples include Phenolic acids (Fernandez *et al*., 1996), alkaloids (Vanbeek *et al*., 1985), flavanoids, (Watcher *et al*., 1999), terpenes (Coveney *et al*., 1985), terpenoids (Habibi *et al*., 2000) and napthoquinones (Cai *et al*., 2000). It is clear that the chemical structure of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plant secondary metabolites.

Sumathi and Pushpa (2007) reported the aqueous extract of *A. indica* shows 9mm inhibition zone to *Escherichia coli* and no zone were showed against *Staphylococcus aureus, Salmonella typhi* and *Shigella flexneri*. Alcoholic extract of *Acalypha indica* shows 10mm inhibition zone towards *Staphylococcus aureus* and *Salmonella typhi*. Studies by Rajaselvam *et al.* (2012), acetone and aqueous extract assayed for its antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella sp*. Zone of inhibition to acetone against *Staphylococcus aureus* (22mm), *Bacillus subtilis* (18 mm), *Klebsiella sp*. (16 mm) and *Escherichia coli* (15 mm). In aqueous, *Staphylococcus aureus* (13 mm), *Bacillus subtilis* (15 mm), *Escherichia coli* (16 mm).

Results of the current study also reveals the antibacterial activity of *A. indica* and *P. maxima* against *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Vibrio cholera*. Basha and Sudarshanam (2011) reported the antimicrobial activity of *A. indica* plant leaves due to the presence of phytochemical compounds like tannins, alkaloids, saponins, phenolics and flavonoids. In our present study we identified *A. indica* leaves contain alkaloids, tannins, saponins and proteins.

According to the studies by Ishak *et al.* (2013), all tested bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, were resistant towards petroleum ether crude extract of *A. indica* stem bark which could indicate that the extract does not contain active compounds that could be responsible for antimicrobial activities. It is also noted that all Gram negative bacteria were more resistant towards all the extracts tested compared to the Gram-positive bacteria. According to a study conducted by (Govindarajan *et al.,* 2008), *A. indica* extracts produced active results against all the Gram-positive bacteria tested while one of the Gram-negative bacteria, *Pseudomonas aeruginosa* was only susceptible towards the extracts at a

higher concentration. This result could be attributed to the difference in wall compositions that exist in both Gram-positive and Gram negative bacteria. While the Gram-negative bacteria possess wall that consists of lipopolysaccharide layer along with proteins and phospholipids that may impede the entry of active compounds of *A. indica* crude extracts, the Gram-positive bacteria contains a very active area of cell metabolism called periplasmic space that carry many digestive enzymes and transport proteins which could attribute to the susceptibility of the microorganisms.

The antibacterial compounds extracted from the plants might inhibit bacteria by a different mechanism to that of currently used antibiotics and have therapeutic values as antibacterial agents. All types of extracts of all the plants showed varied antibacterial efficacies against all the reference bacteria. The ethanolic extracts showed best result followed by the other extracts. In general, the plant antibiotic substances appear to be more inhibitory to gram-positive organisms than gram-negative organisms. Unlike gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of gram-negative bacteria (Raja *et al.,* 2011). The outer lipopolysaccharide layer hinders access of most compounds to the peptidoglycan layer of the cell wall. This explains the resistance of gram negative strains to the lytic action of most extracts.

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds, as well as tannins. The components with phenolic structures were highly active against the microorganisms. Members of this class are known to be either bactericidal or bacteriostatic agents depending upon the

concentration used (Rasooli *et al.,* 2002). The antimicrobial activity of the rhizome may be due to presence of phenolic active compounds in *C. orchioides* (Xu *et al.,* 1992).

Essential oil fraction from *Curculigo orchioides* possesses significant ($P < 0.001$) antibacterial activity at very low concentrations (20 µg/disc) against the pathogenic Gram-positive *S. aureus* (CI) bacteria (Nagesh and Shanthamma, 2009). The demonstration of antimicrobial activity against both Gram-negative and Gram-positive bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also support the traditional application of the plant and suggest the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents. The MIC of crude extracts of individual plants varies against different test strains. The relationship between zone of inhibition and MIC value may or may not be related. The crude extracts have mixture of phytoconstituents.

Secondary metabolites the main components of both purified extract alkaloids and phenol. They represent the high content compounds and its unique components, which recommended as an index in pharmaceutical drug manufacturing. FTIR identified that the leaf extract contained strong C-H out-of-plane bending (oop bend) vibration for substituted benzene ring, presence of phenols and flavonoids in the crude *Enhalus acoroides* extract. Flavonoids are polyphenols characterised by two benzene rings joined by a linear carbon chain. The identification of benzenoid compounds via FTIR spectrophotometry supported the findings from the phytochemical screening, which detected the presence of phenols and flavonoids. The amines, imines, alkanes and phenols present were considered the major functional groups of bioactive compounds. *Enhalus acoroides* has the potential to be used for biomedicinal applications, as phenolic compounds, tannins, flavonoids, chlorophyll and carotenoids are known

to have antioxidant activities. The capsules were produced and characterized by encapsulation efficiency, functional groups, thermal stability and morphology, the capsule that presented the best parameters was used for the evaluation of cell cytotoxicity and antitumor activity. The capsule with 50 % of the extract showed good results of the efficiency, morphology and thermal stability and was used to evaluate the antitumor activity, since the addition of extract in proportions greater than 60 % promoted saturation of the active sites and lower encapsulation efficiency, and directly affects the morphology and thermal stability. The encapsulated and unencapsulated extracts showed strong selective antitumor effect against glial tumor cells without toxicity to non-tumor cells.

The results of the study showed various phytochemical constituents with numerous reported pharmacological activities. The bioactive compounds like methyl ester of hexadecanoic acid act as an antifungal agents (Beulah *et al.,* 2018), n-hexdecanoic acid acts as anti-inflammatory, antimicrobial, and antioxidant (Abdullah *et al.,* 2020). 1, 2-Benzenedicarboxylic acid possesses antifouling and antimicrobial properties (Abubacker and Deepalakshmi, 2013). Hexadecanoic acid methyl ester, a fatty acid exhibits the antifungal and antibacterial, anti-inflammatory, and anticancer properties. Literatures reported its antibacterial activity against both gram-positive and gram negative bacteria such as *Bacillus subtilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Novak *et al.,* 1961; Davoodbasha *et al.,* 2018). 9,12-Octadecadienoic acid (Z,Z)- methyl ester, is a linoleic acid ester with reported anticancer and antiinflammatory properties. 9,12,15-Octadecatrienoic acid (Z,Z,Z) methyl ester, showed anti-inflammatory activity (Yu *et al.,* 2005). Phytol is a diterpene acting as a precursor in the preparation of vitamin K1 and vitamin E and have found to possess biological activities including antioxidant, diuretic and anticancer (Sen and Batra, 2012; Netscher, 2007).

Decanoic acid methyl ester, was found to possess antibacterial and anti-inflammatory properties. Dodecanoic acid methyl ester is an hypocholesterolemic agent that decreases the blood cholesterol level. Methyl tetradecanoate identified in chloroform and ethanol fractions was found to possess larvicidal activity (Ser, 1988; Sivakumar *et al.,* 2011). 9-Octadecenoic acid (Z) methyl ester is a linoleic acid ester showed the anticancer and antioxidant activities (Yu *et al.,* 2005).

n-Hexadecanoic acid ($C_{16}H_{32}O_2$) is a saturated fatty acid witness antioxidant, anti-inflammatory, anti-androgenic and potent mosquito larvicide. The antiinflammatory activity is due to the significant inhibitory activity of the enzyme PLA2 (Aparna *et al.,* 2012; Rahuman *et al.,* 2000). Trans-13- Octadecenoic acid methyl ester (C19H36O2) showed reported biological activities like antiandrogenic, antiinflammatory, anticancer (Krishnamoorthy and Subramaniam, 2014). (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, is a carboxylic acid consisting of sulfoxide group is helpful in treatment of bruises and skin eruption (Ogunlesi *et al.,* 2010). Tetradecanoic acid ($C_{14}H_{28}O_2$) is an unsaturated fatty acid that showed antioxidant, anticancer, and larvicidal activities (Rajeswari *et al.,* 2012).

Limonene and other antioxidants such as pinene, careen, and phelandrene obtained quite good separation in coconut plants. A protein and polypeptide rich surface chemistry of *Azadirachta indica* GNP might have increased cellular association of the nano-conjugate; enhancing their ability to deliver drugs more efficiently and thus increasing the overall efficacy of the anti HIV therapy. In *Phyllanthus amaras*, have more amount of carbonyl Group, it will be used for antijauntice activity. The GC-MS analysis of *Pleiospermium alatum* study revealed that that the presence of some chemical compounds were identified, such as 2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one, Benzamide, N-(1,3-dihydro-2- oxo-4-isobenzofuryl,

n-Hexadecanoic acid, (Z)6,(Z)9-Pentadecadien-1-ol and 2-Methoxy-4-vinylphenol in the leaves of *P. alatum*. The compounds may be acted as highly responsible agent for their antimicrobial and antioxidant activity. *Morinda citrifolia* have proanthocyanidins, as a whole, cause many bioactivities that produce positive, healthful changes in the human body. It had been demonstrated that thesecompounds exhibit antioxidant properties and help the body ward off cardiovascular disease, various immune disorders, and neurodegenerative disease (Packer *et al.,* 1999).

The major compound present in the ethanolic root extract of *Plumbago zeylanica* as identified by GC-MS was phenol, 2,4-bis(1,1-dimethylethyl). The GCMS spectrum gives the structure of the compound molecular formula as $C_{14}H_22O$, molecular weight as 206 and biological activity as antioxidant. Other components also identified in the root of *P. zeylanica* are cyclopentadecane fenoprofen, Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6.7-tetrahydro- Anthranilic acid, 3-chloro-N-(Ochlorophenyl)-(RT: 12.443), 2-Methyl-7-phenylindole. Because the presence of anti-aging properties.

Among the identified phytochemicals, n-Hexadecanoic acid (6.11%), hexadecanoic acid, ethyl ester (0.93%)- Palmitic acid have the property of antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5- alpha is a reductase inhibitors (Jegadeeswari *et al.,* 2012). n-hexadecanoic acid as the major compound in the leaves of *Cleistanthus collinus* (Parasuraman *et al.,* 2009). 9,12-Octadecadienoic acid (Z,Z)- is one among the phytocompounds in leaf (8.36%) and stem bark (18.81%) of *P. alatum* was found to have potential cancer preventive, antiinflammatory and antiarthritic activities. Similar report was made in *Croton tiglium* seed and found to have potential antioxidant and anticancer activity (Mangunwidjaja *et al.,* 2006) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9,

12- Octadecadienoic acid. These reports are in accordance with the result of this study (Devi *et al.,* 2009). Phytol is detected in *P. alatum* leaf which was also found to be effective in different stages of arthritis. Similar results were also observed in the leaves of *Lantana camera* (Rani *et al.,* 2011), *Mimosa pudica* (Sridharan *et al.,* 2011) and aerial parts of *Flueggea leucopyr*us (Sudha *et al.,* 2013). Phytol was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al.,* 2009). Lupeol is detected in *P. alatum* leaf part have the properties of antioxidant, anti-inflammatory, antimalarial and antitumour activity (Gallo and Sarachine, 2008).

Oleic acid is another compound present in stem bark of *P. alatum* which can act as antioxidant and antiinflammatory property. Oleic acid may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil (Terés *et al.,* 2008). Several other compounds were also detected through GC/MS chromatogram having notable medicinal property. The above said compounds found in the ethanol extract of *P. alatum* leaf and stem bark are being used for the pharmacological work. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *P. maxima* contains various bioactive compounds. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

SUMMARY AND CONCLUSION

Acalypha indica and *Physalis maxima* was studies for their histochemical properties and reveals the presence of starch, crystals, tannins and alkaloids. Further subjected to phytochemical studies, using different solvents like n-butyl alcohol, isopropyl alcohol, ethanol and aqueous extract. Phytochemical analysis reveals the presence of different constituents such tannins, saponin, alkaloids, flavonoids, coumarins, terpenoids, quinine, sterols, carbohydrate, glycoside, protein and phenolic compounds. Phytochemical analysis shows that ethanolic extract has the more active compounds, aqueous extract and isopropyl alcohol extracts having moderate active compounds and n-butyl alcohol extract has least active compounds.

MIC reveals that the inhibition was noticed between 2000 µg/mL in *P. maxima* and 4000 µg/mL in *A. indica.* Whereas the MBC was 4000 µg/mL in *P. maxima* and 8000 µg/mL in *A. indica.* Antimicrobial activity shows that ethanolic extract has high zone of inhibition against *Bacillus subtilis, S. aureus, E. coli* and *V. cholerae.* Maximum zone of inhibition was observed against all the tested bacterial strains using the extracts of *P. maxima.* The zone of inhibition ranged between 16 -20 mm. Whereas in *A. indica* the zone of inbition was recorded between 12-16 mm inhibiting all the tested bacterial strains.

Medicinal plants were of great importance to the health of individuals and communities. Our study concludes that the phytochemical analysis of the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Additional understanding about the plant will be useful in the finding of various drugs in treating many dreadful diseases. Hence, the bioactive compound has to be isolated for further studies to explore the potential activity of *Physalis maxima* fruit.

P. maxima is a valuable herb that could be evaluated more and more for its medicinal used besides its conventional. Based on the present analysis and previous reports, it can be concluded *that P. maxima* is a valued reservoir of useful phytoconstituents like, phytosterols, phenols, flavonoids, and alkaloids. The use of four different solvents also indicates the significance of ethanol as a solvent to be used in future studies. The fruits appeared more valuable in terms of phytochemicals. During this analysis, nine phytoconstituents with bioactivity were also identified, showing their potential as an antimicrobial agent against drug-resistant pathogens. Hence, this common herbe would be a special candidate for the search for future pharmaceuticals and nutraceuticals.

The FTIR results revealed more number of peaks were detected, informing the complex structure of the compound. The results conclude the occurrence of 15 peaks of which 3 major peaks, 3 medium peaks and three low peaks. Based on the analysis, the compound showed the presence of peak indicating the C-Br stretching alicyclic axial. The peak value 928.66 and 993.27 shows the presence of groups asymmetric stretching ring and C-H out of plane bending (aldehyde group). The peak values 1054.99, 1139.85, 1250.75, 1395.4 and 1632.63 confirms the presence of groups primary alcohol, asymmetric Dialkyl saturated cyclic, diaryl anti symmetric epoxides, phenol and associated primary amides. The group is imides cyclic in the peak 1727.14, C=O stretching vinyl or phenolic esters in 1784.03, O-H Stretching carboxylic acid in 2360.71, symphonic acid -O-H stretching in 2888.2, C-H stretching cyclobutane in 2981.74, Single bridge polyvalent alcohols in the peak 3448.49.

GC-MS chromatogram of ethanol extract of *P. maxima* fruit showed the presence of several active principle compounds. GCMS data confirmed the presence of 20 different compounds like 2-Methyl-7-phenylindole, at Propiophenone, 2'-(trimethylsilo..., 1,2-

Bis(trimethylsilyl)benzene, Octasiloxane, 1,1,3,3,5,5,7,7,9,..., Propiophenone, 2'-(trimethylsilo..., 2-Ethylacridine, Tetrasiloxane, decamethyl-, 2-Methyl-7-phenylindole, Indolizine, 2-(4-methylphenyl)-, Tris(tert-butyldimethylsilyloxy)..., 9H-Fluorene-4-carboxylic acid, 9..., Cyclotrisiloxane, hexamethyl-, 2,4-Cyclohexadien-1-one, 3,5-bis..., Benzo[h]quinoline, 2,4-dimethyl-, 9H-Fluorene-4-carboxylic acid, 9..., 5-Methyl-2-trimethylsilyloxy-ace..., Methyltris(trimethylsiloxy)silane, Silicic acid, diethyl bis(trimet..., 2,4,6-Cycloheptatrien-1-one, 3,5..., Hexasiloxane, 1,1,3,3,5,5,7,7,9,... and 1H-Indole, 1-methyl-2-phenyl-.

The importance of the study is due to the biological activity of some of these compounds present in the fruit. The present study, which reveals the presence of active components in *P. maxima* suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

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PHYTOCHEMICAL, ANTIOXIDANT, FT-IR, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF JATROPHA PODAGRICA HOOK. L. AND JATROPHA GLANDULIFERA Roxb.

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CERTIFICATE

This is to certified that this dissertation entitled,'**PHYTOCHEMICAL, ANTIOXIDANT, FT-IR, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF JATROPHA PODAGRICA HOOK. AND JATROPHA GLANDULIFERA Roxb.**' submitted by **MuthuLakshmi MReg.No.20APBO07** to ST.MARY'S COLLEGE (Autonomous) Thoothukudi- in partial fulfillment for the award of the degree of '**Master of Science in Botany**' is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

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I do here by declare that this dissertation entitled **"PHYTOCHEMICAL, ANTIOXIDANT, FT-IR, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF JATROPHA PODAGRICA HOOK.AND JATROPHA GLANDULIFERA Roxb."**submitted by me in partial fulfillment for the award of the degree of **'Master of Science in Botany'**,in the result of my original and independent work carried out under theguidance of **Dr. Mrs. B.Maria SumathiM.Sc, M.Phil., Ph.D.** Assistant Professor. Department ofBotany, St. Mary's College (Autonomous) Thoothůkudi and it has not been submittedelsewhere for the award of any other degree.

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CONTENTS

INTRODUCTION

"If you want to be happy for a lifetime, plant a garden." - Chinese proverb

According to a chinese proverb 'Every plant is a medicinal herb'. So, the plant kingdom is a treasure house of potential drugs and used for prevention and treatment of ailments. A disorder or a disease can be compared through various remedial measures like Naturopathy, Homeopathy, Ayurveda, Allopathic and Unani are some such measures. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs (De Pasqual 1984).

The medicinal plants play an important role in supporting health care in India. The use of herbs to treat disease is almost universal and is now recognized by WHO as an essential building block for primary health care. According to World Health Organization, 80% of the people living in the rural areas depend on medicinal plants as primary health care system, particularly the developing countries. Out of the total 4,20,000 flowering plants reported from the world more than 50,000 are used for medicinal purposes. The medicinal plants are important therapeutic aid for the alleviation of ailments of humankind. Historically, plants (fruits, vegetables, medicinal herbs, etc.) have provided a good source of a wide variety of compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and some other secondary metabolites, which are rich in valuable bioactivities like antioxidant, anti-inflammatory, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, or antiviral activities. [Maridass and Britto, 2008].

Antioxidants are capable of preventing oxidative damage, the wide use of natural antioxidants as a replacement of conventional synthetic antioxidants in food and food supplements has been employed, owing to the fact that natural products are considered to be a promising and safe source [Mandal *et al.*, 2011]. Moreover, these natural antioxidants have easy and unlimited access to metabolic processes in the body, and produce virtually none of the side effects associated with synthetic antioxidants [Beevi *et al.*, 2010]. The most commonly used antioxidants at present are Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), Propyl Gallate (PG) and Tert-Butyl Hydroquinone (TBHQ). However, they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals. Therefore, the development and utilization of more effective antioxidants of natural origin are desirable [Raja and Pugalendi, 2009].

Many infective diseases are treated with a chemotherapeutic agent, such as antibiotics, that selectively inhibit or kill the pathogen with little or no effect on the host. Ideally, antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function or blocking metabolic pathways through the inhibition of key enzymes (Prescott *et al.*, 2008). Conventional antimicrobial agents face a lot of resistance problems in recent times as microorganisms are losing sensitivity to some of these drugs. Recently, concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the old generation, and to the newer or modern antibiotics that have been produced in the last decades (Cohen, 1992; Nascimento *et al.*, 2000; Okesola and Makanjuola, 2009). Also, the problem posed by the high cost; adulteration and increasing toxic side

effects of these synthetic drugs coupled with their relative inadequacies in disease treatment, especially in the developing countries portends serious limitations (Shariff, 2001). Consequently, the continuous acute need for novel and effective antibiotics for antimicrobial chemotherapy is clearly evident. Phytochemicals derived from plants have shown great promise in the treatment of intractable infectious diseases (Nascimento *et al.*, 2000; Rios and Recio, 2005) with lesser side effects compared to the synthetic drug agent (Iwu *et al.*, 1999).

To promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use. Some phytochemicals produced by plants have antimicrobial activity and are used for the development of new antimicrobial drugs. GC-MS and FT-IR have played an important role in pharmaceutical analysis in recent years. For the present study, the two taxa - *Jatropha podagrica* Hook. and *Jatropha glandulifera* Roxb. were selected.

Jatropha podagrica HOOK. belongs to the family of Euphorbiaceae, is common shrub of the Asia, Africa, Latin America and Nigeria. Due to fancy red flowers induction, this plant is maintained in the gardens and house for decorative purposes. *Jatropha podagrica*possess several phytochemical like alkaloids, steroids, flavonoidsand diterpenoidsto exhibits various biological activities likeantiinsect, molluscicidal, antitumour and antimicrobial (Aiyelaagbe *et al.*, 2007). It is known as Buddha belly plant, Bottle plant shrub, Gout plant, Lapalapafunfun in Nigeria and traditionally used to treat skin infections, nosocomial infection, gonorrhoea, fever, jaundice, pyretic, diuretic, choleretic and purgative (Aiyelaagbe *et al.*, 2007; Bhaskarwar *et al.*, 2008). This import shrub showed a multipurpose utility in the

horticulture and traditional medicine system for various biological activities (Kosasi *et al.*, 1989; Das *et al.* 2008; Aiyelaagbe *et al.*, 2007 and Bhaskarwar *et al.*, 2008).

Jatropha glandulifera Roxb. is a medicinal plant distributed in the black cotton soil of Deccan and also found in the plains of Northern India [Anonymous, 2001]. In Tamilnadu, it is found distributed in Chengalpattu, Dharmapuri, Pudukottai, Ramanathapuram, South Arcot, Trichy, Tirunelveli and Thoothukudi. The Seed oil of *Jatropha glandulifera* is used in chronic ulcerations, foul wound ringworm, rheumatism and paralysis. Plant juice is used to remove film from the eyes. Water extract of root is given to children suffering from abdominal enlargement. [Senthilkumar *et al.*, 2006]. Stem of *Jatropha glandulifera* used to arrest bleeding from wounds, cuts and ulcers [Jothi *et al.*, 2008]. The roots of *Jatropha glandulifera* are boiled and taken to treat diabetics [Kadhirvel *et. al.*, 2010].

The current study of phytochemical analysis, antioxidants and antimicrobial analysis will contribute to the knowledge of the medicinal properties of selected plants and it will create a path to travel for the researchers. Through this research, I could share the therapeutic uses of these plants to my society.

SCOPE AND OBJECTIVE

The aspiration of current study was to assess the biochemistry and bioactivities of the plants extract (*Jatropha podagrica* and *Jatropha glandulifera*). In this work the following objectives are focused.

i. Collection of the stem of*Jatropha podagrica* and root of *Jatropha glandulifera* plants for extract preparation.

ii. To qualitatively screen the presence of phytochemicals by using different solvents (acetone, methanol, ethanol,) and aqueous extracts of stem of *Jatropha podagrica* and root of *Jatropha glandulifera*.

iii. To quantitatively analyses and compare the total phenols, flavonoids, vitamin C, Tannin and vitamin E of*Jatropha podagrica* stemand *Jatropha glandulifera* root.

iv. To identify and compare the functional group ofstem of*Jatropha podagrica* and root of *Jatropha glandulifera* by Fourier transform infrared spectroscopy (FTIR) analysis.

v. To identify the bioactive compounds ofstem of*Jatropha podagrica* and root of *Jatropha glandulifera*plants extract using GC-MS analysis.

vi. To assess the antioxidant potential of*Jatropha podagrica* stemand *Jatropha glandulifera* root using aqueous extract against DDPH radical scavenging activity.

vii. To evaluate the anti-bacterial potential of acetone, methanol, ethanol and aqueous extracts ofstem of*Jatropha podagrica* and root of *Jatropha glandulifera.*

REVIEW OF LITERATURE

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "Secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent unconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Many herbaceous and medicinal plants contain important photochemical and vitamins such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignins, vitamin C, vitamin E and carotenoids, which are utilized both by humans and animals as important components of diets (Hussain *et al.*, 2011). The medicinal effects of plants are considered to be due to metabolites, especially secondary compounds, produced by plant species. Phytochemical analysis suggests that the presence of various biologically active compounds [alkaloids, phenols, flavanoids, proteins-lectin, carbohydrates, indigo, steroids etc.] and could be correlated to various therapeutic purposes (Vinoth *et al.*, 2011). Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al.*, 2007).

The phytochemical screening of different parts of the *Jatropha curcus* revealed the presence of tannins, saponins, carbohydrates, sterols, diterpenes, alkaloids, flavanoids and various enzymes. Root contains di-terpenoid, Jatrophol and Jatropholones A and B, taraxerol b-sito-sterol. The bark contains tannins, resins,

saponins, reducing sugar and traces of a volatile oil. Leaves contain Steroid, alkaloids triterpene (Rajore and Batra, 2004). Musa *et al.*, (2000) studied the phytochemistry of powdered leaves of *Acalypha recemosa* (Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes.

Sivaraj *et al.*, (2011) conducted preliminary phytochemical screening using five different solvents extracts of *Aegle marmelos, Ruta graveolens, Opuntia dillenii, Euphorbia royleana*and *Euphorbia antiquorum*. Phytochemical profiling of *Mimosa pudica*was carried out by Sriram *et al.*, (2011). Sukumaran *et al.*, (2011) identified the phytochemical constituents of methanol extract of flower of *Peltophorumpterocarpum*. Phytoconstituents found in *Tridax procumbens* were isolated and characterized by Surendra and Talele (2011).

Nwokocha *et al.*, (2011) studied the comparative phytochemical screening of *Jatropha curcus*, *Jatrophagossypifolia, Jatropha multifida* and *Jatropha podagrica* on leaf, stem root and seeds and the results revealed that tannins were found to be the most abundant followed by saponins and flavanoids and phenols. Vindhya K *et al.*, (2014) conducted the preliminary phytochemical study in *Gardenia latifolia* and *Gardenia gummifera*, using different solvents. The petroleum ether extract of both the plants were found to contain glycosides, phytosterols, fats and oils, resins, phenols and triterpenes. Flavonoid was found to be present in *Gardenia latifolia* and not in *Gardenia gummifera*. Alkoloids, carbohydrates, saponins, tannins, proteins, amino acids and diterpenes were absent in both the plants. Ethyl acetate extracts of the plant was found to contain glycosides, phytosterols, resins, phenols, flavonoids and triterpenes. Alkoloids, carbohydrates, saponins, fats, oils, tannins, proteins, amino acids and diterpenes were absent in both the plants.

Ved Prakash *et al,* (2015) investigated phytochemical screening and antioxidant activity of *Adina cordifolia* leaf. The plant extracts were screened for presence of flavonoids, carbohydrate, alkaloid, saponin, phenol, tannins, phlobatannins, terpenoids, and cardiac glycosides. Total flavonoid content, phenols content was estimated. Antioxidant activity was determined using nitric oxide scavenging assay, DPPH assay, hydrogen peroxide scavenging and ferric reducing methods, also MIC was calculated against a set of bacteria (*S. aureus, B. subtilis, E. coli, V. cholarae*). Ravindranath (2003) has been isolated a novel macrocyclic diterpene–Jatrophenone from the whole plant of *Jatropha gossypifolia*. This compound possesses significant antibacterial activity.

FTIR

A large number of medicinal plants are used as alternate medicine for diseases of man and other animal since most of them are without side effects when compared with synthetic drugs. Identification of the chemical nature of phytochemical compounds present in the medicinal plant will provide some information on the different functional groups responsible for their medicinal properties. Iqbal Ahamed *et al.*, (2006) detected major groups of compounds as the most active fraction of four plants extract by infrared spectroscopy.

Ramamoorthi and Kannan (2007) screened the bioactive group of chemicals in the dry leaf powder of *Calotropis gigantea* by FTIR analysis Karerue*t al.*, (2008) detected saponins in crude dry powder of 11 plants using FTIR spectroscopy.

Muruganantham *et al.*, (2009) carried out the FTIR spectroscopic analysis in the powder samples of leaf, stem and root of *Eclipta alba* and *Ecliptaprostratea*. The FTIR analysis of aqueous methanolic leaf extracts of *Bauhinia racemosa* for

phytochemical compounds was done by Gauravkumar *et al.*, (2010). Ragavendran *et al.*, (2011) detected the functional groups in various extracts of *Aervalanata* using spectroscopic method .

Thangarajan Starlin *et al.*, (2012), analyzed the ethanolic extracts of *Ichnocarpus frutescens*, by FTIR, revealed the presence of functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Parag A. Petnekar and Bhanu Raman (2013) carried out the FTIR spectroscopic analysis of methanolic leaf extract of *Ampelo cissuslantifolia* for antimicrobial compounds.

FTIR analysis for five selected green leafy vegetables (GLVs) viz., *Hibiscus cannabinus* , *H. sabdariffa* , *Basella alba* , *B. rubra* L. and *Rumex vesicarius* confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching (Sravan Kumar and Manoj., 2015).

The functional group identification is made by FTIR analysis and the active components based on the peak value in the region of infrared, radiation. The ethanolic flower extract of *Erythrina variegata* L. is passed into the FTIR spectroscopy and the functional groups of the components are separated based on the peak ratio. The results of FTIR analysis confirm the presence of functional groups such as non-bonded, O-H stretch, carboxylic group, acidic, H bonded, C-H stretch, asymmetric stretching of –CH (CH2) vibration, C=N (stretch), carbon-carbon triple bond, multiple bonding, carbonyl compound frequency, C=O stretch, C=C stretch, O-H bend, alcoholic group, C-N stretch, C-O stretch, PO3 stretch, =C-H bending and C-Cl (Priyanga S *et al.*, (2017).

GC-MS

The chemical composition of the essential oils from leaves and wood of *Ocotea brenesii* growing wild in Costa Rica was determined by capillary GC/FID and GC-MS. From the leaves, 64 compounds were identified, corresponding to 85.9% of the oil, and from the wood 57 compounds were identified corresponding to 69.0% of the oil (Carlos and Jose, 2005). The chemical compositions of the essential oils of *Ocimum basilicum*L. *cv*. purple and *Ocimum basilicum*L. *cv*. green cultivated in Iran were investigated by GC-MS (Seyed, 2006).

GC-MS analysis of *Jatropha curcas* leaves revealed the presence of 16 compounds. The most abundant components were 22, 23-dihydro-stigmasterol (16.14%) alpha-tocopherol (15.18%), beta amylin (7.73%) and dotriacontanol (7.02%) The content of gamma tocopherol reached 2.88% and Vitamin E reached 18.06% in the extract (Wang *et al*., 2009). The GC-MS analysis of *Strobilanthes crispus*oil revealed the presence of 28 components. The main constituents were found to be phytol, α-cadinol, Megastigmatrienone, 2,3-dihydrobenzofuranand eugenol (Asmah*et et al*., 2006).

Nithya Narayanaswamy and Balakrishnan (2011) evaluated the antioxidant properties of 13 important medicinal plants and it showed that *Ocimum basilicum* leaf, *Alpinia calcarata* leaf, *Jatropha mulitifida*flower,*Hyptissua veolens* leaf, *Solanum indicum* leaf and *Clitoria ternatea* leaf and flower possessed higher DPPH scavenging activity. Moussa *et al*., (2011). The aqueous leaf extracts of 124 Egyptian plant species belonging to 56 families were investigated and compared for their antioxidant activity by DPPH scavenging assay. Safi *et al*. (2012) studied the biological activities of aqueous extract of the root of *Jatropha curcas* like

antimicrobial and free radical scavenging activities. In the evaluation of DPPH free radical scavenging activity. Olabinri *et al.*, (2013) investigated *in vitro* antioxidant and nitric oxide radical scavenging capabilities of *Jatropha gossypifolia* extract.

Sermakkani M. And V. Thangapandian (2012) evaluated GC-MS analysis of *C. italica* leaves revealed the presence of seventeen compounds. The identified compounds possess many biological properties. For instance, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- Linolenic acid (R/T 20.06) possesses anti-inflammatory, insectifuge, hypocholesterolemic, cancer preventive, nematicide, hepatoprotective, antihistaminic, antieczemic, antiacne, 5-alpha reductase inhibitor, antiandrogenic, antiarthritic and anticoronary properties. n-Hexadecanoic acid - palmitic acid (R/T 17.25) can be an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities.

Fenghuan Wei *et al.*, (2015) identified thirty compounds in *Jasminum grandiflorum* by using GCMS. The major volatile components of the flower were 3,7,11,15- tetramethyl-2-hexadecen-1-o (phytol) (25.77 %), 3,7,11-trimethyldodeca -1,6,10-trien-3-ol (12.54 %) and 3,7,11,15- tetramethyl -1-Hexadecen-3-ol (12.42 %). The results show that phytol is the major volatile component of *Jasminum grandiflorum.*

Praveen Kumar P *et al.*, (2018) studied the identification of bioactive compounds from the Neem sap by Gas chromatography and Mass spectroscopy (GC-MS). The GC-MS analysis of the Neem sap revealed the presence of 30 volatile compounds. Among the 30 compounds, the most predominant compounds are fatty acids like Hexadecanoic acid and Pentadecanoic acid. Hence, this current attempt

forms a basis for the biological characterization and importance of the compounds which could be exploited for future development of drugs.

Seventy six kinds of chemical compounds were found in methanol extract of *E.cephalotes* including aldehydes (7.9%), phenols (7.5%), fatty acids (5.8%) and furfural (5.4%) and 86 kinds of chemical compounds found in *M.anisodan* extract. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M.anisodan* by S. Mohammadi*et al.*, (2019).

Antioxidant activity

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Natural antioxidants can also be replaced by commercially available, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are quite unsafe to use and is restricted due to their carcinogenic effect (Velioglu*et al.*, 1998). Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton and Brown, 1999). Carotenoids, flavonoids, cinnamic acids, folic acid, ascorbic acid, tocopherols, tocotrienols *etc.*, are some of the antioxidants produced by this plant for their sustenance. Beta-carotene, ascorbic acid and alpha tocopherol are the widely used as antioxidants (McCall and Frei, 1999).

Flavonoids are polyphenolic compounds, which are ingredients of many vegetables and fruits. They are classified into flavanols, flavanones, flavones, iso-flavones, catechins, anthocyanins, proanthocyanidins, etc. (Huy*et et al.*, 2008). They are among the most bioactive plant secondary metabolites which outperform well-known antioxidants.

Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, anti-allergic, anti-thrombic and vasodilatory activities. Antioxidant activity gives rise to anti-carcinogenicity, anti-immunogenicity and anti-aging activity (Gulcin*et al.*, 2010).

Flavonoids serve as ROS scavengers by locating and neutralizing radicals (Gill and Tuteja, 2010). Bioactive properties such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action of flavonoids is known (Njoku *et al.*, 2011). The antioxidant activity of the dietary phenolics considered to be superior to that of the essential vitamins and is ascribed to their high redox potential, which allows them to interrupt free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups (Beevi*et al.*, 2010).

Phenolics are secondary metabolities that behave as antioxidants due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring). The antioxidant activities of phenolic compounds are also attributed to their ability to chelate transition metal ions, such as those of iron and copper, which have been proposed as the catalyst for the initial formation of ROS (Knezevic*et al.*, 2011).

Ascorbic acid (vitamin C) is a vital component in human diet with the highest concentrations in animal organs like the liver, leukocytes, and anterior pituitary. It is used for its antioxidant effect (Ensafi*et al.*, 2010). Vitamin C is a major ubiquitous non-enzymatic, water soluble antioxidant (Ueta*et al.*, 2003). It acts as ROS scavenger, thus potentially protecting cells from harmful oxidative products (Fossati *et al.*, 2011). Vitamin C functions in enzyme activation, oxidative stress reduction, and immune function. There is considerable evidence that vitamin C protects against

respiratory tract infections and reduces risk for cardiovascular disease and some cancers (Schlueter and Johnston, 2011).

Tannins are group of polymeric phenolic substances. Consumption of tannin containing beverages, especially green teas and red wines can cure or prevent a variety of illness including heart related diseases (Van-Burden and Robinson, 1981).

Swamy *et al.*, (2004) tested the leaf extracts of medicinal plant, *Leptadenia reticulata* for AgNPs production and antioxidant activity studies. He observed that, 500 µg/ml of green synthesized silver nanoparticles showed maximum (64.81 %) radical scavenging activity. The silver nanoparticles were synthesized using aqueous *Piper longum* fruit extract and the aqueous *P. longum* fruit extract and the green synthesized silver nanoparticles showed powerful antioxidant properties *in vitro* antioxidant assays. Haes *et al.*, (2002).

Pourmorad *et al.*, (2006) carried out a comparative study on the antioxidant potentials of some selected Iranian medicinal plant extracts. The antioxidant properties of 25 edible tropical plants were studied by Wong *et al.*, (2006). Badami and Channabasavaraj (2007) studied the *in vitro* antioxidant activities of thirteen medicinal plants collected from Western Ghats of India.

Ademiluyi and Oboh (2008) studied the antioxidant activity of methanol leaf extract of *Viscum album* by using linolenic acid peroxidation and DPPH methods. Effat *et al.*,(2008) screened thirteen medicinal plant extracts for antioxidant activity. MoniRani *et al.*,(2008) evaluated antioxidant activities of methanol extract of *Ixora coccinea* by DPPH free radical scavenging activity, reducing power and total antioxidant activity assays.

Gayatri *et al.*, (2011) observed that the piperine, an alkaloid found naturally in *Piper nigrum* and *Piper cubeba*. It is widely used in various herbal cough syrups and anti-inflammatory, antimalarial, anti-leukemiatreatement. Ethanol extract of *Piper cubeba* showed high antioxidant activity.

Inbathamizh *et al.*, (2013) studied in vitro evaluation of antioxidant and anticancer potential of *Morindapubescens* synthesized silver nanoparticles. The decolorization from purple DPPH radical to yellow DPPH molecule by the sample in a dose-dependent manner with an IC50 value of 84±0.25 µg/ml indicated the sample's high radical scavenging activity, which was closer to that of the standard whose IC50 value was found to be 80±0.69 µg/ml.

Niraimathi *et al.*, (2013) investigated on biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) leaf extract and determined antioxidant activities. Free radical scavenging activity of the AgNPs on DPPH radical was found to increase with increase in concentration, showing a maximum of 62% at 500 µg/ml. The standard gallic acid, however, at this concentration exhibited 80% inhibition. The IC50 value was found to be 300.6 µg/ml.

The silver nitrate extract of *Annona squamosa* and *Sapium macrocarpum* showed two times more DPPH scavenging activity than the commercial antioxidant butylated hydroxyl anisole. (Ruiz *et al.*, 2008). The silver nitrate extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citrates* were found to possess DPPH scavenging activity. (Pereira *et al.*, (2009). Sowndharajan *et al.*, (2010) studied the antioxidant capacity and total phenolic contents present in the silver nitrate extracts of leaves, stem, and roots of *Melothria maderaspatana* were evaluated. Sathisha *et al.*, (2011) determined antioxidant potentials in silver nitrate extract of

some plants, *Curcuma longa, Coffea Arabica, Tribulus terrestris, Bacopa monnieri* and *Trigonella foenumgraceum* using various *in vitro* assays.

Iwalewa *et al.*, (2005) studied the pro and antioxidant effects of silver nitrate extracts of nine edible vegetables in southwest Nigeria using 1, 1-diphenyl-2-picrylhydrazyl free radical assay. The silver nitrate extract of *Helichrysum plicatum* had been reported to have antioxidant activity using two *in vitro* methods, namely DPPH and -carotene linoleic acid assays . (Tepe *et al.*,(2005)

The silver nitrate extracts of *Chlorophytum borivilianum* had been shown to scavenge DPPH radical and decrease TBRAS (Thiobarbituric Acid Reactive Substances), revealing that it is a promising anti-stress agent as well as a potential antioxidant. (Kenjale*et al.*, 2007).

Antibacterial Activity

Musa *et al.*, (2000) studied the phytochemistry of powdered leaves of *Acalypha recemosa* (Euphorbiaceae). This study revealed the presence of alkaloid, tannin, flavanoid and terpenes. Antimicrobial activities of cold water, hot water and methanolic extracts were studies against *Staphylococcus aureus* was more than *Escherichia coli* but *Candida albicans* was completely resistant to the extracts. The cold water extracts showed activity with MIC range from 3.0 mg/ml (against *S. aureus*) to 4.0 mg/ml *Escherichia coli* for cold water and 7.0 mg/ml for the two isolates (methanolic extract). The MBC of cold water extract (6.0 mg/ml) was able causes 2 log cycle reduction of cell population in 90 minutes. Prema (2004) studied the antibacterial activity in eleven medicinal plants. The acetone extract of *Acalypha indica* was more effective against *Staphylococcus aureus*. Ethanol extract of *A. indica* and *Eucalyptus globulus* were highly sensitive to *S. aureus* and *P. Aeruginosa*.

Poonkothai *et al.*, (2005) worked on antibacterial activity of chloroform, ethanol and aqueous extracts of the leaves of *Gymnema sylvestre* on *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* on Muller Hindon agar plates. Commercially available chloramphenicol disc (30 mg) was used as control and discs impregnated with DMSO were also used in this technique. *Klebsiella pneumoniae* was resistant to both chloroform and ethanol extracts exhibiting a zone of inhibition of 12 and 11 mm respectively. *Pseudomonas aeruginosa* (16 and 21mm) and *Salmonella typhi* (17 and 19mm) were found to be sensitive to both the extracts. This indicates that gymnemic acid, an active component of *Gymnemasylvestre* double in both chloroform and ethanol was found to have a strong antibacterial activity. There was no significant effect of aqueous extract because there was no zone of inhibition.

Akinpelu *et al.*, (2009) studied the medicinal plants *Jatropha curcas*and *Newboulda laevis*. Methanolic leaf extract of *J. curcas, N. laevis* exhibited antibacterial activity against 8 of the thirteen tested bacterial isolates at a concentration of 20 mg/ml. The zones of inhibition exhibited by *J. curcas*ranged between 18 and 17mm. *N. laevis* varies between 10 and 23 mm.

Dhale and Birari (2010) studied the antimicrobial effect of *Jatropha gossypifolia* leaf extracts on gram positive species *Staphylococcus spp.* and *Bacillus spp.* and gram negative species like *Escherichia spp.* and *Pseudomonas spp.*, in solvents like petroleum ether, alcohol and chloroform. The method employed was disc diffusion method, standard was Amphicillin, the alcoholic extract of leaves showed maximum antibacterial activity.

Dipankar Choudhury *et al.*, (2011) studied phytochemical screening and antimicrobial activity of extracts from leaves and stem of *Ecbolium linnean* .The bacterial pathogens were strongly inhibited by leaf extracts but acetone extracts of stem have failed to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* even at the highest concentration. The results revealed that leaf extracts were found to be more effective than stem extracts. *E. linneanum* possesses antimicrobial activity against most commonly encountered human pathogens.

Yusha'u, *et al.*, (2011) studied antibacterial activites of ethanolic extracts of *Annona squamosal (L.)* leaves were studied against clinical respiratory tract isolates of *Klebsiella pnemoniae, Proteus species, Pseudomonas species, Staphylococcus aureus, Streptococcus pnemoniae* and α- haemolytic *Streptococci* using disc diffusion and micro broth dilution techniques. Sensitively test results showed that water fraction of the plant was active on *Stephylococcus aures and Streptococcus pnemoniae* (10mm) at 50µg/disc concentration while ethanolic extract of the plant was active , *Streptococcus pnemoniae*and *Proteus species* at 200µg/disc concentration with zone diameter formed by *Klebsiella pnemoniae*(11mm) being wider than that formed in response to standard Augmentin disc (06mm).

Nidhi uttamkumar and sumitkumar (2013) evaluated antibacterial activity of rhizome of *Barleria prionitis*. The methanol extract showed antibacterial activity against two Gram's positive *(S. aureus and B. cereus*) and two Gram's negative (*E. coli and S. typhi*) bacteria. The antibacterial potential was measured by agar disc plate method. The active phytocomponents of *Barleria prionitis* were revealed using Gas chromatography with mass spectrophotometric detector and 27 constituents identified, Phthalazine was the most abundant phytocompound in

methanol extract. All the results supported that the extract can be used to prevention of bacterial infection and may have role in pharmaceutical medicine evolution.

Nayan R. Bhalodia and V.J.Shukla (2014) reported extracts obtained from *Cassia fistula* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. The results show that the activity of hydroalcohol extracts of *Cassia fistula* shows significant antibacterial and antifungal activities.

Niveditapatel *et al.*, (2014) reported phytochemical analysis and antibacterial activity of *Moringa oleifera*. The result showed that the plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. To evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutans, Bacillus subtilus,* and *Staphylococcus epidermidis bacteria* were used. Phytochemical analysis of the leaf in solvents of varying polarity; viz., aqueous, ethanol were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc. in the both extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximum activity against *Streptococcus mutant* and aqueous extract shows maximum activity against *Proteus vulgaris*.

Hassan Waseem *et al.*, (2016) detected the antimicrobial activity of *C. tamala* against a number of organisms. The Plant extract from *C. tamala* was found to have antimicrobial activity against only one tested bacterium, *S. aureus* (ATCC 25293). They found different degrees of antimicrobial activity against all tested gram

positive and gram negative bacteria contrary to our result where only *S. aureus* was found to be effective.

MATERIALS AND METHODS

Plant material

Botanical name - *Jatropha podagrica* Hook.

Classification:

Division	spermatophyte
Sub-Division	phanerogams
Class	Dicotyledons
Sub-Class	Monochlamydeae
Series	Unisexuales
Order	Euphorbiales
Family	Euphorbiaceae

Distribution:

Jatropha podagrica HOOK. belongs to the family of Euphorbiaceae, is common shrub of the Asia, Africa, Latin America and Nigeria. Due to fancy red flowers induction, this plant is maintained in the gardens and house for decorative purposes.

Description:

Jatropha podagrica HOOK. - small shrub, up to 1 m height, native of panama. Often found in conservatories, gardens and parks but thrive well in rich soil fully exposed to the sun and regularly watered. 'podagrica' is a Latin word meaning 'gouty' and refers to the swollen base of the plant. Branches are soft and succulent, deeply scarred where the leaves, peltate, long petiolated, glaborous, glauccus 3-5 lobed, lobe subovate with margins devoid of serration, orange red or scarlet flowers on terminal, long stalked cymes, unisexual, monoecious , male flower are more in number than female flowers, stamens 6-8, seated in a yellowish disk, furnished with 5 yellow glands, filaments red. Fruits: 3cm long, initially green turning brownish on maturity and dehiscent. The seed shell to kernel ratio by dry weight 25: 75, seed yield is 400 kg/hg with oil content upto 54%. Flowering and fruiting through the year and propagated by division or seed

Plant material

Botanical name - *glandulifera*Roxb.

Classification:

Division	spermatophyte
Sub-Division	phanerogams
Class	Dicotyledons
Sub-Class	Monochlamydeae
SeriesUnisexuales	
Order	Euphorbiales
Family	Euphorbiaceae

Distribution:

Jatropha glandulifera Roxb. is a medicinal plant distributed in the black cotton soil of Deccan and also found in plains of Northern India [Anonymous, 2001]. In Tamilnadu, it is found distributed in Chengalpattu, Dharmapuri, Pudukottai, Ramanathapuram, South Arcot, Trichy, Tirunelveli and Thoothukudi.

Description:

The Plant is an evergreen bushy shrub, upto 1-2 m in height, stem, green, stout and thick, bark pale green to cream in colour, branching erect, dichotomous. Leaf simple, alternate, 6-7cm, 3-5 lobed bright green, in colour; leaf tip-acute micronulate; margin serrate, petioles 5-7cm long; stipulated. Stipulesdissected,filiform and glandular. Inflorescence: cymose, peduncle glabrous. Flowers: unisexual, greenish- yellow; sepals-5; petals-5. Stamens-8, diadelphous; Ovary superior. Fruit-capsule; 1-2cm long, trilocular; one seed in each locule. Seed brown, 0.3-0.6cm long. [Plate 1, 2].

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The fresh plant materials of *root Jatropha glandulifera* Roxb. and stem of *Jatropha podagrica* HOOK. are collected from Thoothukudi. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for extraction of active constituents of the plant materials.

QUALITATIVE ANALYSIS

Water soluble extractive

Two gram of the shade dried powder of root of *Jatropha glandulifera* and stem of *Jatropha podagrica*was macerated with 50 ml water in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Methanol soluble extractive

Two gram of the shade dried powder ofroot of *Jatropha glandulifera* and stem of *Jatropha podagrica* was macerated with 50 ml methanol in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Acetone soluble extractive

Two gram of the shade dried powder ofroot of *Jatropha glandulifera* and stem of *Jatropha podagrica* was macerated with 50 ml acetone in a closed flask for

24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Ethanol soluble extractive

Two gram of the shade dried powder of root of *Jatropha glandulifera* and stem of *Jatropha podagrica* was macerated with 50 ml ethanol in a closed flask for 24 hours. Shaking frequently during first 6 hours and allowed to stand for 18 hours. It was filtered using a muslin cloth and used for phytochemical analysis.

Test for tannins (Ciulei I.)

To 1 ml of the extract, 2 ml of 5% $FeCl_3$ was added. A dark blue or green - black indicates the presence of tannins.

Test for saponins (Harbronejb)

Foam test

The crude extract is mixed with 5 ml of distilled water and shaken vigorously, resulting in the formation of stable foam which is a positive indication for saponins.

Test for Flavonoids (Savithramma*et al* and selvaraj *et al.*,)

For identification of flavonoids, 2ml of plant extract, 1ml of 2N sodium hydroxide (NaOH) was added. Formation of yellow colour indicates the presence of flavonoids.

Test for Coumarins (Harbrone JB)

For identification of coumarins, 1ml of plant extract, 1ml of 10% NaOH was added. Formation of yellow colour indicates the presence of coumarins.

Test for Terpenoids (Harbrone JB)

For identification of terpenoids, 0.5 ml of the plant extract, 2ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

Test for Quinines (P. D. Egwaikhide and C. E. Gimba)

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Alkaloids (E. C. G. Clarke)

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

Test for Sterols (P. D. Egwaikhide and C. E. Gimba)

Extract (1 ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour. No dark pink or red colour precipitate, absence of sterols.

Test for Carbohydrate (Harbrone JB)

Fehling's test

5 ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing sugars.

Test for Glycosides (E. C. G. Clarke)

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaoH solution was added. Formation of yellow colour indicates the presence of glycosides.

Test for Protein (Harbrone JB)

Ninhydrin test:

0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids.

Test for phenol (Harbrone JB)

To 1 ml of the extract, 2 ml of distilled water was added and followed by few drops of 10% aqueous ferric chloride. Appearance of blue or green colour indicates the presence of phenols.

Quantitative analysis of antioxidant

Total phenolic content :(Duan *et al.*, 2006)

Reagents

- 50%Folin – ciocalteau reagent
- 20%sodium – carbonate
- Gallic acid – standard

Procedure

100mg of samples was homogenate with 10 ml of distilled water and filtered through a muslin cloth. 1ml of the filtrate was added to 1.5 ml of deionized water and 0.5 ml of 50% folinciocalteau reagent and the contents were mixed thoroughly. After 1min, 1 ml of 20% sodium carbonate solution was added mixed the control contained all the reagents except the sample. After 30 minutes of incubation at 37°c, the absorbance was measured at 750nm. Total phenolics were calculated as Gallic acid equivalent (GAE) per gram fresh weight.

Total flavonoid content (Zhinshen *et al.*, 1999)

Reagents

- 5% sodium nitrate (NaNo2)
- 10% Aluminium chloride (Alcl3,H2O)
- 1N sodium hydroxide (NaoH)
- Quercetin standard

Procedure

100mg of plant material was homogenized with 10ml of distilled water and filtered through a muslin cloth. 0.5 ml of the extract was added with 2.5 ml distilled water and mixed. After 6 minutes 0.15 ml NaOH, was added and again after 6min 0.3 ml of 10% $Alcl^3$ was added. After 5 minutes 1ml of 1M NaOH and 0.5 ml of water were added. Following through mixing of the solution the absorbance against blank were recorded at 510nm. Quercetin was used as standard and the results were expressed as my quercetin equivalents (QE) 1g fresh weight.

Vitamin C [Ascorbic acid] (Baker and Frank, 1968)

Reagents

- 5% of TCA
- Indophenols reagent
- 20mg of dichlorophenol indophenols was dissolved in 10ml of warm distilled water
- DT reagent 2g of 2, 4 dinitraphenyl hydrazine and 1g of thiourea were dissolved.
- 85% sulphuric acid
- L-ascorbic acid - standard

Procedure

100 mg of plant material was homogenized with 10ml of 5% Trichloro acetic acid (TCA). The homogenate was centrifuged. To 2 ml of indophenols reagent and 0.5ml of DT reagent was added and incubated at 10c for 1hour and then cooled in ice bath and 2.5 ml of 85% sulphuric acid was added and shaken well for 30 minutes (until) red colour appeared. The absorbance was measured at 540nm. 1-ascorbic acid was used as standard and the results were expressed as mg/1g/FW.

Estimation of Tannin (Julkunen-Titto, 1985)

Procedure

100 mg of sample homogenized with 10 ml of distilled water and filtrated through a muslin cloth. 1ml of aliquot of aqueous extract was mixed with 1.5ml of 4% vanillin (prepared with methanol) and 750 µl of concentrated HCL was added the solution was shaken vigorously and left to stand at room temperature for 20 minutes

in darkness the absorbance against blank was read at 500nm using UV-Visible spectrophotometer. Results were expressed as mg catechin equivalent (CE) 1g tissue.

Vitamin E (Tocopherol): Rosenberg, 1992

Procedure

The plant sample (2.5g) was homogenized in 50ml of 0.1 N sulphuric acids and allowed to stand overnight the content in the flask was shaken vigorously and filtered through what man No.1 filter paper. Aliquots of the filtrate were used for estimation.

In stoppered centrifuge tubes 3ml of extract and 3ml of water were pipette out separately. To both the tubes, 3ml of ethanol and 3ml of xylene were added, mixed well and centrifuged. Xylene (2.0ml) layer was transferred into another stoppered tube. To each tube, 2.0 ml of dipyridyl reagent was added and mixed well, the mixture (3ml) was pipette out into a cuvette and the extinction was read at 460nm. Ferric chloride solution (0.66 ml) was added to all the tubes and mixed well. The red colour developed was read exactly after 15min at 520nm. Tocopherol was used as standard.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infra -red spectra were recorded as KBr pellets on a Thermo Scientific NicotiS5ID1 transmission, between 4000-400 cm^{-1} (Kareru et al., 2008).

GC-MS Analysis:

Extract Preparation

The 50g root powder of*Jatropha glandulifera* and stem *Jatropha podagrica* was serially extracted with 250 ml of Methanol with the help of soxhlet apparatus. The extraction procedures were continued for 3-4 hours at 60°C -80°C[15]. These extracts were concentration under reduced pressure evaporator and stored in air tight vials at 4°C for further study.

Phytochemical analysis by GC-MS

Gas chromatography-Mass spectrometry (GC-MS) analysis of the ethanolic extracts was performed by using a GC-MS (Model; QP2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia.and0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature was set at 200 and 240°C respectively. The oven temperature was programmedfrom70to220°Cat10°C/min, held isothermal for 1 min and finally raised to 300°C at 10°C/min. 2 µ1 of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass 18 scan of 50-600 amu. Total running time of GC-MS is 35min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of phytochemical components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s. LIB and WILEY 8.

LIB library sources were used for matching the identified components from the plant material.

ANTIOXIDENT ACTIVITY

Crude samples extracts were prepared by pouring 100ml of distilled water in a conical flask containing 10g of each samples separately in the ratio of 10:1 (V/W). After 24 hours, the mixture was filtrated through whatman no: 1 filter paper and the filtrate were evaporated to dryness. Crude (aqueous) extracts of all samples (1mg/ml) were used for the determination of free radical scavenging activity.

Free radical scavenging assays (Hatano *et al.*, 1998).

Free radical scavenging assay was measured by 2-2 Diphenyl, 1-picryl hydrazine (DPPH) method proposed by with slight modifications. 1ml of aliquot of test sample was added to 3ml of 0.004% DPPH solution prepared in methanol. The mixture was vortexes for 1min and kept at room temperature for 30 minutes in darkness the absorbance was read at 517 nm. Allow absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid was used as standard.

DPPH scavenging activity (%)

A control –A test / A control * 100

Where, a control is the absorbance of the DPPH solution without test solution. A test is the absorbance of DPPH with the test solution. Aqueous extract was used as blank.

Antibacterial studies

Extraction of plant materials

The plant powder was extracted with methanol, ethanol, acetone and water.25 gms of plant powder was extracted with methanol, acetone and water solution individually in soxhelt apparatus continuously for about 4-6 hours, which was again concentrated till it become semi solid. It was evaporated to dryness and stored at 0 C, until the time of the experiment.

Bacterial strains used

The test organisms were obtained from the Department of Microbiology; St. Mary's College (Autonomous), Thoothukudi. The one gram positive bacteria viz; *Bacillus subtilis* G-ve MTCC 1133 and four gram negative bacteria *Escherichia coli*, G-ve, MTCC 50, *Staphylococcus* G-ve, 737. *Vibrio cholera* G-ve MTCC 3906, were used in the present study.

Broth Medium:

- Nutrient broth Himedia MOO1
- Nutrient broth 1.3 gm
- Distilled water 100 ml

2-3 ml of sterilized broth medium was taken in the culture tube. The inoculating loop was flamed and after a few minutes a loopful bacterial colony was transferred to the broth medium. This microbe culture was incubated at room temperature for 24 hours.

Agar medium:

- Nutrient broth Himedia MOO1
- Nutrient broth 1.3 gm
- Distilled water 100 ml

To prepare the agar medium all the above ingredients were dissolved and sterilized.

Disc diffusion method

Anti- bacterial activity was evaluated by agar disc diffusion method (Kirby – Bauer *et al.*, 1986). Test solution was prepared with known weight of methanol, ethanol, acetone and water extracts dissolved in 5% dimethyl sulphoxide (DMSO). What man No.1 filter paper disc (5mm) was impregnated with 20 of these extracts and allowed to dry at room temperature. The spread plates were prepared by proper concentration of inoculate. Each sample loaded discs was placed in the seeded agar plate. 24-48 hours of + 37^0c incubation, the diameter of the inhibition zone was for positive control, amoxicillin discs (100g/ml) was used, whereas for negative control; respective solvents loaded on the sterile discs.

Plant have been major sources of bioactive principles employed in drug formulations both modern and traditional medicine. According tothe World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as a primary health care system. (Sakarkar and Deshmukh, 2011)

Jatropha podagrica and *Jatropha glandulifera* are the important medicinal plants in the family E*uphorbiaceae.* Both the plants are selected for the present study. *Jatropha podagrica* is used as a diuretic, gonorrhoea, anti-inflammatory, anti-microbial (Ramadevi and Kanapathi , 2012). *Jatropha glandulifera* is used for tonic, intestinal ulcer and cooling, useful in dysentery and diuretic. Leaves, Flowers and Fruits of *Jatropha glandulifera* are used in gonorrhoea and leprosy. In the present study, active constituents of the plants were analyzed and evaluated (Devmurari *et.al.,* 2010)

QUALITATIVE ANALYSIS

Preliminary phytochemical analysis of the various solvent by the stem of *Jatropha podagrica* and root of *Jatropha glandulifera* showed different results. The alkaloids, phenols, tannin, saponins, glycosides, quinones, flavonoids, terpenoids and coumarins were predominantly present in the solvent extracts. Tables (1&2)

Johnson *et.al.,* (2012). reported the methanol extracts of some medicinal plants to contain tannin, saponin, flavonoids, phenol, betacyanin and coumarin. Sukumaran *et.al.,* (2011) reported the presence of alkaloids, flavonoids, tannins, saponins, phenol and terpenoids in *Peltrophorum pterocarpum* flowers.

TOTAL PHENOL

Phenolics are the most widespread secondary metabolites and are believed to be responsible for antioxidant activity. The phenol contents of thestem extract of *Jatropha podagrica* (3.867±0.323mg GAE/g) were higher than that of the root extract of *Jatropha glandulifera* (2.874±0.0143mg GAE/g).(Table-3). Phenolic compounds are a class of antioxidant agents thatact as free Terminators (Shahidi and Wanasundra, 1992). Phenolic compounds have a variety of beneficial activities. They have potent antioxidants and free radical scavengers. (Meenakshi *et .al.,* 2010) the

Table 1: Preliminary phytochemical screening and distribution of secondary constituents in stem of *Jatropha podagrica*

Phytochemical Test	Acetone	Methanol	Ethanol	Water
Alkaloids	+	+	-	-
Flavanoids	+	+	+	+
Sterols	+	+	+	+
Carbohydrates	-	-	+	-
Glycosides	-	+	+	+
Saponin	+	+	+	+
Protein	-	+	+	-
Quinone	-	-	-	+
Phenol	+	-	+	+
Coumarin	-	-	+	+
Tannin	+	+	+	-
Terpenoid	+	+	+	+

Table 2: Preliminary phytochemical screening and distribution of secondary constituents in root of *Jatropha glandulifera*

Phytochemical Test	Acetone	Methanol	Ethanol	Water
Alkaloids	+	+	+	+
Flavanoids	+	+	+	+
Sterols	+	-	+	-
Carbohydrates	-	+	+	+
Glycosides	+·	+	-	+
Saponin	+	-	+	+
Protein	-	+	+	+
Quinone	+	+	-	-
Phenol	+	+	+	+
Coumarin	+	+	+	-
Tannin	+	+	+	+
Terpenoid	-	+	-	+

Table : 3

TOTAL FLAVANOID CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*	
Sample	**Amount of flavanoid mg (GAE)/g**
Jatropha podagrica	3.675±1.009
Jatropha glandulifera	3.453±0.451

Table: 4

TOTAL PHENOL CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*	
Sample	**Amount of phenol mg (GAE)/g**
Jatropha podagrica	3.867±0.451
Jatropha glandulifera	2.874±0.0143

Table: 5

TOTAL TANNINS CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*	
Sample	**Amount of Tannins mg (GAE)/g**
Jatropha podagrica	1.124±0.025
Jatropha glandulifera	1.119±0.239

Table: 6

TOTAL VITAMIN C CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*	
Sample	**Amount of Vitamin C mg (GAE)/g**
Jatropha podagrica	1.389±0.004
Jatropha glandulifera	0.654±0.032

Table: 7

TOTAL VITAMIN E CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*	
Sample	**Amount of Vitamin E mg (GAE)/g**
Jatropha podagrica	24.876±8.965
Jatropha glandulifera	21.453±0.234

Figure: 3 TOTAL FLAVANOID CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*

Figure:4 TOTAL PHENOL CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*

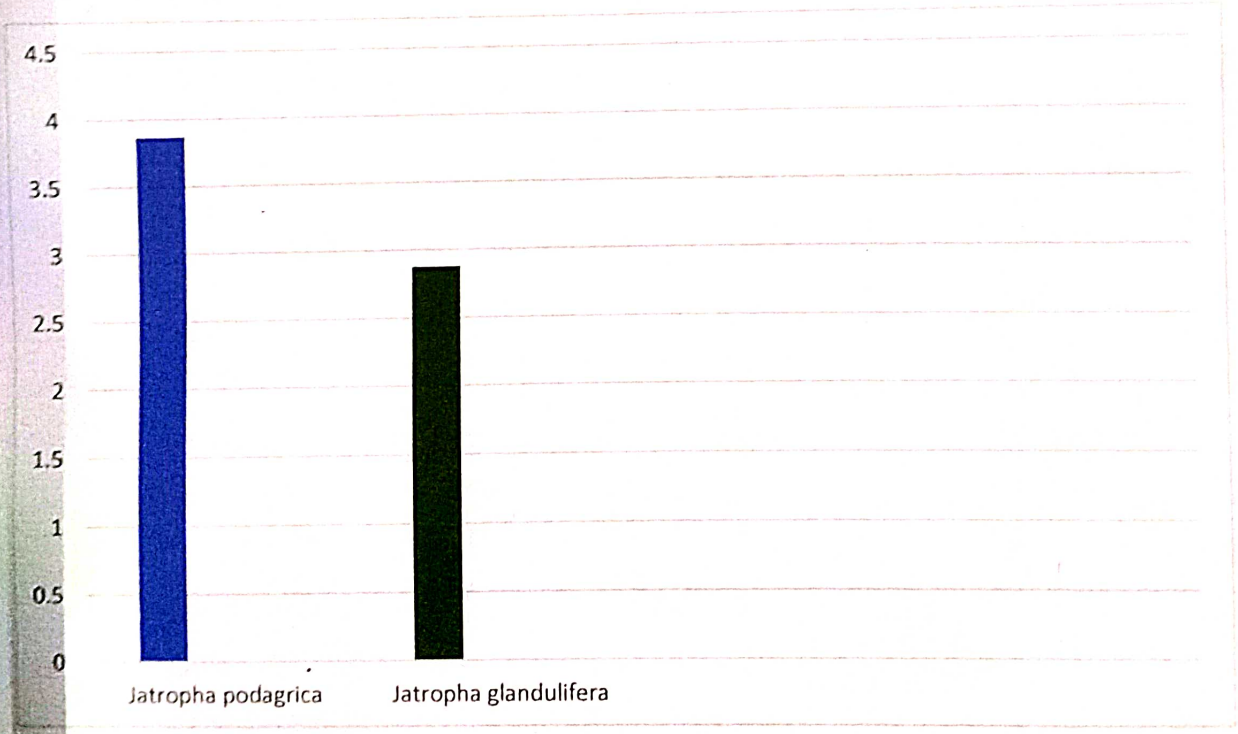


Figure:5 TOTAL TANNINS CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*

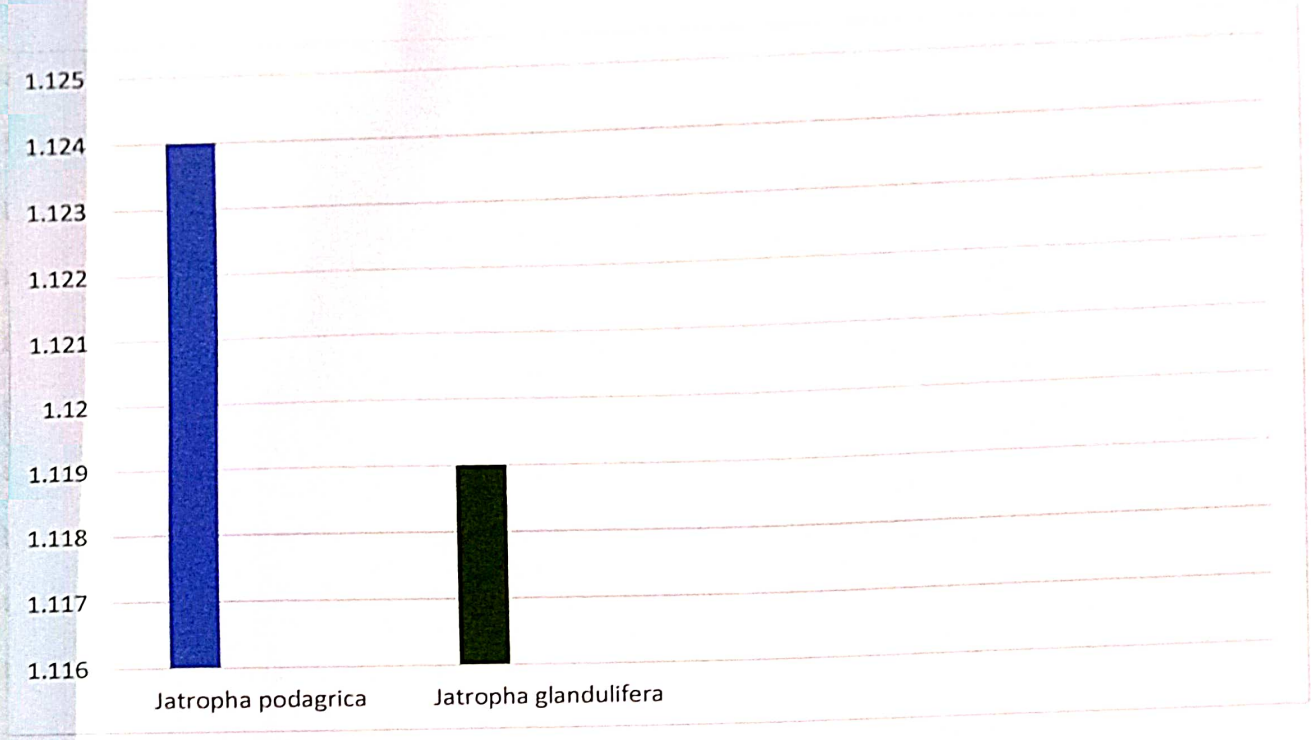


Figure:6 TOTAL VITAMIN C CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*

Figure:7 TOTAL VITAMIN E CONTENT OF *JATROPHA PODAGRICA* AND *JATROPHA GLANDULIFERA*

antimicrobials (most of the phenolics) may provide a microbe-free environment within the body.

TOTAL FLAVONOIDS

Flavonoids are secondary metabolites and have responsible for antioxidant activity in the medicinal field. The total flavonoid contents of *Jatropha podagrica* (3.675 ± 1.009 mg (GAE) -g were higher than that of *Jatropha glandulifera* (3.453±0.451 mg (GAE) – g)(Table-4). Flavonoids are potent antioxidants and epidermic studies indicate that high flavonoids in taking are correlated with decreased risk of lifestyle diseases like diabetes and cardiovascular diseases (Kaur *et.al.*,2008). Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity (Havsteen,2002)

TOTAL VITAMIN-C

Table-5 shows *Jatropha podagrica* (1.389±0.004) and *Jatropha glandulifera* (0.654±0.032) contains a significant amount of vitamin C. vitamin C is a vital component in human.Diet with the highest concentration in animal organs. Vitamin C is a non-enzymatic, water-soluble antioxidant (Ueta *et al.*, 2003). Vitamin C function in enzyme activation, oxidative stress reduction, and immune function. It protects against respiratory tract infection and reduces risk for cardiovascular disease and cancer.

TOTAL TANNINS:

(Table 6) shows stem extract of *Jatropha podagrica* (1.124±0.025mg/g) and root extract of *Jatropha glandulifera* (1.119±0.239mg/g) contain a significant amount of tannin (Table-6). Tannins are present primarily in the leaves of trees growing in stressful conditions. They are accumulated in the vacuoles, especially those of the epidermal layer and the palisade layer and the palisade mesophyll. Tannins are useful in treating inflammation, ulcers and remarkable activity in cancer prevention and anticancer activities (Li *et al.*, 2003; Akinpeluet *al.*, 2009).

TOTAL VITAMIN- E

Total vitamin E contains in thestem extract of *Jatropha podagrica* (24.876±8.965mg/g) is the highest and the stem extract of *Jatropha*

glandulifera (21.453±0.234mg/g) is the lowest (Table-7). Vitamin E is a fat-soluble nutrient found in many foods (Jacob, 1995). In the body, it acts as an antioxidant, helping to protect cells from the damage caused by free radicals are compounds formed when our bodies convert the food we eat into energy(Havsteen, 1983).

FT-IR

Fourier Transform Infrared Spectroscopy was to analyse the functional group present in *Jatropha podagrica* and *Jatropha glandulifera*. The FTIR spectroscopy analysis of *Jatropha Podagrica*stem obtained peaks at 443.6 cm^{-1}, 652.86 cm^{-1}, , 924.8 cm^{-1}, 1244.97 cm^{-1}, 1745.46 cm^{-1}, 3834.22 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for C-I in Aromatic, C-BR in Alicyclic Axial, N-O in Stretch, C-O-C in Asymmetric, C=O in Ester, N-H in Urethanes.

The FTIR spectroscopy analysis of *Jatropha glandulifera*root obtained peaks at 516.89 cm^{-1}, 661.54 cm^{-1}, 946.02 cm^{-1}, 1160.1 cm^{-1}, 1318.25 cm^{-1}, 2849.63 cm^{-1}, 3503.45 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for C-Br in Aromatic, N-H in Amines, C=O in Esters, N-O in Stretch, C=C in Symmetric stretch, C-O in Hydroxyl group, H-C-H in Asymmetric Stretch, O-H in Medium.

From the spectral data presence of C-I, C-BR, N-O, C-O-C, C=O, N-H, N-O, C=C, H-C-H and O-H were identified. These bonding are responsible for the presence of Aromatic, Alicyclic Axial, Stretch, Asymmetric, Ester, Urethanes, Symmetric stretch, Asymmetric Stretch, Medium. Carboxylic acid present in the medicinal plant serves as the main pharmaceutical product in curing ulcers, jaundice, headache, stomatitis, hemicranias, fever, pain in the liver and treatment of rheumatic joint pain. Amides, amine and amino acids are the main groups, involved in protein synthesis. The study revealed that the stem of*Jatropha Podagrica* and root of *Jatropha Glandulifera* contains a considerable amount of secondary metabolites and it may be considered in future to be used in human disease management.

Figure 8: FTIR spectroscopy analysis of *Jatropha Glandulifera*

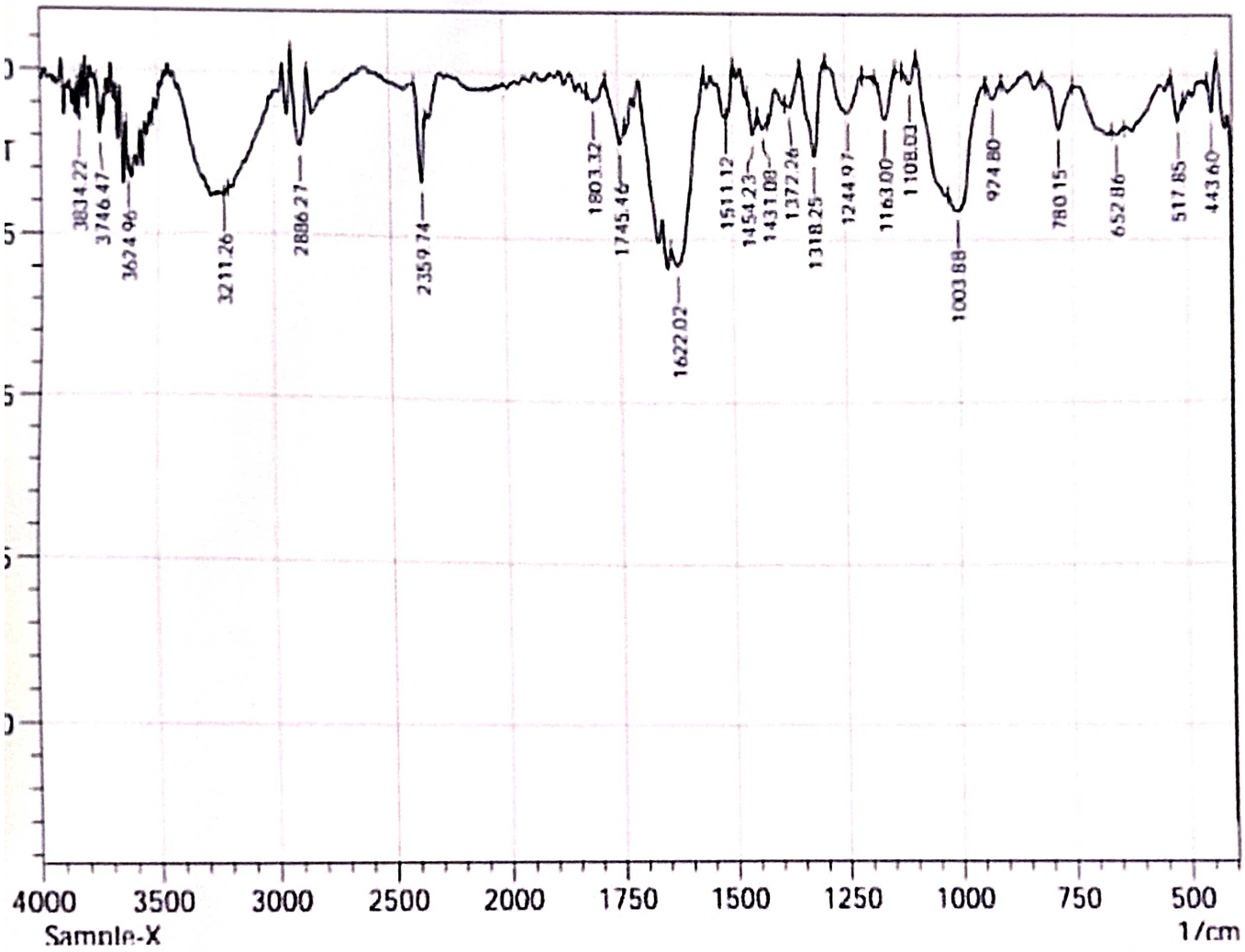


Figure 9:FTIR spectroscopy analysis of *Jatropha Glandulifera*

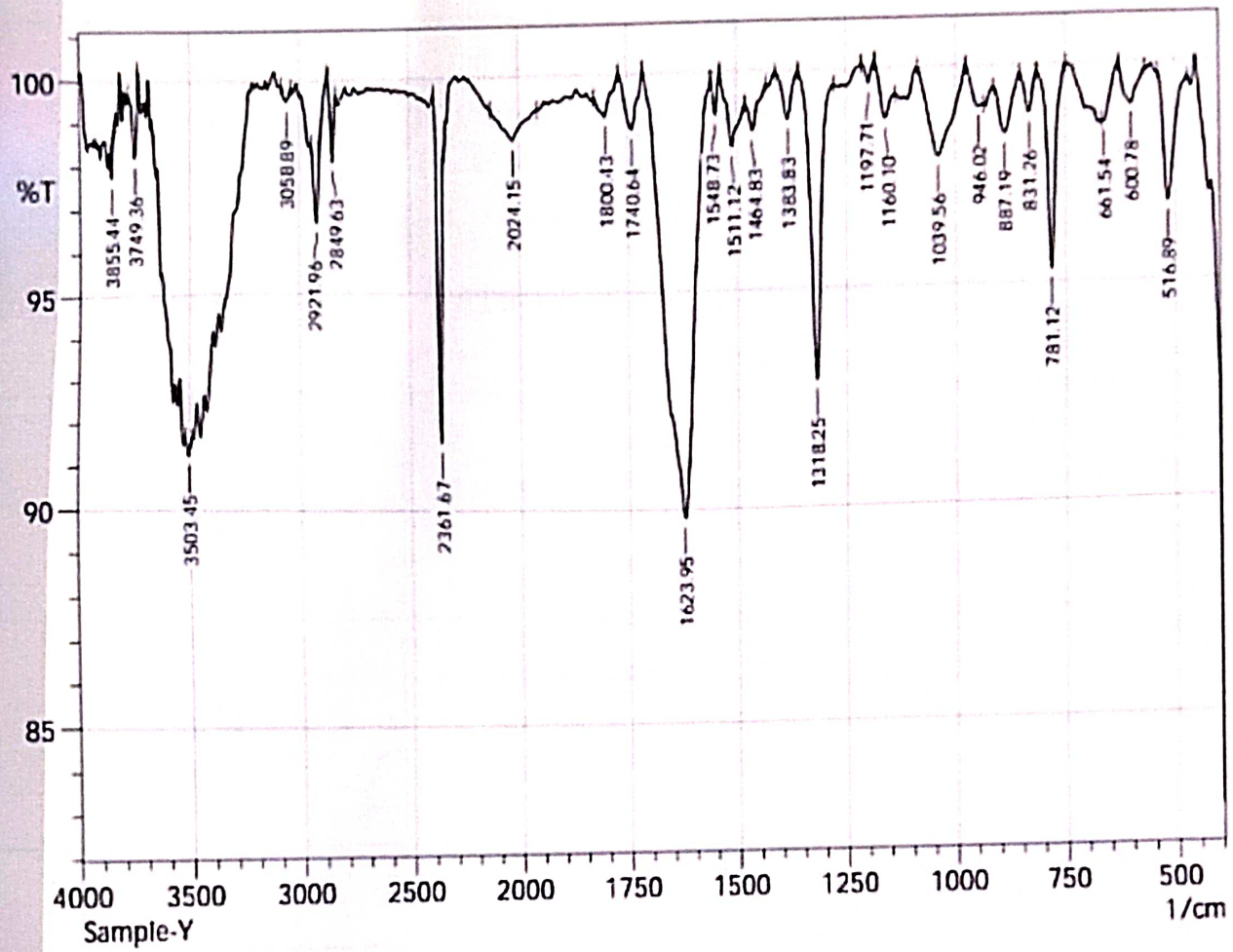


Table 8: FT-IR spectroscopy analysis of *Jatropha podagrica*

PEAK VALUE	BOND	FUNCTIONAL GROUP
443.6	STRONG,STRETCH,AROMATIC	C-I
517.85	STRONG,WEAK,STRETCH	C-BR
652.86	STRETCH,STRONG,ALICYLIC AXIAL	C-BR
780.15	STRONG,STRETCH,MEDIUM, WEAK	C-CL
924.8	STRETCH	N-O
1003.88	MEDIUM, STRETCH	N-N
1108.03	WEAK, STRONG, MEDIUM	C-O-C
1163	WEAK, MEDIUM, STRETCH, STRONG	C-O
1244.97	STRONG, WEAK, ASYMMETRIC, STRETCH	C-O-C
1318.25	STRETCHING	C-N

1372	STRETCH, AMIDE I BOND	C=S
1131	STRETCH, BENDING VIBRATION	CH_2
1454.23	WEAK, NITRATE GROUP	N-O
1511	ASYMMETRIC, STRETCH, AROMATIC ALIPHATIC NITRO GROUP,	NO_2
1622.02	STRONG, WEAK, MEDIUM, NITRATE GROUP	NO_2
1745.46	STRONG, STRETCH, ESTER	C=O
1803.32	WEAK, STRONG, STRETCH, AROMATIC	CH
2359.74	MEDIUM, STRONG, ASYMMETRIC, STRETCH	N-H
2886.27	MEDIUM, STRONG, SYMMETRIC STRETCH	CH_3
3211.26	STRONG, MEDIUM, ASYMMETRIC STRETCH	N-H
3624.96	MEDIUM, UREATHANES	N-H
3746.47	STRONG, MEDIUM, UREATHANES	N-H
3834.22	MEDIUM, UREATHANES	N-H

Table 9: FT-IR spectroscopy analysis of *Jatropha glandulifera*

PEAK VALUE	BOND	FUNCTIONAL GROUP
516.89	STRONG,AROMATIC	C-Br
600.78	STRONG,IODO COMPOUND	C-I
661.54	STRONG,PRIMARY AMINES	N-H
781.12	STRONG,HYDROGEN ATOMS	C-H
813.26	STRONG,SULPHINIC ACID GROUP	S-O
887.19	ALIPHATIC NITRO GROUP	C-H
946.02	STRONG,STRETCH	N-O
1039.56	VERY STRONG,RING STRETCH	C-O
1160.1	STRONG,SYMMETRIC STRETCH	C=C
1197.71	VERY STRONG,ESTERS GROUP	C-O
1318.25	STRONG,HYDROXYL GROUP	C-O

1383.83	STRONG,SYMMETRIC NITRO GROUP	N=O
1464.83	STRONG,AMINES	N=O
1511.12	WEAK,SECONDARY AMIDE	N-H
1548.73	ASYMMETRIC STRETCH	N=O
1623.95	MEDIUM,PRIMARY AMIDES	N-H
1740.64	STRONG,ESTER	C=O
1800.43	STRONG STRETCH	C=O
2024.15	MEDIUM	C-H
2361.67	MEDIUM,ASYMMETRIC STRETCH	H-C-H
2849.63	MEDIUM,ASYMMETRIC STRETCH	H-C-H
2921.96	MEDIUM,ASYMMETRIC STRETCH	H-C-H
3058.89	MEDIUM, URETHANES	N-H
3503.45	MEDIUM	O-H

GC-MS Analysis:

The GC-MS analysis of stem extract of *Jatropha podagrica* confirmed the presence of 5 compounds with retention time. Interpretation of the mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of these 5 compounds, 3 compounds are majorly present in the stem extract of *Jatropha podagrica* respectively 2-Ethylacridine(52.89%), Benzo(h)quinoline,2,4-dimethyl(52.89%) and 5-methyl 2- phenylindolizine (35.86 %).

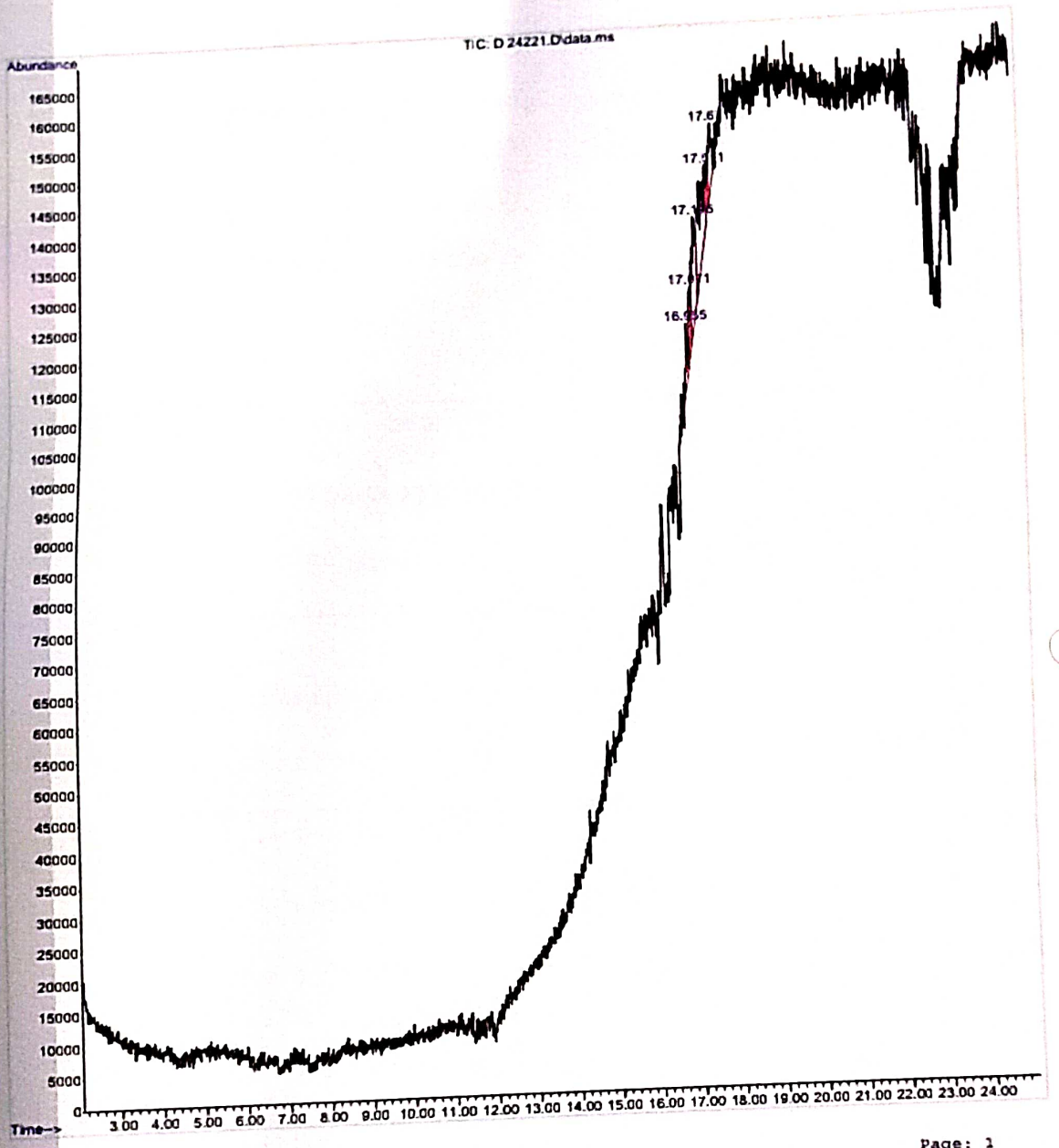
The two minor compounds as Indole-2-one, 2,3- dihydro-N-hydroxy-4-methoxy-3, 3-dimethyl (52.89%) and 1- H-Indole, 1- methyl-2 phenyl- (35.86%) were also reported from the stem extract of *Jatropha podagrica.* The chemical constituent's analysis result of *Jatropha podagrica* was reported in table-8 and their GC-MS chromatogram is presented in Fig-8 table-8&9.

The first compound identified with less retention (16.941 min) was 2-Ethylacridine, Indole-2-one,2-3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl, Benzo(h) quinoline, 2-4-dimethyl whereas 1H-Indole,1-methyl-2-phenyl and 5-Methyl-2-phenylindolizine was the last compound which took longest retention time (17.111 min.) to identify. At (16.941 min) retention time 2-Ethylacridine, Indole-2-one, 2,3- dihydro-N- hydroxyl-4-methoxy-3, 3-dimethyl and Benzo (h) quinoline, 2,4- dimethyl was found to be high (52.89 %) and the lowest percentage (11.25%) was found to be 1H-Indole,1-methyl-2-phenyl- and 5- Methyl -2-Phenylindolizine.

The GC-MS analysis of root extract of *Jatropha glandulifera* confirmed the presence of 13 compounds with retention time. Interpretation of the mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of these 13 compounds 2 compounds were majority present in the root extract of *Jatropha glandulifera* respectively 1- H-Indole, 1- methyl-2-phenyl (8.63%) and cyclotrisiloxane, hexamethyl.

The eleven minor compounds such as tert-Butyl (5-isoprophyl-2-methyl phenoxy) dimethyl silence (8.63%), 1,2,4-Benzenetri carboxylic acids ,4-dodecyl dimethyl ester (9.13%), 5-Methyl-2-Phenylindolizine (9.13%), Benzo (h) quionoline, 2,4-dimethyl-(9.13%), 2-Ethylacridine (28.85%), Methyltris (trimethylsiloxy) silane (38.37 %) Tetra siloxane, decamethyl-(38.37%), Trimethyl

Fig:10 GC-MS chromatogram of root extract *Jatropha glandulifera*



Page: 1

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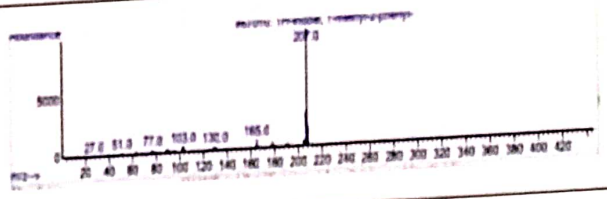
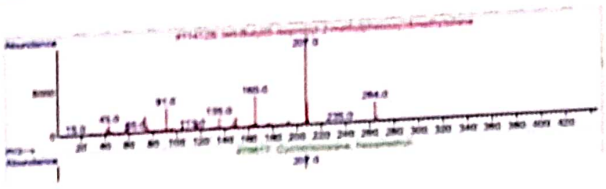
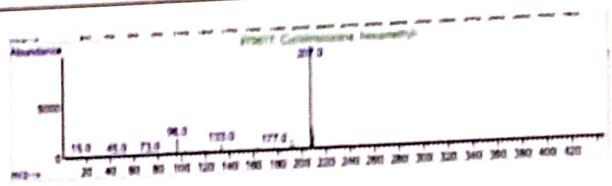
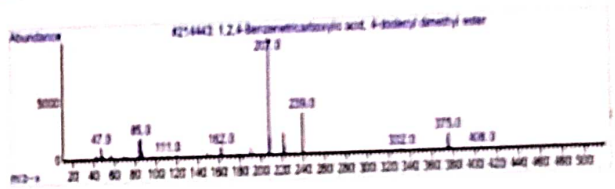
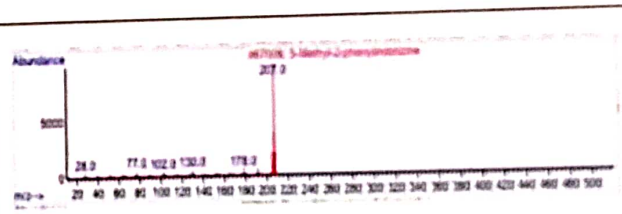
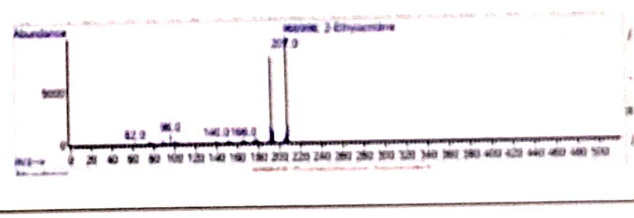
Table:10 ***Jatropha glandulifera*** **root spectrum**

S.No	RT	Name of the compounds	Area%	Mass spectrum
1.	16.951	1H-Indole, 1-methyl-2-phenyl	8.63	
2.	16.951	tert-Butyl(5-isopropyl-2-1methyl phenoxy)dimethyl silane	8.63	
3.	16.951	Cyclotrisiloxane,hexamethyl	8.63	
4.	17.074	1,2,4-Benzene tricarboxylic acid, 4-dodecyl dimethyl ester	9.13	
5.	17.074	5-Methyl-2-phenylindolizine	9.13	
6.	17.074	Benzo(h)quinoline, 2,4-dimethyl-	9.13	

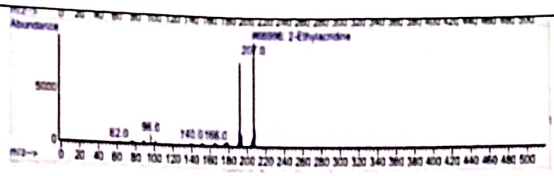
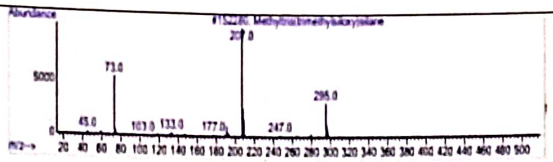
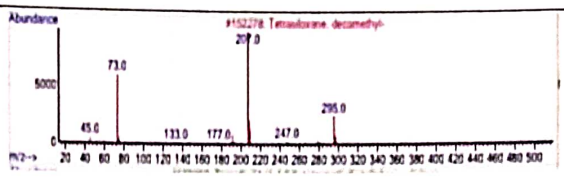
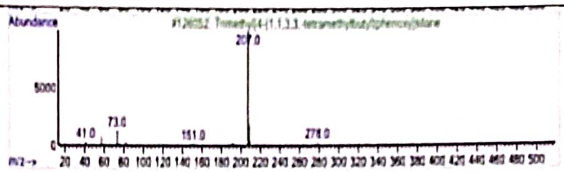
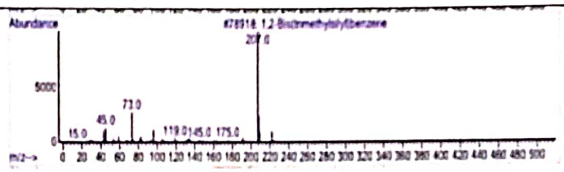
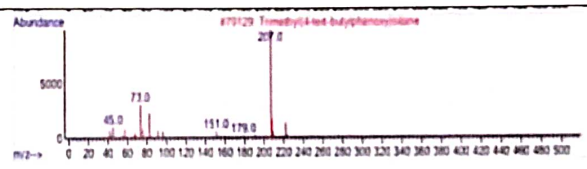
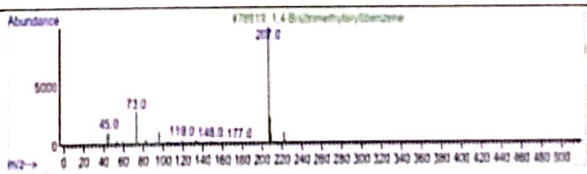
	17.197	2-Ethylacridine	28.85	
	17.509	Methyltris(trimethylsiloxy) Silane	38.37	
	17.509	Tetrasiloxane,decamethyl-	38.37	
).	17.509	Trimethyl[4-(1,1,3,3,-tetramethyl butyl) silane	38.37	
.	17.669	1,2-Bis (trimethyl silyl) benzene	15.01	
.	17.669	Trimethyl (4-tert-butyl phenoxy) silane	15.01	
.	17.669	1,4-Bis (trimethyl silyl) benzene	15.01	

Figure:11 GC-MS chromatogram of stem extract (ethanol) *Jatropha podagrica*

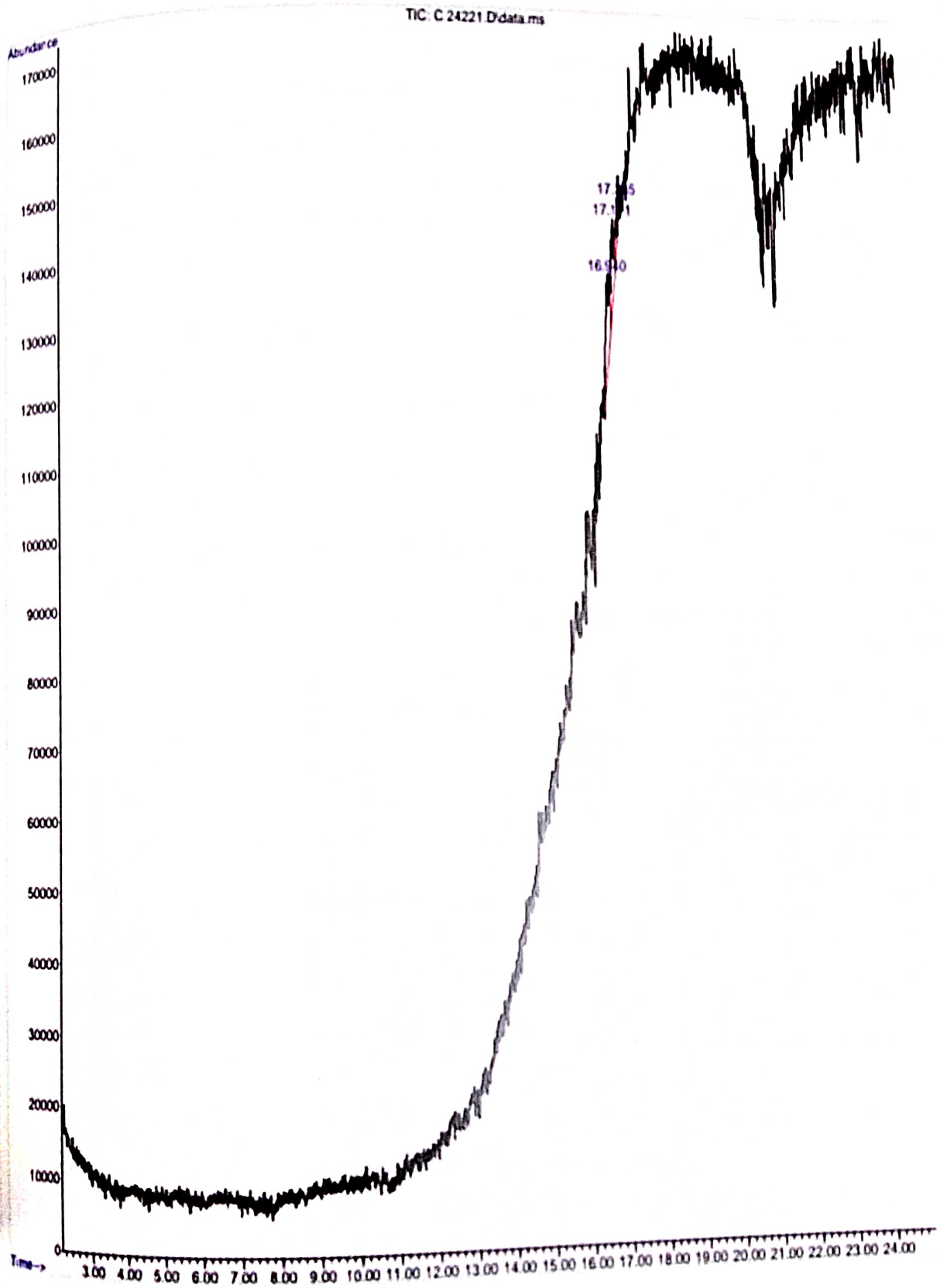
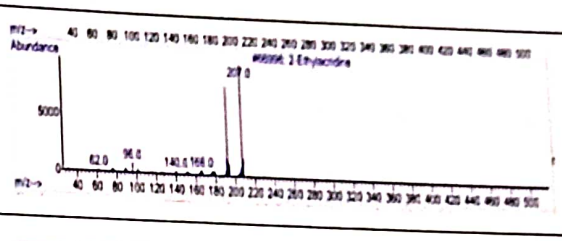
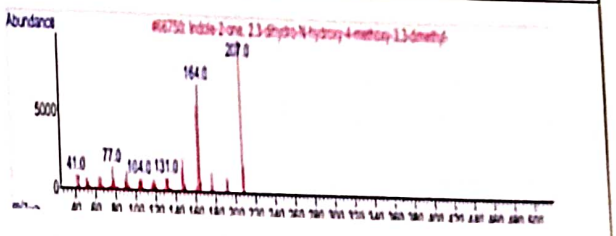
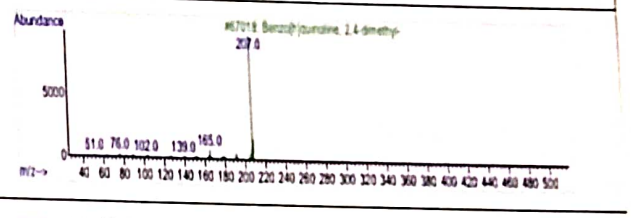
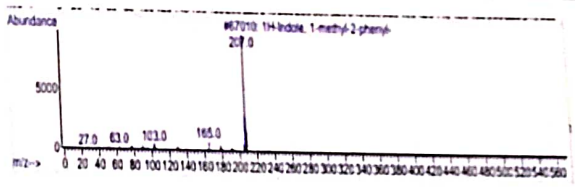
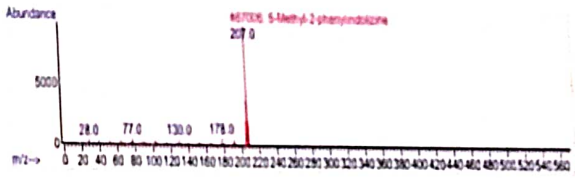


Table:8 Jatropha podagrica stem **mass spectrum**

S.No	RT	Name of the compounds	Area%	Mass spectrum
1.	16.941	2-Ethylacridine	52.89	
2.	16.941	Indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	52.89	
3.	16.941	Benzo[h]quinoline,2,4-Dimethyl-	52.89	
4.	17.111	1H-Indole,1-methyl-2-Phenyl-	35.86	
5.	17.111	5-Methyl-2-Pheylindolizine	35.86	

Jatropha glandulifera **root spectrum**

S. No	R. T	Name of the compound	Area (%)	biological activity
1.	16.951	1H-Indole, 1-methyl-2-phenyl	8.63	Antiviral antiBacterial,
2.	16.951	tert-Butyl(5-isopropyl-2-1methyl phenoxy)dimethyl silane	8.63	Antineoplastic, antimicrobial, HIV activity
3.	16.951	Cyclotrisiloxane,hexamethyl	8.63	Antioxidants
4.	17.074	1,2,4-Benzene tricarboxylic acid, 4-dodecyl dimethyl ester	9.13	Muscle stimulant, kindney functions
5.	17.074	5-Methyl-2-phenylindolizine	9.13	Antimicrobial, anti fungal, antiviral
6.	17.074	Benzo(h)quinoline, 2,4- dimethyl-	9.13	Antiviral, antioxidants
7.	17.197	2-Ethylacridine	28.85	Antibacterial, antineoplastic, antifungal
8.	17.509	Methyltris(trimethylsiloxy) Silane	38.37	AntiViral, antioxidants
9.	17.509	Tetrasiloxane,decamethyl-	38.37	Antifungal
10.	17.509	Trimethyl[4-(1,1,3,3,-tetramethyl butyl) silane	38.37	Antioxidants
11.	17.669	1,2-Bis (trimethyl silyl) benzene	15.01	Antifungal
12.	17.669	Trimethyl (4-tert-butyl phenoxy) silane	15.01	Antibacterial
13	17.669	1,4-Bis (trimethyl silyl) benzene	15.01	Antineoplastic, antimicrobial, HIV activity

Table: 10 Jatropha podagrica stem **mass spectrum**

S. No	R. T	Name of the compound	Area (%)	Biological activity
1.	16.941	2-Ethylacridine	52.89	Antiviral, antineoplastic, anti bacterial
2.	16.941	Indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	52.89	Antineurotic,Cholesterol antigonestic, antiviral
3.	16.941	Benzo[h]quinoline,2,4-Dimethyl-	52.89	Staphylococcus, kidney function stimulant
4.	17.111	1H-Indole,1-methyl-2-Phenyl-	35.86	Antimicrobial activities, antitumour
5.	17.111	5-Methyl-2-Pheylindolizine	35.86	Antineurotic, cholesterol antigonestic, antiviral

[4-(1,1,3,3-tetramethylbutyl) phenoxy] silane(38.37%), 1,2-Bis(trimethysilyl) benzene (15.01%), Trimethyl (4-tert-butyl phenoxy) silane (15.01%) and 1,4-Bis (trimethysilyl)1 Benzene (15.01%)were also reported from the stem extract of *Jatropha glandulifera*. The chemical constituents's analysis result of *Jatropha glandulifera*root were reported in Table 8 and their GC-MS chromatogram is presented in Fig :9, Table-10&11

The first compound identified with less retention (16.951 min) was 1-H-Indole, 1-methyl-2 –phenyl, tert-Butyl (5-isopropyl-2- methyl phenoxy) dimethyl silane, cyclotrisiloxane, hexamethyl- whereas Methyltris (trimethyl siloxy) silane, tetrasiloxane, decamethyl, trimethyl [4-(1,1,3,3-tetramethyl butyl)phenoxy] silane was the last compound which took longest retention time (17.509 min) to identify. At (17.509 min.) (trimethylsiloxy) silane, tetrasiloxane, decamethyl, Trimethyl[4-(1,1,3,3-tetramethyl butyl) phenoxy] silane was found to be high (38.37%) and lowest percentage (8.63%) was found to be 1- H-Indole, 1- methyl-2-phenyl, tert-Butyl (5-isopropyl-2-methyl phenoxy) dimethylsilane, cyclotrisil oxane, hexamethyl, The above mentioned isolated compounds from the root extract of *Jatropha glandulifera*and stem extract of*Jatropha podagrica* have a medicinal important.

Benzo (h) quinolone, 2,4-dimethyl in the stem extract of *Jatropha podagrica* is a main antiviral compound (WWW.Pharmaexpert.ru/pass online predict.php).Quinolines are important compounds because of their bioactive properties and medicinal uses such as antimalarial (Larsen *et al.*, 1996), anti-inflammatory (Chen *et al.*, 2001), antiasthmatic (Roma *et al.*, 2000), antibacterial (Dube *et al.*, 1998) and tyrosine kinase inhibiting agents (Billker *et al.*, 1998).

1,2- Bis (trimethylsilyl) benzene is found in the root extract of *Jatropha glandulifera* is a main antimicrobial compound.Phytocompound 1,2-Bis (trimethylsilyl) benzene at retention time15.651 and 16.055 min have antioxidant, antimicrobial, anticanserous and antitumerous activity (Alok prakash and Suneetha,2014).Tetrasilaxane identified in the ethanolic stem extracts of *Jatropha glandulifera*is a main antimicrobial compound.(Cai *et al.*, 2018).

Cyclotrisiloxane and hexamethyl found in the root extract of *Jatropha glandulifera*are the main antioxidant compounds that help remove harmful toxins and free radicals in the body.(Anju Krishna*et al.*, 2015).

ANTIOXIDENT ACTIVITY

An antioxidant is a molecule capable of showing or preventing the oxidant of other molecules. In a biological system, they protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants terminate the chain reaction by removing free radical intermediates, and inhibit other oxidant reactions by being oxidized themselves. They are believed to play a role in preventing the development of chronic diseases like cancer, heart diseases, stroke, AD, RA and cataracts (chakraborty et al., 2010).

Antioxidant chemicals found in nature inhibit or prevent the oxidation of substrate leading to the formation of reactive oxygen species and reactive nitrogen species and thus protect the biological system (Hwang *et al.*, 2007).

Fruits and vegetables are endowed with antioxidants and consumption of these, prevent and protects from oxidative stress-related diseases, inflammatory diseases viz. , arthritis, autoimmune disease, carcinogenesis, neurodegenerative diseases, inflammatory diseases, cardiovascular disorders etc. several food industries use butylated hydroxyl, butylated hydroxynol toluene and tertiary butyl hydroquinone, the common synthetic antioxidants for preventing lipid oxidation in food products while processing and storage. These synthetic antioxidants have been suspected to be carcinogenic and hence their use as food ingredients has been prohibited (hung and Wang 2004). Natural antioxidants comprised non –detrimental decimal combinations are considered to be rather safer for use in food products. Further, uncared wastes if exploited as a resource of antioxidants, will be more beneficial to human kind and protect the environment. Flavonoids are water soluble polyphenolic molecules with antioxidant activity which has many beneficial effects on the cardiovascular system (Evans ,1989).Vitamin C acts as a ROS scavenger, thus potentially protecting cells from harmful oxidative products (Fossati et al.,). Vitamin E supplement elevates the activities of antioxidant tazymes(Kiron et al., 2004).,

DPPH FREE RADICAL SCAVENGING ACTIVITY

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of a specific compound or plants extracts (Wei et al, 2012) DPHH solution has a strong absorption band at 517nm appearing as a deep violet colour, The absorption vanishes and the resulting decolourization is stoichiometric

DPPH FREE RADICAL SCAVENGING ACTIVITY

Anti-oxidant activity in aqueous extract of *Jatropha podagrica and Jatropha glandulifera* (table 14)

S.No	Aqueous Extract	DPPH free radical assay %
1	*Jatropha podagrica*	25.43
2	*Jatropha glandulifera*	28.33

Anti-oxidant activities in aqueous extract of *Jatropha podagrica and Jatropha glandulifera* (figure 10)

with respect to the degree of reduction. The stem extract of *Jatropha podagrica* androot extract of *Jatropha glandulifera* was able to reduce stable DPPH radical to yellow colour diphenyl picryl hydrazine. The degree of reduction in absorbance is the reflection ofthe radical scavenging power of the compound.

The antioxidant activity of aqueous stem extracts of *Jatropha podagrica* androot extract *ofJatropha glandulifera* was evaluated by using DPHH scavenging assay. Aqueous extract using *Jatropha podagrica stem* has higher scavenging activity (28.33%) followed by *Jatropha glandulifera*root(25.43%), as shown in figure (10) and Table (14)

This result indicated aqueous stem extract of the*Jatropha podagrica* plant shows higher scavenging activities. It has been reported that the antioxidant activity of aqueous stem extract of *Jatropha podagrica and* root extract of *Jatropha glandulifera* was due to the presence of phenolics and it is responsible for redox properties, which allow them to act as reducing agent, hydrogen donors ringlet oxygen quenchers. (Arasali and kadimi 2009)

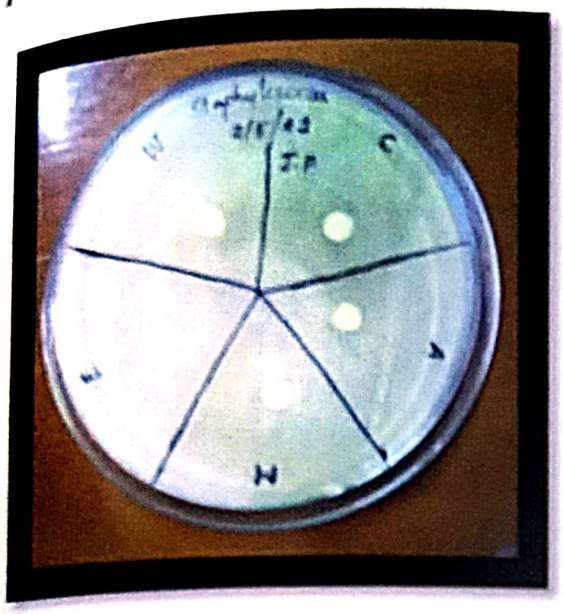
ANTIBACTERIAL ACTIVITY

In the present study, the antibacterial activity of different solvents (acetone, ethanol, methanol and water) using *Jatrophapodagrica*stem and *Jatropha glandulifera* root were tested against four human pathogenic bacteria (*Bacillus substilis*, *Escherichia coli*, *Staphylococcus sp, Vibiryo cholerae)* presented in table (15 & 16). The diameter of the inhibition zones against these species ranged from (3 to 11)

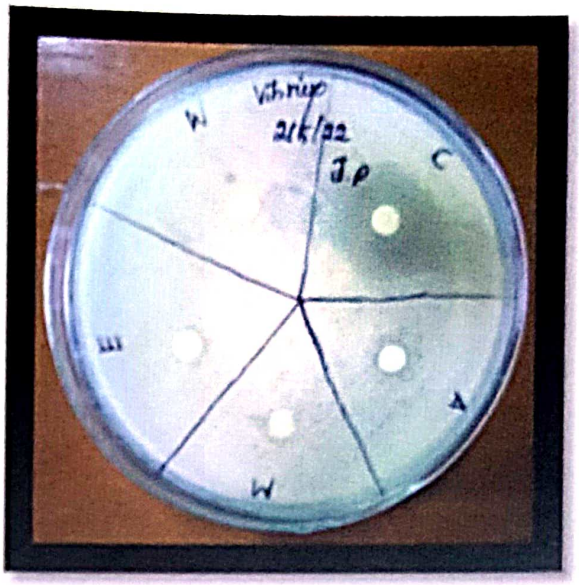
The different solvents (acetone, ethanol, methanol and water) extract of *Jatropha podagrica*stem exhibited maximum activity against different bacterial species, *E. Coli* (1-11mm), *Bacillus substilis* (3-10mm), *Vibriocholorae*, (1-14mm) *staphylococcus sp (*3-8mm),

The different solvents (acetone, ethanol and water) extracts of *Jatropha podagrica*stem exhibited maximum activity against different bacterial species, *E.coli* (3-11mm), *Bacillus substills*(4-9mm), vibrio cholera(1-13), *Staphylococcus sp*(2-15mm) inhibition zone .(plate:3),(Fig 11).

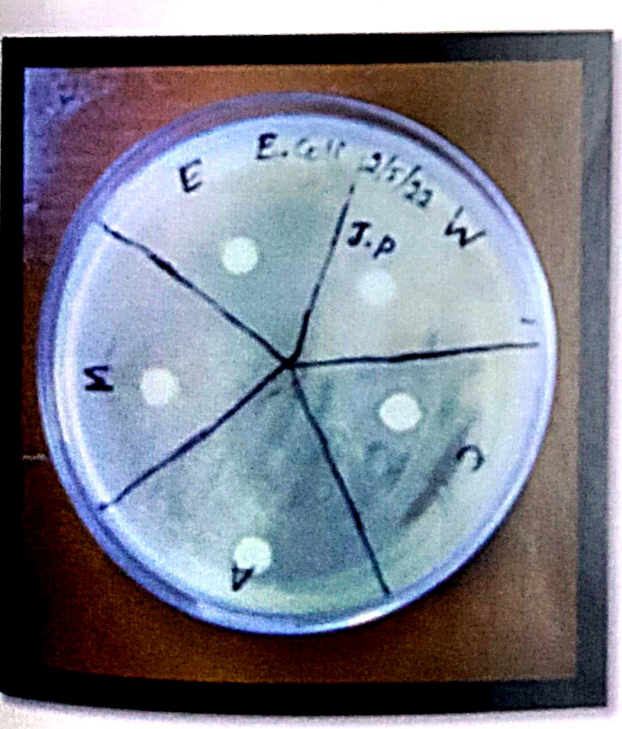
Antibacterial activity of different solvents extract of *Jatropha podagrica*(plate 3)



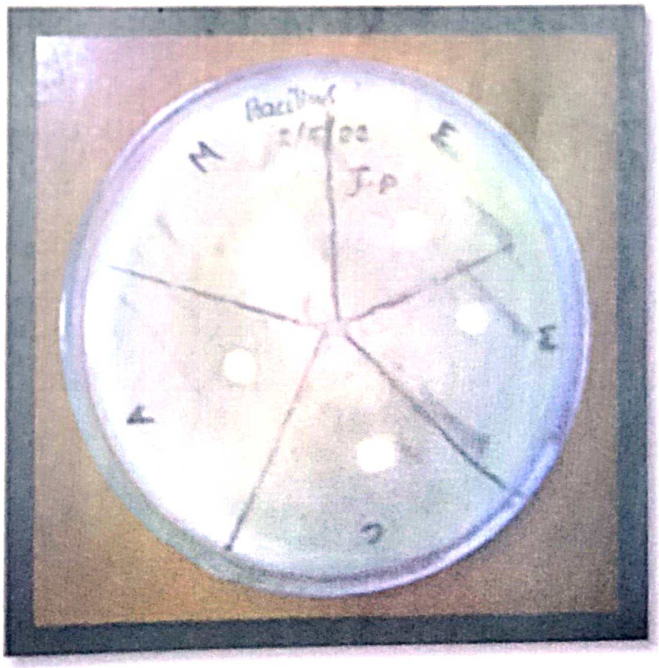
Staphylococcus sp.



Vibrio cholerae

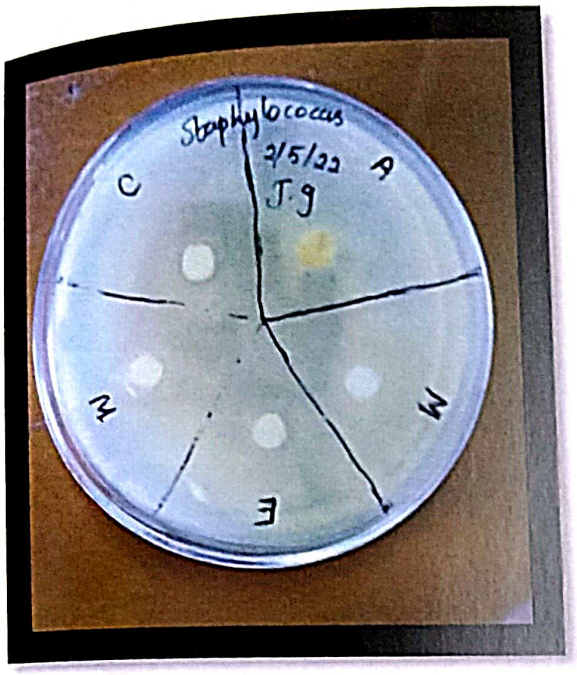


E.coli

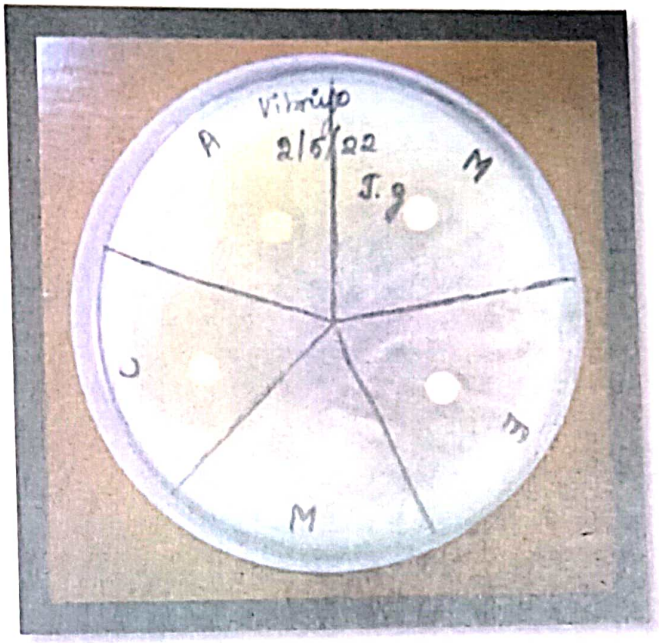


Bacillus

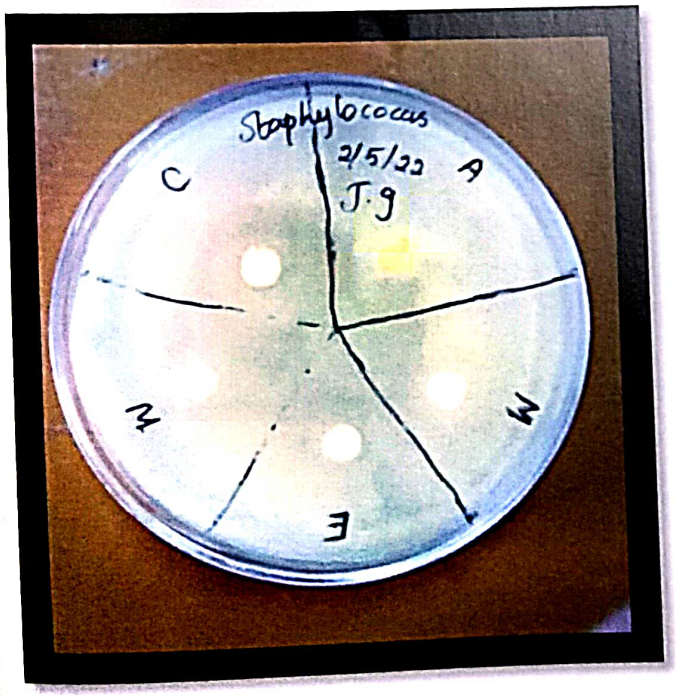
Anitibacterial activity of different solvent root extract of *Jatropha glandulifera*(plate 4)



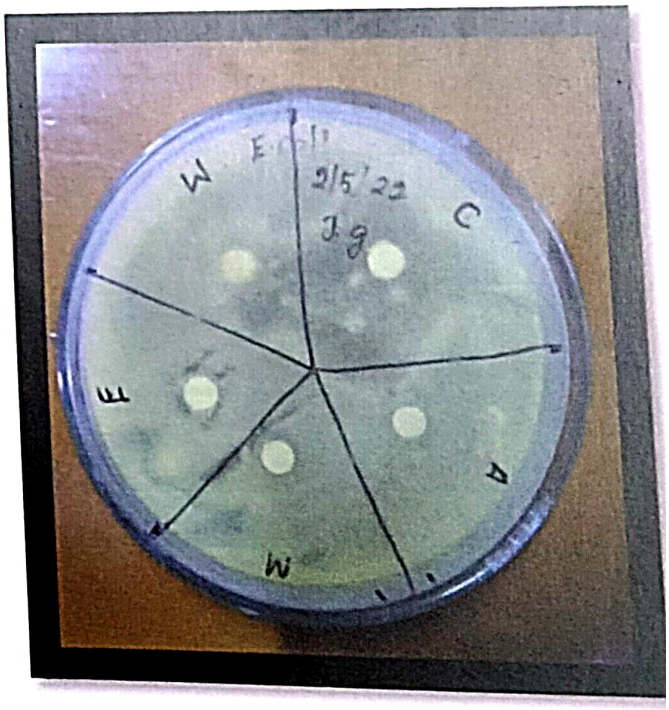
Staphylococcus aureus



Vibrio cholerae



Bacillus substilis



E.coli

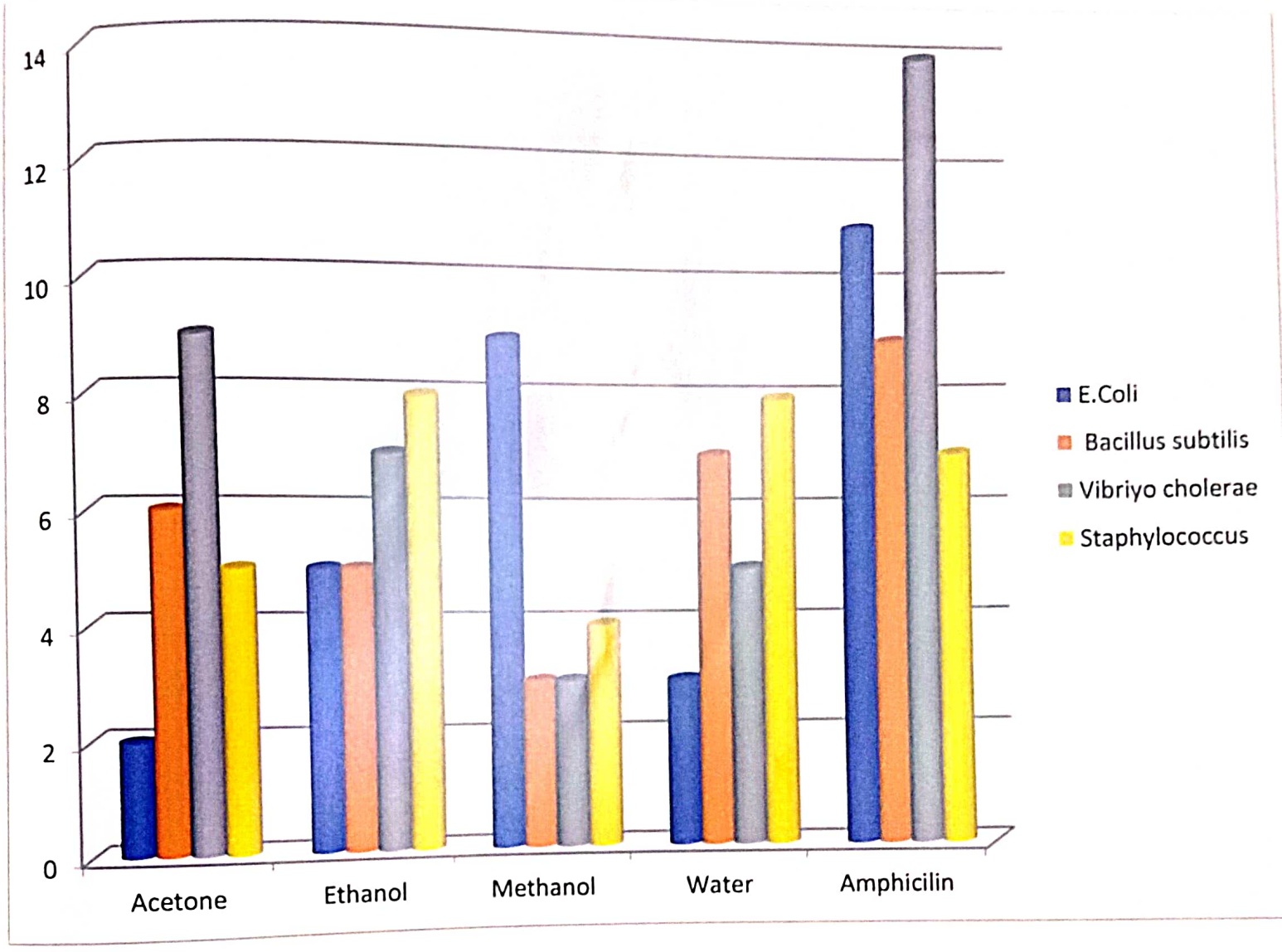
Table 15: Antibacterial activity - stem extract of *Jatropha podagrica* with different solvent against human pathogen

Samples	*Jatropha podagrica*				
Microorganisms	Acetone	Ethanol	Methanol	Water	Amoxicillin
E.coli	3	7	8	6	9
Bacillus subtilis	4	3	3	5	6
Vibrio cholerae	9	11	7	3	10
Staphylococcus	3	3	4	3	5

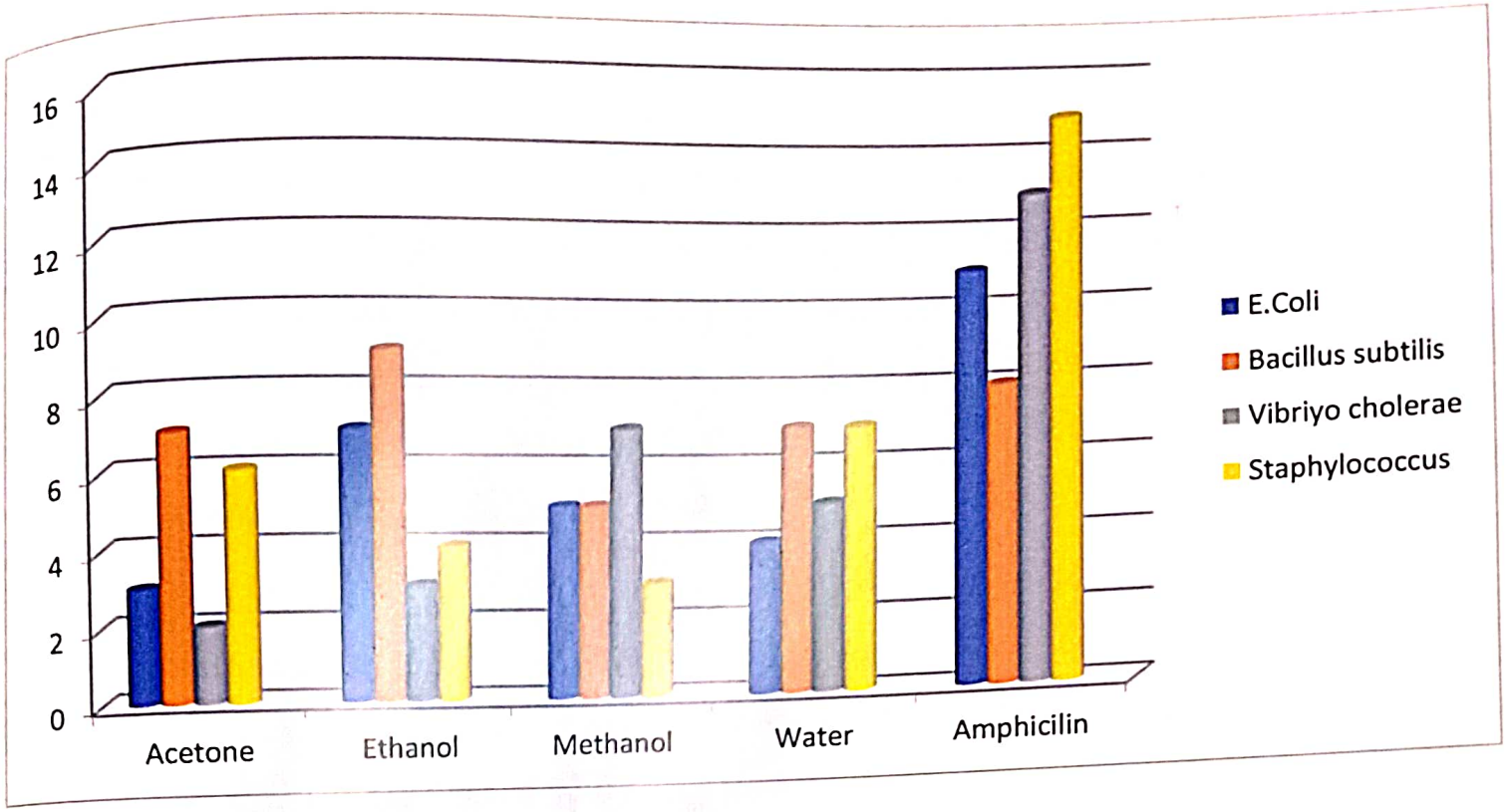
Table 16: Antibacterial activity – root extract of *Jatropha glandulifera* with different solvent against human pathogen

Samples	*Jatropha glandulifera*				
Microorganisms	Acetone	Ethanol	Methanol	Water	Amoxicillin
E.coli	3	4	7	5	8
Bacillus subtilis	4	6	10	3	11
Vibrio cholerae	3	5	4	4	6
Staphylococcus	3	4	5	3	5

Anitibacterial activity of *jatropha podagrica* with different solvent against human pathogen(figure 11)



Anitibacterial activity of jatrophaglandulifera plant extract with different solvent against human pathogen (figure 12)



The different solvents (acetone, ethanol, methanol and water extracts of *Jatropha glanduliferaroot*exhibited maximum activity against different bacterial species, *E.coli*(3-9mm),*Bacillus substilis*(3-10mm), *vibrio cholera* (3-6mm), *Staphylococcus sp*(5-8mm) inhibition zone.(plate:4),(Fig 12).

The maximum activity was found to be an 11mm zone of inhibition obtained by ethanol stem extract of *Jatropha podagrica*against *Vibrio cholerae*. The ethanol extract of the stem of*Jatropha podagrica*exhibited high antibacterial activity against *Vibrio cholorae*, The diameter of the inhibition zone was 11mm. The ethanol extract of *Jatropha podagrica*stem exhibited more or less same zone of inhibition compared to standard antibiotics Amoxicillin. Maximum bacterial effect was found in *vibrio cholerae* for ethanolic stem extracts of *Jatropha podagrica* .

The maximum activity was found to be a 10 mm zone of inhibition obtained by ethanol extract of *Jatropha glandulifera* against*Bacillus substilis*. The ethanol extract of *Jatropha glandulifera* exhibited high antibacterial activity against *Bacillus substilis*, The diameter of inhibition zone was 10mm. The ethenolextract of *Jatropha glandulifera* exhibited more or less the same zone of inhibition compared to standard antibiotics Amoxicillin. Maximum bacterial effects were found in *Bacillus substilis* for ethanol extracts of*Jatropha glandulifera.*

The antibacterial activity of *Jatropha podagrica*stemand *Jatropha glandulifera* root extract were nearly similar to Amoxicillin. Maximum bacterial effects were found in *Vibrio cholerae* in *Jatropha podagrica* stem and *Bacillus substilis* in *Jatropha glanduliferaroot* extract. The effects were significant in *Jatropha podagrica* and *Jatropha glandulifera*. The antibacterial activities of the stem of *Jatropha podagrica* androot of *Jatropha glandulifera* may be due to the presence of various phytochemicals which are known to be synthesized by plants in response to microbial infection (Cowan,1999). The mechanism of action of saponins as antimicrobial agents may be due to mebranolytic properties, rather than simply altering the surface tension of the extracellular medium(Killeen,1998). In our study stem of *Jatropha podagrica* and root of *Jatropha glandulifera* showed the extracellular saponins. The presences of tannins were also reported in *Jatropha podagrica* and*Jatropha glandulifera* .

The antibacterial activity of tannins may be due to their intercalation with enzymes, cell envelope transport proteins and also complex with cell wall polysaccharides(Ya *et al.*,1998). Hence these plants stand as a potential candidate as a source of ingredients in drug formulation for the treatment of bacterial infection.

SUMMARY AND CONCLUSION

Plants have been an important source of medicine for thousands of years. Medicinal plants are a source of great economic value. The plants *Jatropha podagrica and Jatropha glandulifera* were collected from Thoothukudi, Tamil Nadu. For the present study, the two taxa *Jatropha podagrica* (stem) *andJatropha glandulifera* (root) *were* selected. Both are known plants of the family Euphorbiaceae. The plant *Jatropha podagrica* exhibits various biological activities like antiinsect, molluscicidal, antitumour and antimicrobial (Aiyelaagbe *et al.*, 2007). Traditionally used to treat skin infections, nosocomial infection, gonorrhoea, fever, jaundice, pyretic, diuretic, choleretic and purgative. The Seed oil of *Jatropha glandulifera* is used in chronic ulcerations, foul wound ringworm, rheumatism and paralysis. Plant juice is used to remove the film from the eyes. Water extract of the root is given to children suffering from abdominal enlargement. [Senthilkumar *et al.*, 2006]. The stem of *Jatropha glandulifera* is used to arrest bleeding from wounds, cuts and ulcers [Jothi *et al.*, 2008]. The roots of *Jatropha glandulifera* are boiled and taken to treat diabetics [Kadhirvel *et. al.*, 2010]. The medicinal effects of plants are considered to be due to metabolites, especially secondary compounds, produced by plants. The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, saponins, terpenoids, phenol and tannins. The preliminary phytochemical tests are helpful in finding chemical constituents in the plants materials that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds. The information obtained

from the preliminary phytochemical screening will be finding out the genuinity of the drug.

In this study, total phenol, flavonoid, tannin, vitamin C and vitamin E content were quantitatively analyzed in *Jatropha podagrica* stemand *Jatropha glandulifera* root using spectrophotometric methods. The results of this study showed that the*Jatropha podagrica stem* has a significant amount of phenol, flavonoids, tannins, vitamin C and vitamin E and ascorbic acids compared to*Jatropha glandulifera* root.

The FTIR spectroscopy analysis of *Jatropha Podagrica* stem obtained peaks at 443.6 cm^{-1}, 652.86 cm^{-1}, , 924.8 cm^{-1}, 1244.97 cm^{-1}, 1745.46 cm^{-1}, 3834.22 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for C-I in Aromatic, C-BR in Alicyclic Axial, N-O in Stretch, C-O-C in Asymmetric, C=O in Ester, N-H in Urethanes.

The FTIR spectroscopy analysis of *Jatropha glandulifera* root obtained peaks at 516.89 cm^{-1}, 661.54 cm^{-1}, 946.02 cm^{-1}, 1160.1 cm^{-1}, 1318.25 cm^{-1}, 2849.63 cm^{-1}, 3503.45 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for C-Br in Aromatic, N-H in Amines, C=O in Esters, N-O in Stretch, C=C in Symmetric stretch, C-O in Hydroxyl group, H-C-H in Asymmetric Stretch, O-H in Medium.

From the spectral data presence of C-I, C-BR, N-O, C-O-C, C=O, N-H, N-O, C=C, H-C-H and O-H were identified. These bonding are responsible for the presence of Aromatic, Alicyclic Axial, Stretch, Asymmetric, Ester, Urethanes, Symmetric stretch, Asymmetric Stretch, Medium. Carboxylic acid present in the medicinal plant serves as the main pharmaceutical product in curing ulcers, jaundice, headache, stomatitis, hemicranias, fever, pain in the liver and treatment of rheumatic joint pain. Amides, amine and amino acids are the main groups, involved in protein synthesis. The study revealed that the stem of *Jatropha Podagrica* and root of *Jatropha Glandulifera* contains a considerable amount of

secondary metabolites and it may be considered in the future to be used in human disease management.

The GC-MS analysis of stem extract of *Jatropha podagrica* confirmed the presence of 5 compounds with retention time. Interpretation of the mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of these 5 compounds, 3 compounds are majorly present in the stem extract of *Jatropha podagrica* respectively 2-Ethylacridine(52.89%), Benzo(h)quinoline,2,4-dimethyl(52.89%) and 5-methyl 2- phenylindolizine (35.86 %).

The GC-MS analysis of root extract of *Jatropha glandulifera* confirmed the presence of 13 compounds with retention time. Interpretation of the mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of these 13 compounds 2 compounds were majority present in the root extract of *Jatropha glandulifera* respectively 1- H-Indole, 1- methyl-2-phenyl (8.63%) and cyclotrisiloxane, hexamethyl. The above mentioned isolated compounds from the root extract of *Jatropha glandulifera* and the stem extract of *Jatropha podagrica* have medicinal importance.

Benzo (h) quinolone, 2,4-dimethyl in the stem extract of *Jatropha podagrica* is a main antiviral compound (WWW.Pharmaexpert.ru/pass online predict.php). Quinolines are important compounds because of their bioactive properties and medicinal uses such as antimalarial (Larsen *et al.*, 1996), anti-inflammatory (Chen *et al.*, 2001), antiasthmatic (Roma *et al.*, 2000), antibacterial (Dube *et al.*, 1998) and tyrosine kinase inhibiting agents (Billker *et al.*, 1998).

1,2- Bis (trimethylsilyl) benzene is found in the root extract of *Jatropha glandulifera* is a main antimicrobial compound. Phytocompound 1,2-Bis (trimethylsilyl) benzene at retention time15.651 and 16.055 min have antioxidant, antimicrobial, anticanserous and antitumerous activity (Alok prakash and Suneetha,2014). Tetrasilaxane identified in the ethanolic root extracts of *Jatropha glandulifera*is a main antimicrobial compound. (Cai *et al.*, 2018).

Cyclotrisiloxane and hexamethyl found in the root of *Jatropha glandulifera* are the main antioxidant compounds that help remove harmful toxins and free radicals in the body. (Anju Krishna*et al.*, 2015).

The antioxidant or free radical scavenging activity of plant extracts of these selected medicinal plants are investigated by using methods like DPPH scavenging activity. The root extracts of *Jatropha glandulifera*and stem extract of *Jatropha podagrica* show maximum antioxidant activity and these extracts are further subjected for antimicrobial studies.

The different solvent extracts of*Jatropha glandulifera root*and*Jatropha podagrica* stem and Amoxicillin were used for antibacterial studies against human pathogenic bacteria, *Bacillus substilis, Escherichia coli, Staphylococcus, Vibrio cholerae.*

The maximum activity was found to be an 11mm zone of inhibition obtained by ethanol stem extract of *Jatropha podagrica* against *Vibrio cholerae*. The ethanol extract of the stem of *Jatropha podagrica* exhibited high antibacterial activity against *Vibrio cholorae*, The diameter of the inhibition zone was 11mm. The ethanol extract of *Jatropha podagrica* stem exhibited more or less same zone of inhibition compared to standard antibiotics Amoxicillin. Maximum bacterial effect was found in *vibrio cholerae* for ethanolic stem extracts of *Jatropha podagrica* .

The maximum activity was found to be a 10 mm zone of inhibition obtained by ethanol extract of *Jatropha glandulifera* root against *Bacillus substilis*. The ethanol extract of *Jatropha glandulifera* root exhibited high antibacterial activity against *Bacillus substilis*, The diameter of inhibition zone was 10mm. The ethenol extract of *Jatropha glandulifera* root exhibited more or less the same zone of inhibition compared to standard antibiotics Amoxicillin. Maximum bacterial effects were found in *Bacillus substilis* for ethanol extracts of *Jatropha glandulifera* root.

The antibacterial activity of stem of *Jatropha podagrica*and root of *Jatropha glandulifera* extract were nearly similar to Amoxicillin. Maximum bacterial effects

were found *Vibrio cholerae* in *Jatropha podagrica* stem extract and *Bacillus substilis* in *Jatropha glandulifera* root extract. The antibacterial activity of various phytochemicals which are known to be synthesized by plants in response to microbial infection.

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BIODIVERSITY OF SEAWEEDS FROM FIVE LOCALITIES IN GULF OF MANNAR COSTAL WATERS OF TAMILNADU

A Short-Term Field Project Submitted to

ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVEI

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN BOTANY

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CERTIFICATE

It is certified that this short-term field project work entitled **"BIODIVERSITY OF SEAWEEDS FROM FIVE LOCALITIES IN GULF OF MANNAR COSTAL WATERS OF TAMILNADU"** submitted to **St. Mary's College (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfillment of the requirements for the degree of **Master of Science in Botany** and is a record of work done in the Department of Botany, **St. Mary's College (Autonomous)**, Thoothukudi during the year 2021-2022 by the following students

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CONTENT

INTRODUCTION

Vegetation in sea is more primitive in the evolutionary scale than that of land. India is one of the richest countries for biodiversity of seaweeds in the world. A myriad of economically and environmentally valuable species are present in the southern coastal region of Tamil Nadu because of different ecosystems. In comparison with the Gulf of Kutch in Gujarat, Lakshadweep in Kerala, Andaman and Nicobar Islands in India, Gulf of Mannar is the major coral reef region that located in the southern part of Tamil Nadu and has a rich diversity of seaweeds.

Seaweeds are the important renewable living resources for human welfare and are highly diversified tropical species commonly found on rocks, pebbles, dead corals and shells with a maximum depth of 180m at the bottom of the shallow coastal region. Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae) are the predominant classes with 900, 1500 and 4000 species respectively in nature. Seaweeds are highly potential of biomass and widely used in various forms and also used as direct food. Products of macroalgae are defensive against various diseases and enhance health.

Seaweeds resources are among the most productive life supporting coastal ecosystems. They also considered as ecologically important component in the marine ecosystems as they contribute substantially to marine primary production and provide habitat for near-shore benthic communities. Seaweeds are also used in animal fodder, used as fertilizer for agriculture and used as food, gels and act as emulsifiers in various sectors. Macroalgae have interesting physiological properties when compared to higher plants.

Moreover, many new records of seaweeds have been added in the recent years by various researchers **(Rani and Bast, 2019)** to the Indian seaweed flora. Even now also, many of the remote localities of the Indian coastline are under explored or unexplored and the actual number of seaweed species may upsurge. Therefore, the Indian coastline exhibits significant diversity of seaweeds.

Seaweeds are under threat in developing countries, where they are being disturbed by a variety of human activities. The decline in algal diversity in recent decades is a matter of great concern **(Hopper *et al*., 2005).** The algal diversity is being lost by natural threats like storms and tidal waves in some years and during monsoon periods the excess fresh water runoff kills many of the fauna and flora in semi enclosed bays and lagoons by lowering salinity and depositing large amounts of sediments and nutrients. The increasing tourist traffic also is one of the causes of disturbing the biodiversity. There is tremendous change in the marine environment due to urbanization and construction activity in addition to industrial pollution and illegal mining of coral reefs in the coastal areas. The biodiversity and density of marine macro algae of Gulf of Mannar have come down gradually over a period of years **(Krishnamurthy, 2006; Kannan and Thangaradjou, 2006; Kaliaperumal, 2007).**

Increasing concern on destruction of seaweed resources due to anthropogenic and climatic disturbances makes it necessary to study their diversity and species richness. Although systematic studies on marine algae and their distribution are known from different coastal parts of India, however not much published information are available about the seaweeds of selected coastal region in the present study. Therefore, in the present

investigation, an attempt has been made to document the seaweed diversity in Gulf of Mannar.

REVIEW OF LITERATURE

The Gulf of Mannar including 21 islands which are situated between Rameshwaram (N 09°13’34.7’’E 078°47’03.2’’) and Kanniyakumari (N 08°08’30.8’’E 077°18’09.7’’) on the south-eastern coast of the country of India. This region has rich diversity of seaweeds due to less pollution, moderate water current and shallow water depth. In Gulf of Mannar coast, seaweeds floral diversity was studied by several researchers. The biodiversity of seaweeds in Gulf of Mannar is due to the large extent of coral reef which provide suitable substrate for its growth**.**

Kalimuthu *et al.* (2000) indicated the availability of 20 species of brown seaweeds at Mandapam. **Kannan and Thangaradjou (2006)** reported a sum of 117 seaweed species consisting of 31 green, 32 brown and 54 red algae from Gulf of Mannar. The rich algal flora (200 species) recorded in Gulf of Mannar in 1970s have become scarce (80 species) after 1980 due to indiscriminate collection of algae in the region. The species which are endangered and facing extinction in that region **(Kaliaperumal, 2007).**

India is endowed with wide range of algal diversity with the country coastal line of 8129 km enjoying the distinct range of habitats supporting rich seaweed biodiversity **(Joshi, 2012). Sathianeson and Samuel (2012)** recorded 32 seaweed species from the Kudankulam region of Gulf of Mannar. Studies carried out to determine seaweed diversity from 42 Indian coastal stations and 14 Gulf of Mannar islands showed a total of 282 seaweed species **(Anon, 2012).** Among these, 80 species were Chlorophyta, 56 species were Phaeophyceae and 146 species were Rhodophyta. The genus *Caulerpa* was

represented by the highest number of species (24) among the green algae, followed by *Codium* with seven species and *Halimeda* and *Ulva* with six species each.

Mary *et al*. (2013) identified 90 seaweed species from the Hare Island in Gulf of Mannar. **Cosman *et al*. (2013)** reported the richness of seaweeds in Muttom coastal waters of southwest coast of India. **Janet Rani** *et al*. **(2013)** studied on the distribution of seaweeds in five selected stations viz., Arockiapuram, Kootapuli, Uvari, Manapad, Punnakayal in the south-east coast of Tamil Nadu. **Sahayaraj *et al*. (2014)** recorded 57 seaweed species from the southern Tamil Nadu. In Gulf of Mannar, the recent survey by Gulf of Mannar Biosphere Trust and **Sahayaraj *et al*. (2014)** reported the availability of ten and nine species of brown seaweeds respectively. **Canciyal *et al.* (2014)** reported a total 86 seaweeds including 32 Rhodophyceae, 27 Chlorophyceae and 27 Phaeophyceae in Tuticorin coast.

Rajusaranam *et al*. (2015) studied seaweed distribution and diversity on the intertidal rocks at Nochiyurani Coast of Gulf of Mannar. The Indian coastlines exhibit very diverse coastal habitats and support 865 taxa of seaweeds **(Rao and Gupta, 2015)**, comprising 442 taxa of Rhodophyceae under 151 genera, 212 taxa of Chlorophyceae under 46 genera and 211 taxa of Phaeophyceae under 50 genera. The Manapad coastal region was recorded with 20 seaweeds **(Doss and Rukshana, 2016).** Perusal of literature pertaining to the works carried on Indian seaweeds revealed that among various maritime states, Tamil Nadu coast showed the highest diversity of seaweeds with 282 species **(Ganesan *et al*., 2019). Mantri *et al*. (2019)** pointed out the gradual increase in diversity of seaweeds from the Indian waters based on the checklists published starting from 167 species in

1970 **(Krishnamurthy and Joshi, 1970)** to 865 in 2015 **(Rao and Gupta, 2015)** and the number of new seaweed taxa reported to 1800 in 2019.

STUDY AREA

Idindakarai (08°12’N, 77°14’E) is a coastal village located about 40 km from Tiruchendur on the way to Cape Comorin. Idindakarai has plenty of rocks that are completely submerged and exposedonly during low tides on one side and rocks that are partially submerged even during high tides on other side. All these rocks host abundant algal vegetation.

Hare Island (**08°44’N, 78°10’E)** is also known as Pandiyan Thivu or Light House Island and is situated 4.5km away from Tuticorin Port Beach with a variety of substrata-rocky, silty, muddy and sandy. Hare island forms a part of the Gulf of Mannar National Park. With an area of 1.29 square kilometers, Hare Island is the largest island in the Gulf of Mannar.

Tharuvaikulam Beach (**08°53’N, 78°10'E)** is located in Tharuvaikulam village in Thoothukudi District. It is calm and sandy beach. In olden days, people walked all the way to Tuticorin by the shore. Tharuvaikulam is located 10km from the town Tuticorin.

Mandapam village is located in Ramanathapuram taluka of Ramanathapuram district in Tamil Nadu, India. It is situated 37km away from Ramanathapuram. Mandapam is situated (**09°16’N, 79°9'E**) on a narrow tongue of land projecting from the southern part of the east coast of India. It is characterized by natural coral reefs.

The coast of **Rameswaram** is the part of Gulf of Mannar Biosphere Reserve, is situated in between the latitude of **9º11’00’N and 9º 19’16’N and the longitude of 79º 10’23’E and 79º 24’00’E.** The coast of Rameswaram encompasses 7 small islands located

an average distance of 4km away from the mainland. These islands are built up of calcareous framework of dead corals and coral reefs. The area is endowed with a combination of ecosystem including mangroves, coral reefs, sea grass and seaweeds.

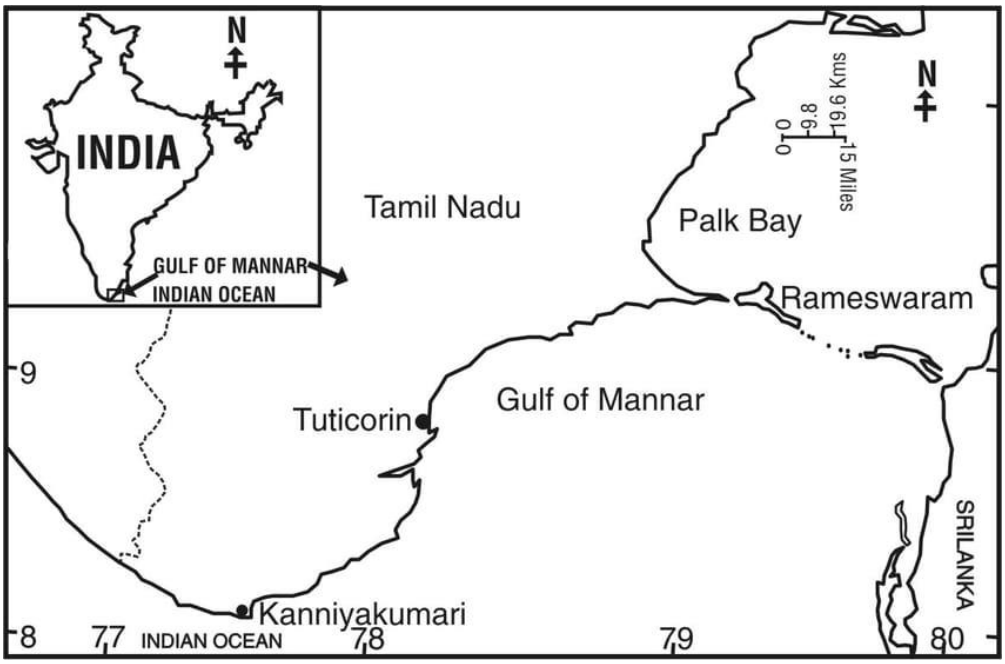


Figure 1: Study Area

METHODOLOGY

The present study was conducted for a period of 3 months (February 2022 to April 2022) to evaluate the seaweed diversity. The study area includes coastal regions of three districts of TamilNadu such as Ramanathapuram, Thoothukudi and Tirunelveli**.** A total of 5 localities (Rameshwaram, Mandapam, Hare Island, Taruvaikulam and Idindakarai) were selected from these three districts and the seaweeds were recorded during the study period. Macroalgae samples were collected from the submerged marine rocks, soft substratum during low tide in theintertidal and sub-tidal regions. Collections were made during dawn and morning hours where the vegetation was discontinuous and also occurs in patches. Fresh samples were preserved in 4% formalin. Voucher specimens and herbarium sheets were prepared and deposited in Botany Laboratory, St. Mary's College (Autonomous), Thoothukudi. Algal samples were micro photographed using Olympus camera (Model E-420) (Olympus Image Corporation, Japan). The algal specimens were identified at Botanical Survey of India (BSI), Coimbatore. The identification was also done using the book Phycolgia. Latitude and longitudes of the study areas were recorded using GPS- map and is given in the **Table 1.**

DISTRIBUTION AND DIVERSITY OF MARINE ALGAE

The diversity study was carried out from the five localities of Gulf of Mannar such as Mandapam, Rameshwaram, Hare Island, Tharuvaikulam and Idindakarai belonging to 3 districts namely Ramanathapuram, Tuticorin and Tirunelveli **(Table 1).** A total of 82 species of macro algae were recorded from the study area of Gulf of Mannar belonging to the three classes such as Chlorophyceae, Phaeophyceae and Rhodophyceae **(Table 2).** The present study revealed the availability of 27 species of green and brown seaweeds and 28 species of red seaweeds from the five selected locations of Gulf of Mannar **(Figure 2a).**

Among the 5 stations, maximum number of 31 species belonging to 18 genera were recorded in Hare Island, Tuticorin and minimum number of 7 and 6 genera were recorded in Rameshwaram. **(Tables 3 & 4).** A total of 14 genera and 16 species from Idindakarai, 15 genera and 19 species from Mandapam and 7 genera and 9 species were recorded from Tharuvaikulam **(Figure 2b & Table 4).** The number of species varied from one station to another among the 5 stations studied. Tuticorin showed highest species diversity (31 species) and Rameshwaram showed low species diversity **(Table 4).**

Maximum number of green algae (12 taxa) and brown algae (12 taxa) was recorded in Hare Island followed by 7 green algae in Idindakarai. Similarly, the highest number of red algae (10 taxa) was noticed in Mandapam followed by 7 red algae in Hare Island **(Table 4).**

TABLE 1: LIST OF THE SELECTED LOCALITIES IN GULF OF MANNAR REGION FOR MARINE ALGAE COLLECTION

S. NO.	NAME OF THE PLACE	LATITUDE	LONGITUDE	MONTH OF COLLECTION
1	Mandapam	$9^0 16^{'}$N	$79^0 9^{'}$E	March
2	Rameswaram	$9^0 13^{'}$N	$78^0 47'$E	March
3	Tharuvaikulam	$8^0 53^{'}$N	$78^0 10'$E	February
4	Hair Island	$8^0 44^{'}$N	$78^0 10'$E	February
5	Idindakarai	$8^0 12^{'}$N	$77^0 44'$E	April

The percentage of distribution of marine algae recorded in the study area is presented in **Figure 3**. In the present study, the maximum percentage of Chlorophyceae members was noticed in Idindakarai and minimum percentage of green algae was observed in Rameshwaram. In contrast to the previous data, the maximum percentage of Phaeophyceae members was noticed in Rameshwaram and minimum percentage of brown algae was observed in Idindakarai. Mandapam constituted the maximum percentage of red seaweeds and Hare Island had the minimum percentage of red algae. In general, Gulf of Mannar is characterized by hot and arid climate most of the year and enriched with seaweeds, seagrass, coral reef, pearl bank and number of endangered and endemic species.

MORPHOLOGICAL CHARACTERS OF THE COLLECTED SEAWEED

CHLOROPHYCEAE (Plates 1 – 4)

***Ulva flexuosa* Wuffen**

Ulva flexuosa has a smooth, tubular thallus that is linear and uniform in width, with numerous branches in the lower third. It has one to several fronds. It ranges from translucent dark to light green, with chloroplasts taking up part to most of the outer region of its cells. It has a very small rhizoidal region.

***Ulva lactuca* Linn**

Ulva lactuca is a thin flat green algae growing from a discoid holdfast. The margin is somewhat ruffled and often torn. It may reach 18 centimetres (7.1 inch) or more in length, though generally much less, and up to 30 centimetres (12 inch) across. The membrane is two cells thick, soft and translucent, and grows attached, without a stipe, to rocks or other algae by a small disc-shaped holdfast. Green to dark green in colour, this species in the Chlorophyta is formed of two layers of cells irregularly arranged, as seen in

TABLE 2: LIST OF MARINE ALGAE WITH CLASS, ORDER AND FAMILY COLLECTED FFROM SELECTED LOCALITIES

S. NO	NAME OF THE SPECIES	CLASS	ORDER	FAMILY	PLACE
1	*Ulva beytensis* Thivy & Sharma	Chlorophyceaae	Ulvales	Ulvaceae	Hare Island
2	*Ulva flexutosa* Wulfen	Chlorophyceaae	Ulvales	Ulvaceae	Hare Island
3	*Ulva lactuca* L.	Chlorophyceaae	Ulvales	Ulvaceae	Hare Island
4	*Ulva prolifera* O. F. Muell.	Chlorophyceaae	Ulvales	Ulvaceae	Hare Island
5	*Chaetomorpha aerea* (Dillwyn) Kuetz.	Chlorophyceaae	Cladophorales	Cladophoraceae	Hare Island
6	*Caulerpa chemnitizia* (Esper) J.V. Lamour.	Chlorophyceaae	Bryopsidales	Caulerpaceae	Hare Island
7	*Caulerpa racemosa* (Forssk.). J. Agardh	Chlorophyceaae	Bryopsidales	Caulerpaceae	Hare Island
8	*Caulerpa scapelliformis* (R. Br. ex Turner) C. Agardh	Chlorophyceaae	Bryopsidales	Caulerpaceae	Hare Island
9	*Caulerpa taxifolia* (M. Vahl) C. Agardh	Chlorophyceaae	Bryopsidales	Caulerpaceae	Hare Island
10	*Caulerpa veravalensis* Thivy & V. D. Chauhan	Chlorophyceaae	Bryopsidales	Caulerpaceae	Hare Island
11	*Halimeda tuna* (J. Ellis & Solander) J. V. Lamour.	Chlorophyceaae	Bryopsidales	Halimedaceae	Hare Island
12	*Codium tomentosum* Stackh	Chlorophyceaae	Bryopsidales	Codiaceae	Hare Island

13	*Padina boergesenii* Allender & Kraft	Phaeophyceae	Dictyotales	Dicyotaceae	Hare Island
14	*Padina tetrastromatica* Hauck	Phaeophyceae	Dictyotales	Dicyotaceae	Hare Island
15	*Dictyota bartayresina* J. V. Lamour	Phaeophyceae	Dictyotales	Dicyotaceae	Hare Island
16	*Stoechospermum polypodioides* (J. V. Lamour.) J. Agardh	Phaeophyceae	Dictyotales	Dicyotaceae	Hare Island
17	*Spatoglossum asperum* J. Agardh	Phaeophyceae	Dictyotales	Dicyotaceae	Hare Island
18	*Colpomenia sinuosa* (Mertens ex Roth) Derbes & Solier	Phaeophyceae	Ectocarpales	Scytosiphonaceae	Hare Island
19	*Sargassum cinereum* J. Agardh	Phaeophyceae	Fucales	Sargassaceae	Hare Island
20	*Sargassum polycystum* C. A. Agardh	Phaeophyceae	Fucales	Sargassaceae	Hare Island
21	*Sargassum tenerrium* J. Agardh	Phaeophyceae	Fucales	Sargassaceae	Hare Island
22	*Sargassum wightii* Greville	Phaeophyceae	Fucales	Sargassaceae	Hare Island
23	*Cystoseira trinodis* (Forssk.) C. Agardh	Phaeophyceae	Fucales	Sargassaceae	Hare Island
24	*Turbinaria ornata* (Turner) J. Agardh	Phaeophyceae	Fucales	Sargassaceae	Hare Island
25	*Gracilaria canaliculata* Sonder	Rhodophyceae	Gracilariales	Gracilariaceae	Hare Island
26	*Gacilaria corticata* (J. Agardh) J. Agardh	Rhodophyceae	Gracilariales	Gracilariaceae	Hare Island

27	*Hydropuntia edulis* (S. G. Gmelin) Gurgel & Fredericq	Rhodophyceae	Gracilariales	Gracilariaceae	Hare Island
28	*Kappaphycus alvarezii* (Doty) L. M. Liao	Rhodophyceae	Gigartinales	Solieriaceae	Hare Island
29	*Solieria chordalis* (C. Agardh) J. Agardh	Rhodophyceae	Gigartinales	Solieriaceae	Hare Island
30	*Solieria robusta* (Greville) Kylin	Rhodophyceae	Gigartinales	Areschougiaceae	Hare Island
31	*Champia compressa* Harv.	Rhodophyceae	Rhodymeniales	Champiaceae	Hare Island
32	*Caulerpa crassifolia* (C. Agardh.) J. Agardh.	Chlorophyceaae	Bryopsidales	Caulerpaceae	Idinthakarai
33	*Caulerpa scapelliformis (R.Br. ex Turner) C. Agardh*	Chlorophyceaae	Bryopsidales	Caulerpaceae	Idinthakarai
34	*Chaetomorpha media* (C. Agardh) Kutzing	Chlorophyceaae	Cladophorales	Cladophoraceae	Idinthakarai
35	*Halimeda tuna (J. Ellis & Solander) J. V. Lamour.*	Chlorophyceaae	Bryopsidales	Halimedaceae	Idinthakarai
36	*Ulva fasciata* Delile	Chlorophyceaae	Ulvales	Ulvaceae	Idinthakarai
37	*Valoniopsis pachynema* (Martens) Boergesen	Chlorophyceaae	Cladophorales	Valoniaceae	Idinthakarai
38	*Ulothrix flacca* (Dillwyn) Thuret	Chlorophyceaae	Ulotrichales	Ulotrichaceae	Idinthakarai
39	*Padina tetrastromatica Hauck*	Phaeophyceae	Dictyotales	Dicyotaceae	Idinthakarai
40	*Hormophysa triquetra* (C. Agardh) Kutzing	Phaeophyceae	Fucales	Sargassaceae	Idinthakarai

41	*Sargassum wightii* Greville	Phaeophyceae	Fucales	Sargassaceae	Idinthakarai
42	*Amphiora anceps* (Lamarck) Decaisne	Rhodophyceae	Corallinales	Corallinaceae	Idinthakarai
43	*Hypnea valentiae* (Turner) Montagne	Rhodophyceae	Gigartinales	Cystocloniaceae	Idinthakarai
44	*Gracilaria confervoides* (Linnaeus) Greville	Rhodophyceae	Gracilariales	Gracilariaceae	Idinthakarai
45	*Gacilaria corticata* (J. Agardh) J. Agardh	Rhodophyceae	Gracilariales	Gracilariaceae	Idinthakarai
46	*Grateloupia filicina* (J. V. Lamouroux) C. Agardh	Rhodophyceae	Halymeniales	Halymeniaceae	Idinthakarai
47	*Gelidium crinale* (Hare ex Turner) Gaillon G	Rhodophyceae	Gelidiales	Gelidiaceae	Idinthakarai
48	*Entermorpha flexuosa* (Wulfen) J. Ag.	Chlorophyceaae	Ulvales	Ulvaceae	Rameshwaram
49	*Padina tetrastromatica Hauck*	Phaeophyceae	Dictyotales	Dicyotaceae	Rameshwaram
50	*Sargassum swartzii* (Turn.) C. Ag.	Phaeophyceae	Fucales	Sargassaceae	Rameshwaram
51	*Sargassum wightii G*rerille.	Phaeophyceae	Fucales	Sargassaceae	Rameshwaram
52	*Turbinaria ornata* (Turner) J. Agardh	Phaeophyceae	Fucales	Sargassaceae	Rameshwaram
53	*Halymenia floresia* (Clem.) C. Ag.	Rhodophyceae	Cryptonemiales	Halymeniaceae	Rameshwaram
54	*Gracilaria edulis (Gmelin) Silva*	Rhodophyceae	Gracilariales	Gracilariaceae	Rameshwaram

55	*Halimeda opuntia* (L) Lamour.	Chlorophyceaae	Bryopsidales	Halimedaceae	Mandabam
56	*Caulerpa peltata* Lamour.	Chlorophyceaae	Bryopsidales	Caulerpaceae	Mandabam
57	*Caulerpa racemosa (Forssk.). J. Agardh*	Chlorophyceaae	Bryopsidales	Caulerpaceae	Mandabam
58	*Chaetomorpha crassa* (C. Ag.) Kuetzing	Chlorophyceaae	Cladophorales	Cladophoraceae	Mandabam
59	*Chaetomorpha antennina (Bony) kutzing*	Chlorophyceaae	Cladophorales	Cladophoraceae	Mandabam
60	*Sargassum myricystum J. Ag.*	Phaeophyceae	Fucales	Sargassaceae	Mandabam
61	*Stoechospermum marginatum* J. Ag	Phaeophyceae	Dictyotales	Dicyotaceae	Mandabam
62	*Padina distromatica* Hauck.	Phaeophyceae	Dictyotales	Dicyotaceae	Mandabam
63	*Turbinaria ornata* (Turner) J. Agardh	Phaeophyceae	Fucales	Sargassaceae	Mandabam
64	*Champia compressa* Harv.	Rhodophyceae	Rhodymeniales	Champiaceae	Mandabam
65	*Haloplegma duperreyi* Mont.	Rhodophyceae	Cermiales	Cermiaceae	Mandabam
66	*Portieria hornemannii* (Lyngb.) P. C. Silva	Rhodophyceae	Gigartinales	Rhizophyllidaceae	Mandabam
67	*Gelidiella acerosa* (Frossk.) Feldmann & Hamel	Rhodophyceae	Gelidiales	Gelidiellaceae	Mandabam
68	*Gracilaria Cylindrica* Boergesen.	Rhodophyceae	Gracilariales	Gracilariaceae	Mandabam

69	*Gracilaria tuticorinensis* Krish .et Rajend.	Rhodophyceae	Gracilariales	Gracilariaceae	Mandabam
70	*Gracilaria verrucosa* (Hudson) Papenfus	Rhodophyceae	Gracilariales	Gracilariaceae	Mandabam
71	*Kappaphycus alvarezii* (Doty) L. M. Liao	Rhodophyceae	Gigartinales	Solieriaceae	Mandabam
72	*Laurencia obtusa* (Hudson) Lamouroux.	Rhodophyceae	Cermiales	Rhodomelaceae	Mandabam
73	*Pterocladia heteroplatos (Boerg.) Rao, M.U. & Kaliap. A.*	Rhodophyceae	Gelidiales	Pterocladiaceae	Mandabam
74	*Caulerpa scalpelliformis* (R. Br. Ex Turner) C. Ag.	Chlorophyceaae	Bryopsidales	Caulerpaceae	Thruvaikulam
75	*Halimeda macroloba Decaisne.*	Chlorophyceaae	Bryopsidales	Halimedaceae	Thruvaikulam
76	*Sargassum wightii G*rerille.	Phaeophyceae	Fucales	Sargassaceae	Thruvaikulam
77	*Sargassum parvifolium* (Turn) J. Ag.	Phaeophyceae	Fucales	Sargassaceae	Thruvaikulam
78	*Turbinaria conoides* (J. Ag) Kutzing	Phaeophyceae	Fucales	Sargassaceae	Thruvaikulam
79	*Turbinaria ornata (Turner) J. Agardh*	Phaeophyceae	Fucales	Sargassaceae	Thruvaikulam
80	*Jania rubens* (L) Lamourox	Rhodophyceae	Corallinales	Corallinaceae	Thruvaikulam
81	*Portieria hornemannii* (Lyngb.) P. C. Silva	Rhodophyceae	Gigartinales	Rhizophyllidaceae	Thruvaikulam
82	*Gracilaria edulis (Gmelin) Silva*	Rhodophyceae	Gracilariales	Gracilariaceae	Thruvaikulam

cross-section. The chloroplast is cup-shaped in some references but as a parietal plate in others with one to three pyrenoids.

***Ulva prolifera* O. F. Muell.**

Thallus light to medium green, erect from a small holdfast and often flaccid, terete or compressed, mostly 5–15 cm high and 0.5–2 cm broad, usually with frequent, proliferous branches but sometimes almost simple. Cells usually arranged in longitudinal rows, often partly disrupted above, and to some extent in transverse rows, typically angular and four sided in upper thallus, 10–14(–16) µm long and 8–12(–14) µm broad, more rounded and larger in the stipe (to 25 µm long); chloroplast occupying most of the cell, with a single, relatively large pyrenoid occupying 20–60% of the cell width.

***Ulva fasciata* Delile**

Frond, large, from 10-30 cm or longer, attached to substratum by circular or oblong hold fasts. Basal attachment disc 2-4 mm in diameter. Thallus divided into many, more or less distinct, narrow lobes. Lobes 1-3 cm wide, flat, linear, lanceolate. Margin, undulate or sinuate or irregularly dentate.

Enteromorpha flexuosa (Wulfen) J. Agardh

Pale green in colour, up to 30 cm long, grown throughout the year, attached by a small, round basal disk, fronds simple or branched, tubular with cylindrical stalks below and expanding blades above, becoming flexuous, ending in an obtuse apex.

***Chaetomorpha media* (Ag.) Kutz.**

Plants attached to hard rocky and similar substrata, dense, tufted, brush like, filamentous, unbranched, erect stiff and rigid below, flexuous above; usually 4-10 cm high, occasionally spread out on substrata, irregularly ramified, branches ending in small

coralliform irregularly shaped disc by which the plants are fastened to rocks and other substrata; rhizoids swell up here and there, filled with starch, ultimately giving new shoots from them.

Chaetomorpha antenina (Bory) Kützing

Thalli about 3–15 cm in tall, firm and crisp in texture, unbranched filamentous, attached, long branches; strong surge, spongy mass, basal holdfasts short rhizoids; light green in colour.

***Chaetomorpha crassa* (C. Ag.) Kuetzing**

Plants dark or yellowish green and 13-18 cm long, thalli are often loosely enlarged, rough, unbranched and contorted filaments. Cells cylindrical, rectangular and square slightly constricted at the nodes. The cells contain a single reticulate chloroplast with many pyrenoids. The cells are 240-440µm wide, 430-560 µm long and the walls are 50-85 µm thick.

***Valoniopsis pachynema* (Martens) Boergs.**

Thallus with cylindrical ramified filaments, 600-700µm thick. Filaments arranged mostly upward and vertical; others growing out in various directions, between the upward directed filaments, thus forming a sort of a felted cushion, up to 3-5 cm high. Wall layer, thick, several layered. Colour green to dark green.

***Caulerpa crassifolia* (C. Ag.) J. Ag.**

Plants creeping by well-developed stolon. Stolon is cylindrical, naked, about 1 mm thick, sending out rhizoids from lower surfaces and assimilators from the upper surface. Assimilators with or without pedicels, several arising from the horizontal stolon, flat, linear, lanceolate, 10-12 cm in length, up to 1 cm broad at the broadest part, pinnate.

***Caulerpa scalpelliformis* (R.Br.) Weber-van Bosse**

Plants in large associations, with prostrate rhizomes-like stolons, rooting from the lower surfaces at intervals and erect branches above. Stolen simple or slightly branched, glabrous, glossy, 15-20 cm or more long with erect assimilators on the upper faces at intervals of 1-2 cm. Assimilators are 1-2 cm long, simple, bilateral, flat, leaf like, 8-24 cm along, 1 cm broad; linear lanceolate in outline.

***Caulerpa racemosa* (Forsskål) J. Agardh**

This plant has erect branches arising from a horizontal stolon attached to the sediment at intervals by descending rhizomes. The erect branches arise every few centimetres, reaching as much as 30 cm in height. A large number of branchlets, resembling ovate or spherical bodies on stalks, arise from each erect branch. Where branches and stolons are close together, the branchlets form a dense mat of seemingly spherical structures. The plants are coenocytic, i.e., the plant is multinucleate and nonseptate.

Caulerpa chemnitizia (Esper) J. V. Lamour

Thallus grass-green to dark green, spreading laterally to 50 cm, with naked stolons 1–2 mm diameter, attached by short pillars with clustered rhizoids. *Assimilators* mostly simple, rarely branched, to 6 cm tall and 10 mm wide, with sparse to crowded radially arranged ramuli. *Ramuli* trumpet-shaped, peltate or clavate, with more than one form often present in the same assimilator, to 6 mm long, lacking constrictions, gradually broadening or with a short stalk and a flat disc (2.0–2.5 mm wide) when the ramuli are mostly peltate.

***Caulerpa taxifolia* (Vahl) C. Agardh**

Branches, feather-like, flattened, and upright, 3 - 10 cm high, rising from a creeping stolon (runner), 1 - 2 mm in diameter, anchored by rhizoids to the substrate. Branchlets oppositely attached to midrib, flattened, slightly curved upwards, tapered at both base and tip, and constricted at point of attachment. Midrib is slightly flattened, appearing oval in cross-section. Dark green to light green.

***Halimeda tuna* (Ell. & Sol.) Lamour.**

Plants about 10-20 cm high moderately calcified branched. Branches, in one plane, segmented. Root, small, somewhat bulbous. Segments, lower ones somewhat thicker, above thinner, thickness up to 1 mm. Segments varying in form, flat, discoid to elongate cuneate, not ribbed. Margin entire. Size of segments, 35 mm long, 22 mm broad. Colour greenish when fresh and young, becoming chalk white with encrustations.

***Halimeda macroloba* Decaisne**

The thallus is erect, flat and moderately calcified and grow up to 19cm tall extending the long, bulbous hold fast which may extend to 5cm. Basal segment is somewhat cuneate (wedged shaped, broad above and tapering to the base) or rectangular with slightly expanded anterior portion supporting two or more separate segments, each of which supports several younger all together forming fan shaped unit; segments are generally flabellate with dull surface measuring 1.2-3.6 cm broad, 1.5 -2 cm tall and 1-2.3 mm thick, upper margin is entire undulate to deeply lobed.

***Halimeda opuntia* (L) Lamour**

Halimeda opuntia forms thick, frequently-branched clumps of calcified, leaf-like segments up to 25 cm (10 inch) high. The segments are flat and kidney- or fan-shaped, up to 8 mm (0.3 inch) high and 10 mm (0.4 inch) broad. They have a distinct central rib and a smooth, sinuous, or lobe-shaped, upper margin. Rhizoids grow where the segments touch the substrate. The plants are often crammed closely together forming a dense mat of herbage in which the individual plants are not easily discernible.

***Codium tomentosum* Stackh**

Mainly on rock in the lower shore; relatively uncommon in Ireland and Britain. A spongy, repeatedly dichotomously branched green alga without distinct flattening at the forks of the branches.

***Ulothrix flacca.* Kutzing**

Ulothrix includes simple, unbranched, filaments of indefinite length, which in younger stages are sessile an attached to the substratum by the basal cell, which is simple or slightly swollen, often broader than long. The cell wall is generally thin, particularly in the wider forms where it becomes lamellate. The chloroplast is a single, girdle shaped parietal band, partly or fully encircling the protoplast. Pyrenoids are present, either one or more in each cell. A small nucleus is present, generally placed internally to the chloroplast.

PHAEOPHYCEAE (Plates 5 – 7)

***Padina tetrastromatica* Hauck**

Plant erect, in several clumps, several blades arising from the same stupose basal attachment to substratum by tufts of rhizoids. Frond stacked, varying in size, numerous,

PLATE 1: CHLOROPHYCEAE

Ulva beytensis

Ulva flexuosa

Ulva lactuca

Ulva prolifera

Chaetomorpha aerea

Caulerpa chemnitzia

PLATE 2: CHLOROPHYCEAE

Caulerpa racemosa

Caulerpa scapelliformis

Caulerpa taxifolia

Caulerpa varavalensis

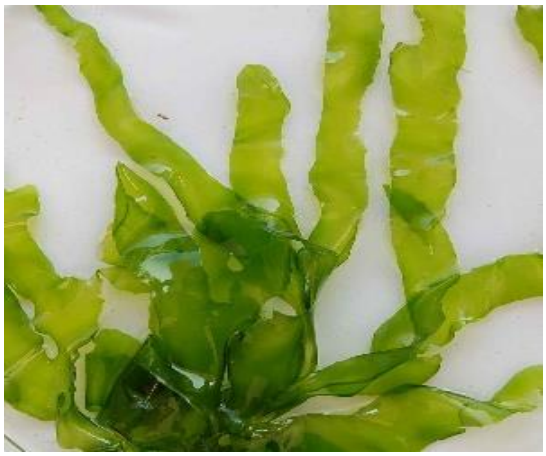
Halimeda tuna

Codium tomentosum

PLATE 3: CHLOROPHYCEAE

Chaetomorpha media

Caulerpa crassifolia



Ulva fasciata

Valoniopsis pachynema

Ulothrix flacca

Entermorpha flexuosa

PLATE 4: CHLOROPHYCEAE

Halimeda opuntia

Caulerpa peltata

Chaetomorpha crassa

Chaetomorpha antennina

Halimeda macroloba

base. Vesicles spherical, about 4 mm diameter, obviate, round, usually mucronate at the apices, subcylindrical.

***Sargassum myriocystum* J. Agardh**

Thalli about 10–80 cm in tall; discoid holdfast, cylindrical with rough out growth, alternately arranged branches, vesicles, stalk about 1–18 mm in diameter; oblong tapered leaves, blades leaf-like; air bladders bulbous, obvious, emarginated or retuse; serrated margin, apices are round, acute outer margin; prominent midrib; cryptostomates blade, pedunculated vesicles; fresh samples are yellowish brown in colour.

***Sargassum swartzii* (Turn) C. Ag.**

Thallus coarse, bushy, to 1.5 m high. Main axis short (to 10 mm high), cylindrical (2.5 mm diameter), smooth, bearing radially arranged several primary branches with thick, elongate-lanceolate phylloids, 4.7-6(-9) cm long, 5-7 mm broad. Phylloids with asymmetrical base, percurrent midrib, entire or shallowly dentate margins and acute apices. Vesicles stalked, solitary or in clusters, up to 8 mm long and 3-(4-5) mm diam., with rounded, pointed or mucronate apices; stalk flattened, cuneate, 8-12 mm long or sometimes longer than vesicle; cryptostomata small, scattered along the midrib and on vesicles. Receptacles 2.5-3.5(-5) mm long, 0.5-1.5 mm broad, compressed; slightly spinulose at apices, 2-3 times forked. Holdfast disc-like, to 12 mm diam. Growing on lower intertidal to subtidal rocks.

***Turbinaria ornate* (Turner). Agardh 1821**

Stiff, erect plant, 2 -20 cm tall when reproductive. Blades conical, hard, thick, with double row of stiff spines around the irregularly triangular margin of the blade when viewing from above. Holdfast bears one terete, erect portion and basal portion is conical

or irregular, usually with several unbranched or dichotomously branched root-like structures growing from basal area of the erect axes. Mostly light yellowish brown to dark brown with dark brown spots. Plant is usually isolated or in small groups, but occasionally forms large, low mats in high intertidal. Rhizoids common in upper intertidal.

***Turbinaria conoides* (J. Ag.) Kuetz**

Thalli about 8–26 cm in tall; erect, subcylindrical, alternative, polystichously; thalli thickness about 10–15 mm long; tape stalk, triangular; vesiculate blade margin or evesiculate, 10–20 mm in long; sharp prominent teeth; fresh samples are dark brown in colour.

***Stoechospermum polypodioides* (J.V. Lamour.) J. Agardh**

Thallus erect, to 17 cm tall, medium to dark brown, dichotomously branched. *Axes* flattened, 4–7 mm wide, generally of a similar width throughout or becoming broader towards the involute apices. *Sporangial sori* narrowly elongate, 10–30 mm long, close to and parallel with the lateral thallus margins, on both surface of the thallus.

***Dictyota bartayresiana* Lamour**

Plants variable, bushy in densely intermingled masses, not stipitate, attached to substratum by irregularly shaped holdfast with rhizoids, or lying loose: base naked. Thallus branched, branches when young complanate, later becoming bent and twisted slightly, dichotomous, equal angled membranous, crisp, relatively fragile. Segments without midrib 2-7 mm broad or usually 4-7 mm broad. Internodes 1-4 times as long as broad; margin entire; apex acute, somewhat broadly rounded. Tetrasporangia on both sides, either solitary

or a few together, scattered over the whole surface. Gametangia in scattered sori. Light brown or yellowish brown in upper portions and blackish brown near basal portions.

***Spathoglossum asperum* J. Ag.**

Plants with an indistinct small holdfast. Thallus flat, Palmate, sub-dichotomously divided into large and small lobes. Lobes elongate, linear, lanceolate, attenuated towards base and summit in large lobes. Apex acute or rounded. Margin sinuate, irregularly dentate with large or smaller proliferations. Fructifications scattered on the surface of the thallus. The plants are dark brown, do not keep fresh long after picking from the sea; they turn dirty-green or bluish green.

***Colpomenia samosa* (Roth) Derbes & Solier**

Plants sessile, when young spherical with even surface, later as they grow becoming irregularly lobed, sinuous hollow vesicles, solitary or several plants growing together closely appressed in small groups or globose masses. Vesicles about 3-12 cm in diameter, light yellow brown. Thallus wall 03-0.4 mm thick, thin walled large colourless cells towards the inferior. Innermost layer cells 180 µ across. Intermediary cells smaller. Outermost 2 or 3 layers small cells, 3.7-75 µ across, with several chromatophores. Hairs superficial growth, ultimately becoming sunk in a depression of the thallus due to repeated division and limited growth to the surrounding cells. The alga is popularly known as the Oyster thief because of the fact that by its rapid multiplication, cause damage and loss to the French oyster fisheries.

PLATE 5: PHAEOPHYCEAE

Padina boergesenii

Padina tetrastromatica

Dictyota bartayresiana

Stoechospermum polypodioides

Spatoglossum asperum

Colpomenia sinuosa

PLATE 6: PHAEOPHYCEAE

Sargassum cinereum

Sargassum polycystum

Sargassum tenerrimum

Sargassum wightii

Cystoseira trinodis

Turbinaria ornata

PLATE 7: PHAEOPHYCEAE

Hormophysa triquetra

Sargassum myricystum

Stoechospermum marginatum

Padina distromatica

Sargassum parvifolium

Turbinaria conoides

RHODOPHYCEAE (Plates 8 – 11)

***Hydropuntia edulis* (S.G. Gmelin) Gurgel & Fredericq**

Thalli erect, cartilaginous, greenish brown to dark brown, or purple in colour, attached by a small discoid holdfast. Branching basically repeatedly dichotomous and divaricate. Branches terete, 1.5 to 2.2 mm in diameter, tapered and characteristically bifurcate at the terminal portions.

***Hormophysa triquetra* (C. Ag.) Kutz.**

Plants erect with a conical well-formed hold fast. Frond generally three sided, up to 30 cm or more in height, 3-10 mm broad, much branched irregularly. Axis at base somewhat terete to 5-6 cm from the basal holdfast, with similar secondary branches. This alga has a very peculiar construction of its thallus which is triquetrous. Membranous wing toothed at margin. Colour brown, turning to paler red on exposure, blackish in dry state.

***Grateloupia filicina* (Wulfen) C. Ag.**

This red alga is a very interesting member of the algal communities in exposed situations, where it grows in larger numbers, several in large tufts. The glossy, and somewhat firm and cartilaginous and slippery nature of the thallus enables the alga with stand exposure and strong even when directly acting on the alga. Holdfast- flat. Frond – numerous, flexuous, linear, attenuated at both ends, usually pinnately branched at base, rarely single, often naked at apices, sometimes slightly bifurcated at tips, flattened in middle.

***Amphiroa anceps* (Lamk.) Decsne.**

Amphiroa anceps is the most common coralline algae found on the East Coast of Australia. It is found in the low inter-tidal zone, in thick mats around the edges of rock

pools. It has a modular branching structure, with obvious cartilaginous nodes joining each section. Individuals on the West Coast of Australia have much wider branches compared to the East Cast individuals. A reddish colour and a repeated modular structure make this species easy to identify. This species turns white when attached by the sun.

***Gracilaria corticata* J. Ag.**

Plants 10-12 cm long, the thallus consists of bundles of flat and much divided blades with 2-3 mm broad segments; branching is dichotomous in young blades; in older plants numerous marginal projections line the edges of the segments in a pinnate fashion; they are half to 2 cm long; the colour of the plants vary from deep purple to grass green.

***Gracilaria confervoides* (L.) Grev.**

The seaweed *Gracilaria confervoides* (L.) Grev. is the only source of agar extraction in Italy. Large populations are present in the coastal Lagoons of the Northern Adriatic Sea. In these habitats (e.g. Bay of Goro near Ferrara) periodical recordings were made during 1968 and 1969 of temperature, salinity, micronutrients, light intensity and O_2 concentration. The lagoon bays offer the best condition for the development of *Gracilaria confervoides* populations, especially near places of fresh water inflow containing large amounts of nutrients. Fresh water in flow also limits critical temperature rise during summer. The natural habitat is shallow (average depth; 1m); since the bottom is muddy, the water is very turbid and the light intensity significantly reduced. *Gracilaria confervoides* lies unanchored on the mud in the vegetative sterile form on long densely branched threads, while the spores settle on shells.

***Hypnea valentiae* (Turn) Mont**

Plants are erect and laxly branched with distinct or percussent cylindrical main axis varying in thickness from 680 to 1700µ; ultimate branchlets are 300 - 1300µ long irregularly disposed around the axis. Branches are simple and filiform (thread like) but occasionally forked and are distinctly oriented at right angle to the axis; stichidia (inflated branches) are seen as swollen bands at the middle, near the base or rarely near the tips of the ultimate branchlets

***Gelidium crinale* (Turn.) Lamour**

The thallus is cylindrical or flattened, pinnately branched on a tough consistency. It is a perennial plant in which new shots are proliferated from the consisting basal portion each growing season. Thalli of *Gelidium* have a single apical cell at each branches apex. Derivatives are cut off at the posterior face of an apical cell and they mature into a single axial filament of the adult portion.

***Solieria robusta (*Grev.) Kylin**

Thallus tufted, irregularly branched on all sides, 10-37 cm in height. Main axis 2-3 mm thick, slender and thinner specimens 1-2mm thick; cylindrical; branches abruptly attenuated at base to arm a short stipe; elsewhere of almost same thickness, cylindrical, tapering slowly to an acute apex. Apex with tuft to short rudimentary branches. Tetrasporangia in cortical layer from terminal cells of branchlets. Tetraspores zonately divvied. Cystocarps producing carpospores.

***Solieria chordalis* (C. Agardh) J. Agardh**

Grows on rocks, stones and pebbles. Generally found in wave-sheltered habitats where silt accumulates but favouring situation with some current movement. Plants firm

and cartilaginous, bright red; fronds initially irregularly dichotomous with may seemingly adventitious laterals giving the thallus an irregularly pinnate appearance. Fronds narrowing at the point of attachment cylindrical or slightly compressed.

Pterocladia heteroplatos (Boerg.) Rao, M.U. & Kaliap. A.

Plants brownish red, 3-6 cm tall, dense tufts, attached, erect, compressed, flattened, 250-800µm thick, simple to distichously, irregularly branched, alternate, terete, compressed at the base, cortex 2-3 layers of pigmented cells, medulla of colourless cells, surface cells elongated, 8-10µm long, 4-8 µm wide, medullary cells 8-24 µm in diameter surrounded by thick walled rhizomes.

***Gracilaria Cylindrica* Boergesen.**

Plant rose red, 15-20 cm height, attached by a small disc, main axis thin below becoming thicker above, cylindrical, branches alternate or irregular, coming out on all the sides, thin at the base becoming thicker above, apex obtuse, cortex of 1-2 layers of cells, small, thick walled irregularly polygonal in surface view, modulla of large, thin walled cells gradually becoming smaller towards the periphery.

***Gracilaria canaliculata* Sonder**

Thallus tough, cartilaginous, 3-7(-30) cm high, greenish, yellowish-red to purple. Main axes and branches commonly cylindrical, 2.5-3 mm in diameter or slightly compressed (to 5 mm width). Branching mostly dichotomous, irregularly dichotomous, alternate, irregular, to 4-5 orders of branches. Axes and branches non-constricted, slightly tapering to bases. Ultimate branchlets simple, with blunt tips. In transverse section, 1-2 rows of small cortical cells to 10 mm high and 5-8 mm broad; medullary cells thin-walled, large, 200-300 mm in diam. and loosely arranged. Terasporangia ovate cruciately divided,

10-15×26 mm. Attachment by basal disk and secondary bundles of rhizoids. Growing on dead corals, stones, in pools, at low intertidal, at sites exposed to wave action.

***Kappaphycus alvarezii* (Doty) L. M. Liao**

Algae are fleshy, firm; up to 2 m tall. Thalli coarse, with axes and branches 1-2 cm diameter, heavy with major axes relatively straight, lacking secondary branches near apices. Frequently and irregularly branches primary, secondary branches intercalated between primary branches or mostly lacking. Shiny green to yellow orange.

***Cystoseira trinodis* (Forssk.) C. Agardh**

The alga main stripes arising from a holdfast are between 1-4 cm long. The stripes bear a few to numerous primary branches (between 20-50 cm long) which are usually formed and lost seasonally. Branchlets are borne on the primary branches. These bear air bladders, egg-holding structures and male gametes. In summer the plant sends up fertile fronds, which float on top of the water at low tide and are easily visible. In late summer these disappear, leaving the basal holdfast.

***Champia compressa* Harvey**

Plants pale to bright red, usually with bright blue or green iridescence when fresh, creeping and branching to form clumps to a few cm tall (rarely as individual tufted plants growing to up to 10 cm tall); axes compressed segmented, pinnately to irregularly branched from margins, up to 3 mm wide and 0.5 mm thick; laterals may be alternate, sub-opposite or regularly bipinnate in arrangement, narrowing towards bases and apices. Thallus hollow with central cavity containing mucus, divided by septa at short intervals, axes slightly constricted at septa; thallus wall of single layer of almost isodiametric cells, 40-50 x 45-55 µm, interspersed with scattered much smaller cells at the outer surface; medulla with

filaments (about 10 µm diameter) bearing spherical gland cells of about 15 µm diameter. Tetrasporangia excised from cells of thallus wall and projecting into central cavity, tetrahedrally divided, up to 100 µm diameter. Cystocarps sessile, about 2 x 1.6 mm when mature, conical to urn-shaped.

***Gracilaria verrucosa* (Hudson) Papenfuss**

Brownish red in colour, up to 30 cm tall, erect, terete, attached to small stones by small circular discs, branching lateral, sub-dichotomous, alternate, branch tips attenuated and ultimate branches small, with branches up to 3rd or 4th order; cystocarps sub-spherical, elevated and scattered over the thallus.

***Gelidiella acerosa* (Frossk.) Feldmann & Hamel**

Thallus yellow to dark red, cartilaginous, with decumbent and erect, terete axes to 2 mm diameter. *Erect axes* simple or sparingly divided, to 35 mm tall, bearing pinnate determinate lateral branches, mostly from the distal region. *Lateral branches*, simple, 1–3 mm long. *Medulla* of densely packed longitudinal filaments, the cells thick-walled and 10–25 µm diam., grading into a smaller-celled cortex of pigmented cells. *Outer cortical cells* ellipsoidal and forming a palisade, in surface view circular to slightly elongate, 3–8 µm diameter.

***Haloplegma duperrevi* Mont.**

Thallus red-brown to grey-red, flat and complanately branched, 1–10 cm high, flabellate to lobed, becoming divided or lacerate, lobes expanding upwards to (1–)2–4 cm broad, mostly 400–600 µm thick, margins smooth to slightly ruffled. Stipe 2–5 mm long, 1–2 mm in diameter, holdfast 2–8 mm across, rhizoidal; epilithic or on shells (one specimen on old axes of Doxodasya bulbochaete). Structure. Marginal apices regularly

fringing the blades, with primary apices and filaments separated by numerous secondary apices from lateral filaments. Apices with alternate laterals from 3–6 cells below the apical cells, walls more-or-less transverse, apical cells 10–18 µm in diameter and L/D 1–1.5. Laterals of apical filaments uniting with next adjacent filaments to form a rectangular network, with other irregular filaments, the outer cells developing short branch clusters. Cells of internal filaments 25–45 µm in diameter and L/D 3–7, outer clusters 45–100 µm long with ovoid basal cells 15–30 µm in diameter, branched 1–3 times with terminal unbranched rows of 2–4 cells, 8–14 µm in diameter, isodiametric to slightly ovoid. Cells multinucleate; rhodoplasts discoid to elongate, ribbon like in larger cells.

***Gracilaria edulis* (S.G. Gmelin) Gurgel & Fredericq**

Thallus stiff, caespitose, cartilaginous, flexuous, to 27 cm high, is forming loose tufts, greenish-brown, brownish-red. Main axes cylindrical (1.0-1.5(-2.0) mm in diam.). Branching abundant, basically irregular, sometimes nearly dichotomous or trichotomous, five to seven orders. Branches and branchlets cylindrical, very slightly constricted basally, tapering gradually to acute tips. Branch intervals gradually becoming shorter to bi- or trifurcate apices. Terasporangia oblong, embedded in the cortex, cruciately divided, 15×8 mm. Cystocarps spherical, markedly projecting. Attachment by small disc-like holdfast with prostrate axes and secondary discs on branch apices. Growing on dead corals, stones, shells, in tide pools, intertidal and upper subtidal (exposed during low tide), in areas with moderate wave action. Abundant in polluted waters.

***Halymenia floresia* (Clem.) C. Ag**.

Algae shows light to medium red or red brown,10-45 cm high, soft and mucilaginous, complanately and profusely branched with 4-5 orders of tapering laterals,

occasionally with small surface leaflets; axes 1-3 cm broad, main lateral, 1-2 cm broad and 300-400µm thick lesser laterals 3-10 mm broad, ultimate ones 1-2 mm broad and tapering to an acute apex. Holdfast discoid, 1-3 mm across, stipe 1-5 cm long, cuneate; epilithic.

***Jania rubens* (L.) Lamourx**

Slender, rose- pink, articulated, calcified fronds, in rounded bunches to 50 mm high. Repeatedly dichotomously branched, luxuriant specimens, to 100 secondarily pinnate. Segments cylindrical to 100 µm diameter, those bearing branches somewhat compressed, to 200 µm diameter. Fixed by the small conical disc, but spreading vegetatively by developing attachment discs from branches in contact with soild substrate.

***Laurencia obtusa* (Hudson) J.V. Lamouroux**

Globose tufts of brittle, cartilaginous, narrow, cylindrical, reddish brown to yellowish red fronds, 150 mm long, from small discoid base. Axis simple, branches patent, often opposite, spirally arranged, shorter towards apex giving irregularly pyramidal outline. Ultimate ramuli very short, truncate.

***Portieria hornemannii* (Lyngb.) P. C. Silva**

Thallus erect pale to dark red (occasionally pale yellow or greenish when dried), to 12 cm tall, richly branched. Branching alternate, distichous, arising at intervals of 0.5-1.0 mm, lateral branches either indeterminate and the branching similar to primary axes, or short and determinate, less than 1 mm long and simple, once or twice divided. Axes flattered, to 1 mm wide, tapering to narrow and circinate apices; distal regions slightly flexuous.

***Stoechospermum marginatum* (C. Agardh) Kutzing**

Rigorously forking plants that may reach a length of 40 cm; usually the plants are 20-30 cm long 8-11 mm broad; thallus flat, erect, spathulate, dichotomously branched respectively, without a midrib; margin entire; apex bifid or flatly truncate; section of thallus, greater part with large parenchymatous cells in the middle and on either side cover by two layers of small cells; fertile plants are easily identified on the marginal dark lines of crowed sporangia

***Gracilaria tuticorinensis* Krish.et Rajend.**

Plants brownish red, flat, foliose, up to 5-10 cm height, main axis about 6 mm wide, slightly flattened, branching irregularly with proliferations on both margins of the branches, upper branches often broader, up to 1 cm wide. Cortex single layered with narrow radially elongated cells. Cells of medulla layer, ovoid at the centre, surrounded with 1-2 layers of small oblong cells.

PLATE 8: RHODOPHYCEAE

Gracilaria canaliculata

Gracilaria corticata

Hydropuntia edulis

Kappaphycus alvarezii

Solieria chordalis

Solieria robusta

PLATE 9: RHODOPHYCEAE

Champia compressa

Amphiroa anceps

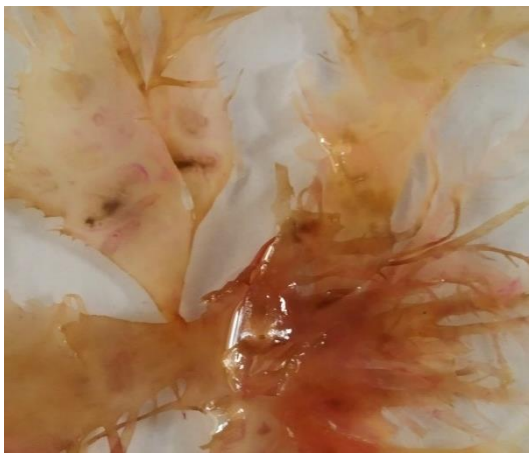
Gelidium crinale

Gracilaria confervoides

Grateloupia filicina

Hypnea valentiae

PLATE 10: RHODOPHYCEAE



Halymenia floresia

Haloplegma duperreyi

Gelidiella acerosa

Portieria hornemannii

Gracilaria cylindrica

Gracilaria tuticoriensis

PLATE 11: RHODOPHYCEAE

Gracilaria verrucosa

Laurencia obtusa

Pterocladia heteroplatos

Jania rubens

TABLE 3: LIST OF MARINE ALGAE RECORDED FROM FIVE LOCALITIES IN GULF OF MANNAR REGION, SOUTH-EAST COAST OF TAMILNADU

S. NO	NAME OF THE SPECIES	M	T	HI	I	R
1.	*Ulva beytensis* Thivy & Sharma			✓		
2.	*Ulva flexutosa* Wulfen			✓		
3.	*Ulva lactuca* L.			✓		
4.	*Ulva prolifera* O.F. Muell.			✓		
5.	*Chaetomorpha aerea* (Dillwyn) Kuetz.			✓		
6.	*Caulerpa chemnitizia* (Esper) J.V. Lamour.			✓		
7.	*Caulerpa racemosa* (Forssk.). J. Agardh	✓		✓		
8.	*Caulerpa scapelliformis* (R. Br. ex Turner) C. Agardh		✓	✓	✓	
9.	*Caulerpa taxifolia* (M. Vahl) C. Agardh			✓		
10.	*Caulerpa veravalensis* Thivy & V. D. Chauhan			✓		

11.	*Halimeda tuna* (J. Ellis & Solander) J.V. Lamour.			✓	✓	
12.	*Codium tomentosum* Stackh			✓		
13.	*Padina boergesenii* Allender & Kraft			✓		
14.	*Padina tetrastromatica* Hauck			✓	✓	✓
15.	*Dictyota bartayresina* J.V. Lamour			✓		
16.	*Stoechospermum polypodioides* (J.V. Lamour.) J. Agardh			✓		
17.	*Spatoglossum asperum* J. Agardh			✓		
18.	*Colpomenia sinuosa* (Mertens ex Roth) Derbes & Solier			✓		
19.	*Sargassum cinereum* J. Agardh			✓		
20.	*Sargassum polycystum* C. A. Agardh			✓		
21.	*Sargassum tenerrium* J. Agardh			✓		
22.	*Sargassum wightii* Greville		✓	✓	✓	✓
23.	*Cystoseira trinodis* (Forssk.) C. Agardh			✓		
24.	*Turbinaria ornata* (Turner) J. Agardh	✓	✓	✓		✓

25.	*Gracilaria canaliculata* Sonder			✓		
26.	*Gacilaria corticata* (J. Agardh) J. Agardh			✓	✓	
27.	*Hydropuntia edulis* (S. G. Gmelin) Gurgel & Fredericq			✓		
28.	*Kappaphycus alvarezii* (Doty) L. M. Liao	✓		✓		
29.	*Solieria chordalis* (C. Agardh) J. Agardh			✓		
30.	*Solieria robusta* (Greville) Kylin			✓		
31.	*Champia compressa* Harv.	✓		✓		
32.	*Caulerpa crassifolia* (C. Agardh.) J. Agardh.				✓	
33.	*Chaetomorpha media* (C. Agardh) Kutzing				✓	
34.	*Ulva fasciata* Delile				✓	
35.	*Valoniopsis pachynema* (Martens) Boergesen				✓	
36.	*Ulothrix flacca* (Dillwyn) Thuret				✓	
37.	*Hormophysa triquetra* (C. Agardh) Kutzing				✓	
38.	*Amphiora anceps* (Lamarck) Decaisne				✓	

39.	*Hypnea valentiae* (Turner) Montagne				✓	
40.	*Gracilaria confervoides* (Linnaeus) Greville				✓	
41.	*Grateloupia filicina* (J. V. Lamouroux) C. Agardh				✓	
42.	*Gelidium crinale* (Hare ex Turner) Gaillon G				✓	
43.	*Entermorpha flexuosa* (Wulfen) J. Ag.					✓
44.	*Sargassum swartzii* (Turn.) C. Ag.					✓
45.	*Halymenia floresia* (Clem.) C. Ag.					✓
46.	*Gracilaria edulis* (Gmelin) Silva		✓			✓
47.	*Halimeda opuntia* (L) Lamour.	✓				
48.	*Caulerpa peltata* Lamour.	✓				
49.	*Chaetomorpha crassa* (C. Ag.) Kuetzing	✓				
50.	*Chaetomorpha antennina* (Bony) kutzing	✓				
51.	*Sargassum myricystum* J. Ag.	✓				
52.	*Stoechospermum marginatum* J. Ag	✓				

53.	*Padina distromatica* Hauck.	✓				
54.	*Haloplegma duperreyi* Mont.	✓				
55.	*Portieria hornemannii* (Lyngb.) P. C. Silva	✓	✓			
56.	*Gelidiella acerosa* (Frossk.) Feldmann & Hamel	✓				
57.	*Gracilaria Cylindrica* Boergesen.	✓				
58.	*Gracilaria tuticorinensis* Krish .et Rajend.	✓				
59.	*Gracilaria verrucosa* (Hudson) Papenfus	✓				
60.	*Laurencia obtusa* (Hudson) Lamouroux.	✓				
61.	*Pterocladia heteroplatos* (Boerg.) Rao, M.U. & Kaliap. A.	✓				
62.	*Halimeda macroloba* Decaisne.		✓			
63.	*Sargassum parvifolium* (Turn) J. Ag		✓			
64.	*Turbinaria conoides* (J. Agardh) Kutzing		✓			
65.	*Jania rubens* (L) Lamourox		✓			

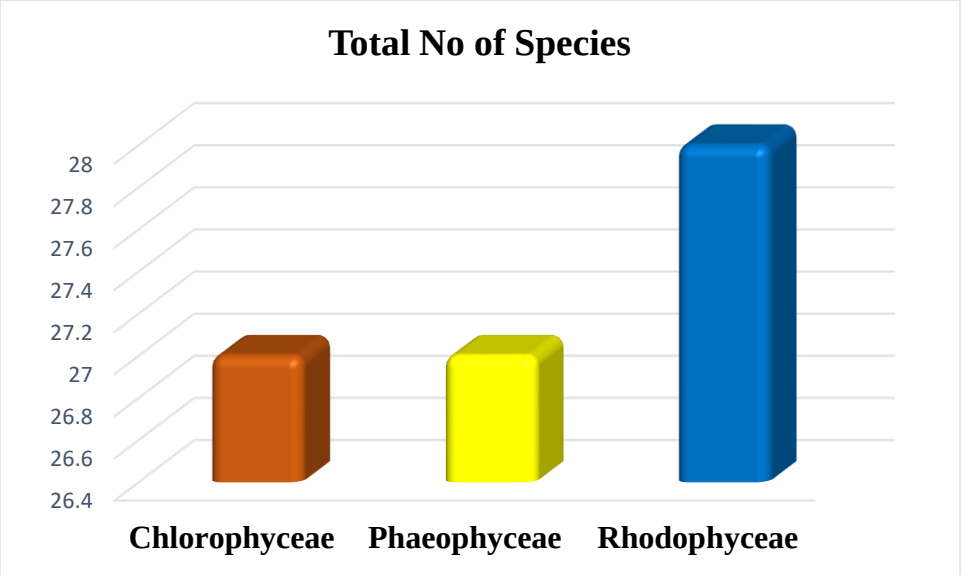


FIG 2A: TOTAL NUMBER OF MARINE ALGAE OF DIFFERENT GROUPS COLLECTED FROM FIVE LOCALITIES OF GULF OF MANNAR

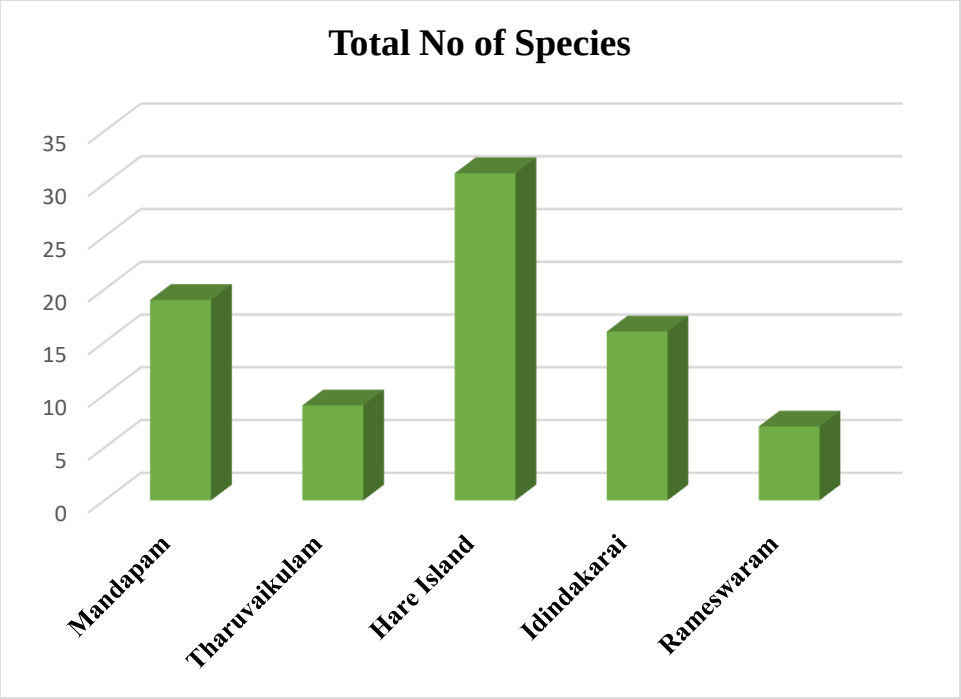


FIG 2B: NUMBER OF MARINE ALGAE COLLECTED FROM FIVE LOCALITIES OF GULF OF MANNAR

TABLE 4: GENERA AND SPECIES OF MARINE ALGAE DISTRIBUTION FROM FIVE LOCALITIES IN GULF OF MANNAR

S. NO.	STATIONS	CHLOROPHYCEAE		PHAEOPHYCEAE		RHODOPHYCEAE		TOTAL	
		GENERA	SPECIES	GENERA	SPECIES	GENERA	SPECIES	GENERA	SPECIES
1	Mandapam	3	5	4	4	8	10	**15**	**19**
2	Tharuvaikulam	2	2	2	4	3	3	**7**	**9**
3	Hair Island	5	12	8	12	5	7	**18**	**31**
4	Idindakarai	6	7	3	3	5	6	**14**	**16**
5	Rameswaram	1	1	3	4	2	2	**6**	**7**
TOTAL		**17**	**27**	**20**	**27**	**23**	**28**	**60**	**82**

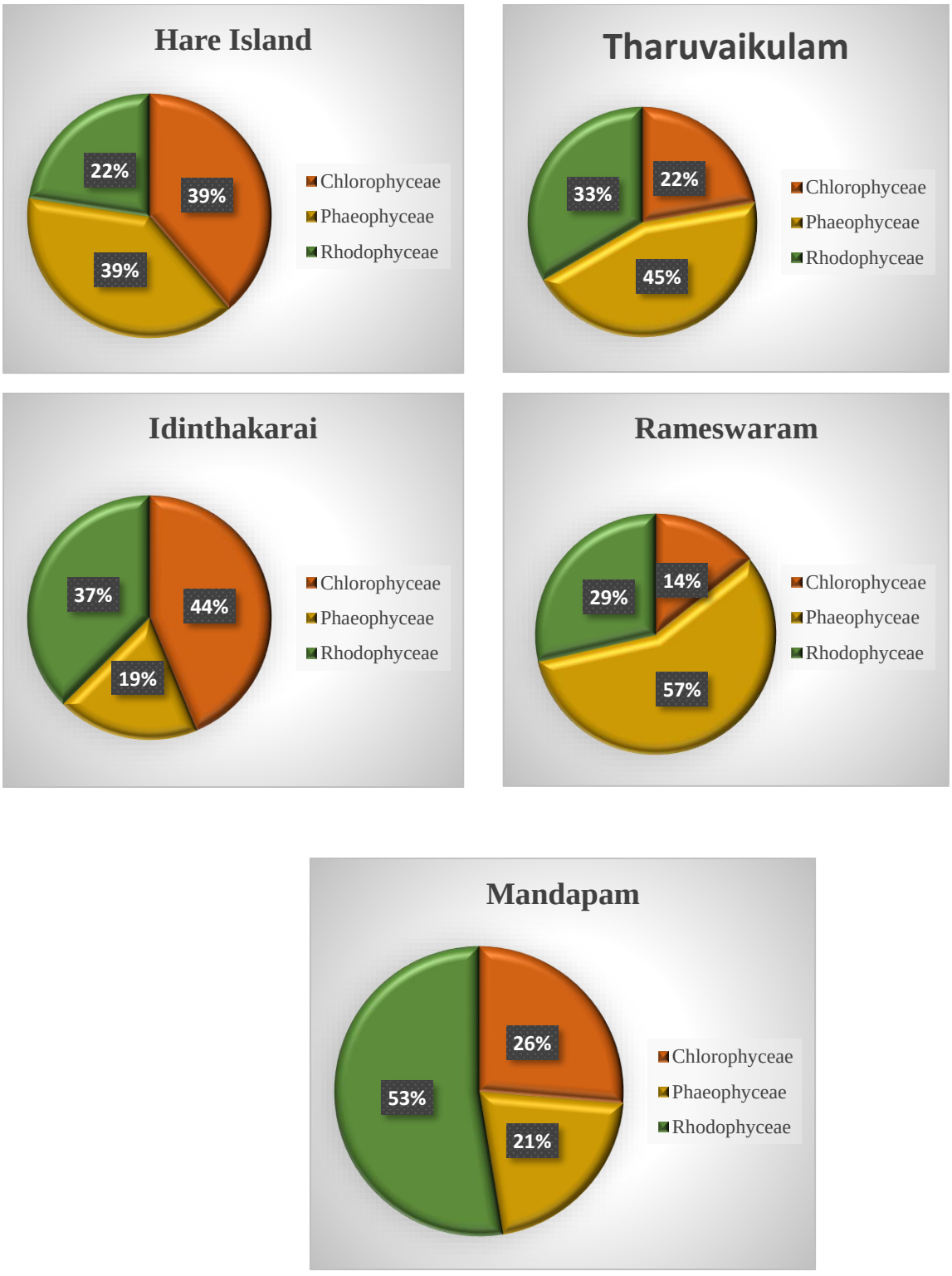


FIG 3: PERCENTAGE OF DISTRIBUTION OF DIFFERENT GROUPS OF MARINE ALGAE IN GULF OF MANNAR

CONCLUSION

Seaweeds are the important marine plants that constitute flora of our coastal habitats. However, information on its diversity is still in a state of infancy when compared with the land plants. Several civilizations around the world have been using seaweeds as a food since time immemorial and recently these marine plants have been showcased as a food for future. While India harbors a rich diversity of seaweeds, neither the extent of its diversity has been documented nor the resources have been utilized effectively. Apart from culinary uses, seaweeds are also used in several industries including cosmetics, nutraceuticals, and pharmaceuticals. Negative effects of seaweeds include marine fouling, biological invasion, and algae blooms. The higher diversity value observed in present investigation clearly showed the healthy nature of the seaweed ecosystems along Gulf of Mannar coastal waters. The data generated through the present research report can provide base-line information for commercial exploitation of seaweed resources along Gulf of Mannar coastal waters.

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21APB002

FLORAL DIVERSITY OF ST.MARY'S COLLEGE

A Short-Term Field Project Submitted to

ST.MARY'SCOLLEGE(Autonomous),THOOTHUKUDI

affiliated to

MANONMANIAMSUNDARANARUNIVERSITY,TIRUNELVEI

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN BOTANY

By

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DEPARTMENT OF BOTANY

ST.MARY'S COLLEGE (Autonomous)

THOOTHUKUDI -628001

2021 -2022

CERTIFICATE

It is certified that this short-term field project work entitled **FLORAL DIVERSITY OF ST.MARY'S COLLEGE** submitted to **St.Mary's College (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfillment of the requirements for the degree of **Master of Science in Botany** and is a record of work done in the Department of Botany, **St. Mary's College (Autonomous)**, Thoothukudi during the year 2021-2022 by the following students

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EXAMINER

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CONTENT

Biodiversity reflects variety and variability within and among living organisms, their associations and habitat-oriented ecological complexes. India is one of the 12 "Megadiversity" countries in the world. Mankind has been utilizing plants for food and medicinal purpose. India is a rich center of plants diversity. The great wealth of biological diversity in tropical regions is due to the environmental conditions existing there. It is well known that floristic composition is determined by environmental factors (Ayyappan and Parthasarathy, 1999); however, the composition influences biodiversity patterns at regional scales and further reflects both anthropogenic and natural disturbances (Pollock, 1997; Ward, 1998). Therefore, floristic characteristics and biodiversity patterns are often influenced by environmental factors and anthropogenic disturbances (Hong, 1999; Liu *et al.*, 2009). Conservation of biodiversity is essential for the proper functioning of ecosystems and for the maintenance of the environmental services they provide (Lopez-del Toro *et al.*, 2010). However, high rates of tropical deforestation and habitat destruction frequently cause the local extinction of plant and animal species. Flowering plants are by far the most numerous, diverse and successful extant plant group containing well over 90%of all land plant species alive today (Simpson, 2006). In India dicots are represented by 2282 genera and 12750 species whereas monocots are represented by 702 genera and 4250 species. Dicots accounts for c.75% flowering plants in terms of both genera and species (Brummit, 1992).

Tamilnadu exhibits great diversity due to the immense variety of climate, altitude and edaphic factors. Vegetation of the state can broadly be classified into four major groups namely i) Island vegetation ii) vegetation of the interior plants iii) vegetation of the hills and mountains and iv) coastal vegetation (Nair &Vivekananthan,1993; Chithra& Nair

1999) Biodiversity and its maintenance are very important for sustaining life on earth. Every species has a specific role in ecosystem. They capture and store energy and also produce and decompose organic matter. The ecosystem supports the services without which human cannot survive. A diverse ecosystem is more productive and can withstand environmental stress. Biodiversity is a reservoir of resources for the manufacture of food, cosmetic products and pharmaceuticals. Crops livestock, fishery, and forests are a rich source of food. Biodiversity preserves different cultures and spiritual heritage. To conserve the biodiversity has three main objectives they are, "To preserve the diversity of species", "Sustainable utilization of species and ecosystem", "To maintain life supporting system and essential ecological process".

SCOPE AND OBJECTIVES

In Tamilnadu the floral diversity was analysed by Nair and Henry in 1963and Henry & al. (1987, 1989), revealed that the state harbours about 5640 species and infraspecific taxa of flowering plants including cultivated species. After about two decades the state flora analysis was revised and a checklist of angiosperms in Tamil Nadu as a floral database was prepared by Narasimhan (2007). According to which, the angiosperms in the state are represented by 5547 taxa. comprising 5239 species,72 subspecies, 548 varieties in 1668 genera and 231 families. However, a recent compilation revealedthere are 5674 angiospermic taxa of which 212 are strict endemics, distributed in 51 families; about 50% of families are represented by single species; families, such as *Poaceae* (30 taxa), *Cyperaceae* (24 taxa), *Apocynaceae*and *Acanthaceae*(13 taxa each) exhibit high level of endemism; out of 212 endemic taxa, 122 are herbs, 51 are shrubs, 36 are trees and 3 are climbers and 85% of the endemic taxa are confirmed to the Western Ghats, 8% from the Eastern Ghats and 6% of the taxa are from coastal regions (Irwin & al., 2013). With 5674 angiosperm taxa. The vallanadu hills was located at 8.6804 N, 77.882 E to Tamilnadu. Vimal kumar in 2007 analysed in rich bio-diversity of that hills. His vegetation study included in Srivaikundam Taluk.

St.mary's college, located in the semi urban city of Thoothukudi(8.8110N, 78.1615E) is considered as the vegetation pocket, rich in verdant park with diversified trees and ornamental plants. This vegetation packet was studied by Dr. Mary josphine formly head of Botany department and their floristic features were documented.

The Botanical garden, maintaining by Department of Botany is also has diversified plants including conserved endangered plants and plant of Red listed. Inspites of its diversified in the garden, the work related to identification, classification and description

of floristic features of the bioreserve are completely lacking. To fill this lacuna our present field study is assigned and executed with the flowwling objective.

- Collection of plant material.
- Identification of plant material using the floras and monographs, plantnet
- Preparation of Herbarium.
- Studying the floristic features.
- Documentation of report.

MATERIALS AND METHODS

MATERIALS:

The source of materials for this work was the periodical collection of plant specimens in St. Mary's College for about six months.

METHODS:

The specimens were collected , processed and mounted following customary methods (fosberg and Sachet,1964). Specimens were identified usingHooker's *Flora of British India.*(1872

–1897) ;Gambles *flora of presidency of madras* (1915-1936); Matthew's *Flora of TamilNadu carnatic* (1983). The data for determining the species as medicinal is based on "*Medicinal plants ofIndia*" – *Tamil Nadu Vol.2* (Yoganarasimhan, 2000).Ornamental and other useful plants are also documented. Photographs were taken.The voucher specimens and photographs are deposited in the Herbarium of Botany department, St. Mary's College, Thoothukudi.

RESULT AND CONCLUSION

Floristic Analysis :

Flowering plants are by far the most numerous, diverse and successful extant plant group containing well over 90%of all land plant species alive today (Simpson, 2006). In India dicots are represented by 2282 genera and 12750 species where as monocots are represented by 702 genera and 4250 species. Dicots accounts for c.75% flowering plants in terms of both genera and species (Brummit, 1992). Species richness is measured on a samples carefully choosen in a particular area. Such data are important for prioritizing conservation strategies since they allow identification of geographic regions of the world wide exceptional or with very poor diversity (Krishnamoorthy 2003). Floristic studies help us to understand the basic aspects ofbiology such as speciation, isolation, endemism and evolution. Flora of any area is not fixed up. It changes from time to time. Various ecological factors, mostly biotic, change the floristic components. The total number of species may be changed; dominant species may be replaced with other species; the floristic composition, i.e.;family: genus: species ratio may be changed.In the following description the plant which was present in St. Mary'scollege botanical garden are collected, identified and photographed.

Equisetum hyemale

Family: Equisetaceae

Genus: *Equisetum*

Species: *hyemale*

Locality: St.Mary's College. Botanical garden

Floristic features:-

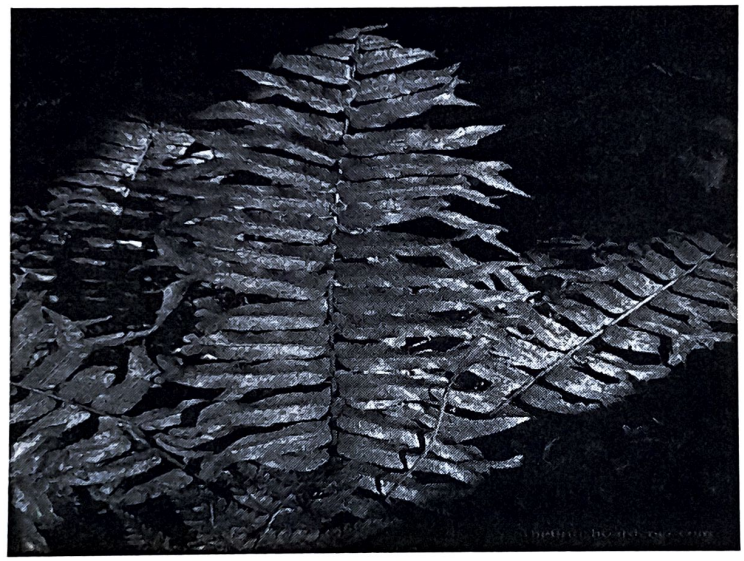
Equisetum hyemale (commonly known as rough horsetail, scouring rush, scouringrush horsetail and, in South Africa, as snake grass) is a perennial herbaceous vascular plant in the horsetail family Equisetaceae. It is a native plant throughout the Holarctic Kingdom, found in North America, Europe, and northern Asia. *Equisetum hyemale* has vertical jointed reed-like stalks of medium to dark green. The hollow stems are up to 3 feet (0.91 m) in height. The stems are seldom branched. The stems themselves have conspicuous ridges, which are impregnated with silica. This makes the ridges feel rough and harsh. The tiny leaves are joined together around the stem, forming a narrow black-green band or sheath at each joint. Like other ferns and their relatives, the plant reproduces by spores and does not produce flowers or seeds.The stems are generally deciduous in cold climates, and remain during winter in warmer climates. It forms dense spreading colonies, in full to partial sun.

Nephrolepis falcata

Family : Nephrolepidaceae

Genus : *Nephrolepis*

Species : *falcata*



Locality : St.Mary's college botanical garden

Floristic features :

Sub-erect fern, with drooping fronds, can grow up to 50 - 90 cm tall and 45 - 60 cm wide. Light green fronds, measuring about 80 - 150 cm long and 5 - 10 cm wide, stipe measuring about 10 - 25 cm long, most pinnae with central division to give 2 short tips and giving a "fish tail appearance". Sori round and arranged submarginal, indusia kidney-shaped with narrow sinus. Genus Nephrolepis means "kidney-scale" and refers to the shape of the indusia of the sori. Species falcata means "with sickle-shaped leaves".

Ocmium basilicum

Family : Lamiaceae

Genus : *Ocmium*

Species : *basilicum*

Locality: St.Mary's College. Botanical garden

Floristic features:

Basil is an annual, or sometimes perennial, herb used for its leaves. Depending on the variety, plants can reach heights of between 30 and 150 cm (1 and 5 ft). Its leaves are richly green and ovate, but otherwise come in a wide variety of sizes and shapes depending on cultivar. Leaf sizes range from 3 to 11 cm (1 to 4+1⁄2 in) long, and between 1 and 6 cm (1⁄2 and 2+1⁄2 in) wide. Basil grows a thick, central taproot. Its flowers are small and white, and grow from a central inflorescence, or spike, that emerges from the central stem atop the plant. Unusual among Lamiaceae, the four stamens and the pistil are not pushed under the upper lip of the corolla, but lie over the inferior lip. After entomophilous pollination, the corolla falls off and four round achenes develop inside the bilabiate calyx.

Coleus amboinicus

Family name : Lamiaceae
Genus : *Coleus*
Species : *amboinicus*

Locality : St. Mary's College botanical garden

Floristic features:

Coleus amboinicus grows up to 1 m (3.3 ft) tall. The stem is fleshy, about 30–90 cm in long or densely covered with soft, short and erect hairs Old stems are smooth. Leaves are 5–7 cm in long fleshy, simple, broad, egg/oval-shaped with a tapering tip (ovate). The margins are coarsely crenate to dentate-crenate except in the base. The petiole is 2–4.5 cm in long. Flowers are shortly pedicelled, pale purplish, in dense 10-20 (or more) flowered dense whorls (cymes), at distant intervals, in a long slender spike-like raceme. Rachis 10–20 cm (3.9–7.9 in), fleshy and pubescent. The bracts are broadly ovate, 3–4 cm (1.2–1.6 in) long, acute. The calyx is campanulate, 2–4 mm (0.079–0.157 in) long, hirsute and glandular, subequally 5-toothed, upper tooth broadly ovate-oblong, obtuse, abruptly acute, lateral and lower teeth acute. Corolla blue, curved and declinate, 8–12 mm (0.31–0.47 in) long, tube 3–4 mm (0.12–0.16 in) long.

Vitex negundo

Family : Lamiaceae
Genus : *Vitex*
Species : *negundo*

Locality : St. Mary's college botanical garden

Floristic features:
Vitex negundo is an erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. The bark is reddish brown. Its leaves are digitate, with five lanceolate leaflets, sometimes three. Each leaflet is around 4 to 10 cm (1.6 to 3.9 in) in length, with the central leaflet being the largest and possessing a stalk. The leaf edges are toothed or serrated and the bottom surface is covered in hair.The numerous flowers are borne in panicles 10 to 20 cm (3.9 to 7.9 in) in length. Each is around 6 to 7 cm (2.4 to 2.8 in) long and are white to blue in color. The petals are of different lengths, with the middle lower lobe being the longest. Both the corolla and calyx are covered in dense hairs.The fruit is a succulent drupe, 4 mm (0.16 in) in diameter, rounded to egg-shaped. It is black or purple when ripe.

Annona Montana

Family: Annonaceae

Genus: *Annona*

Species: *montana*

Locality: St.Mary's College. Botanical garden

Floristic features:

The tree is similar to Annona muricata, but has a more spreading crown and glossy leaves. It is slightly hardier and bears fruit throughout the year.It tolerates brief temperature drops down to 24 °F (−4 °C) when full grown.Its pollen is shed as permanent tetrads.The fruits are nearly round, with dark green skin covered with many short fleshy spines, and are about 15 centimetres (5.9 in) long. Yellow, fibrous pulp – which is aromatic – is sour and bitter, containing many light-brown, plump seeds.There is history of its use as a traditional medicine.Tree, resembling the soursop, but less susceptible to cold.Leaves larger than those of soursop.The fruit a smaller pseudocarp, ca. globose, up to 15 cm diameter, with short yellow prickles (not recurved); pulp is yellow, subacid to bitter, containing many light-brown seeds.It grows up to 700 m altitude.

Nymphaea rubra

Family: Nymphaeceae

Genus: *Nymphaea*

Species: *rubra*

Locality: St.Mary's College. Botanical garden

Floristic features:-

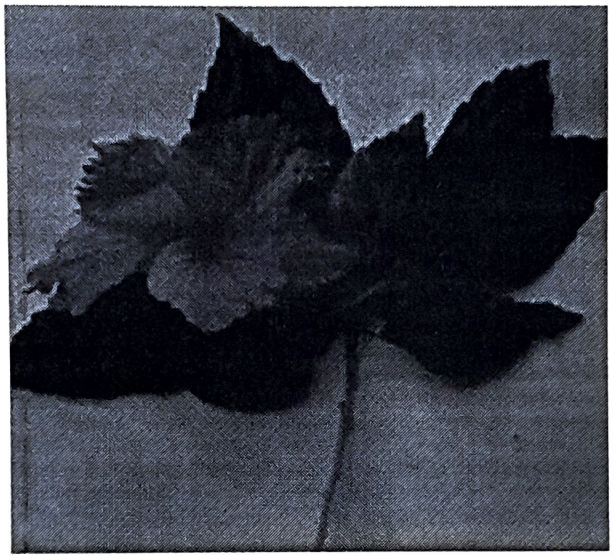
Red Water Lily is a beautiful floating plant native to India. It is widely cultivated in other countries. Flowers are intensely red or rose-colored. Sepals are usually 4 and petals are many. Leaves are around, sharply toothed, downy on the underside. The lobes of the leaves diverge away from each other. Stigma has 10-20 rays. Red Water Lily is found virtually throughout India. Flowering: all year. Nymphaea species are aquatic perennial herbs, laticiferous , rooted. Rhizomes erect or creeping, stoloniferous and sometimes branched. Leaves polymorphic, sublate, hastate, sagittate, deltoid to suborbicular, usually floating or submerged, membranous when young and coriaceous, prominently veined when mature, long petiolate. Flowers bisexual, usually solitary and floating, rarely submerged, yellow, white, pink, red and purple with long peduncles, sepals 4, free, hypogynous, petals 8 to numerous, hypogynous to perigynous. Stamens numerous, inflexed, shorter than petals and sepals, dorsifixed, filaments longer than anthers, anthers partially sunken, carpels 5-35, usually united partially or fully. Ovary superior, ovules numerous. Fruits are irregularly dehiscent, when non-schizocarpic it is a berry, ovoid or globose, crowned, with green filaments, ripened under water, seed globose, enclosed in a fleshy bell shaped aril, smooth or with ridges.

Hibiscus Rosa – sinensis

Family:Malvaceae

Genus: *Hibiscus*

Species: *rosa – sinensis L.*



Locality : St.Mary's college botanical garden

Floristic features:

Hibiscus rosa-sinensis is a dense, multi-branched evergreen shrub, commonly 4–6 ft (1.2–1.8 m) in height, but capable of reaching up to 12 ft (3.7 m) high. It has a rounded form, with a spread that may exceed its height. The glossy, leathery, oblong leaves are about 4 in (10 cm) long, with entire margins, and are carried in opposite pairs or whorled on the stems. Small tubular, scarlet flowers in dense rounded clusters 2–5 in (5.1–12.7 cm) across are produced almost all year long.

Ziziphus mauritiana

Family: Rhamnaceae

Genus: *ziziphus*

Species: *mauritiana*

Locality: St.Mary's College. Botanical garden

Floristic features:

Tree or bushy shrub, up to ca. 15 m tall, erect or spreading with drooping branches; twigs zigzag, tomentose; stipules spinous, solitary and straight (5-7 mm) or in dimorphic pairs, the second shorter and recurved, spines sometimes absent; trees evergreen or semi-deciduous.Leaves alternate, simple, elliptic-ovate to oblong-elliptic, 2-9 cm × 1.5-5 cm, slightly crenate or entire, glossy and glabrous above, densely white-tomentose beneath, with 3 conspicuous longitudinal veins; petiole 8-15 mm long.Inflorescences axillary cymes, 1-2 cm long, with 7-20 flowers, peduncles 2-3 mm long.Flowers 2-3 mm across, yellowish, faintly fragrant; pedicels 3-8 mm long; calyx with 5 deltoid lobes, hairy outside, glabrous within; petals 5, subspathulate, concave, reflexed; stamens 5; ovary 2-celled, styles bifid, disk 10-lobed or grooved.Fruit a drupe, globose to ovoid, up to 6 cm × 4 cm in cultivation, usually much smaller when wild, skin smooth or rough, glossy, thin but tough, yellowish to reddish or blackish; flesh white, crisp, juicy, subacid to sweet, becoming mealy in fully ripe fruits.Seed in a tubercled and irregularly furrowed stone, containing 1-2 elliptic brown kernels.

Mangifera indica

Family : Anacardiaceae
Genuns :*Mangifera*
Species ; *indica*

Locality : St. Mary's college botanical garden

Floristic features :

It is a large green tree, valued mainly for its fruits, both green and ripe. Approximately 500 varieties of mango have been reported in India. It can grow up to 15–30 m (49–98 ft) tall. The tree grows best in well-drained sandy loam; it does not grow well in heavy wet soils. The optimal pH of the soil should be between 5.2 and 7.5. Red-yellow flowers appear at the end of winter, and also at the beginning of spring. Both male and female flowers are borne on same tree. The Climatic conditions have significant influence on the time of flowering of mango. In India, flowering starts in December in the South, in January in Bihar and Bengal, in February in eastern Uttar Pradesh, and in February–March in northern India. The duration of flowering is 20–25 days in Dashehari, while panicle emergence occurs in early December and flower opening is completed by February. The Neelum variety of mango produces two crops a year in Kanyakumari, in South India, but it flowers only once in North Indian conditions. The mango is an irregular, egg-shaped fruit which is a fleshy drupe. Mangos are typically 8–12 cm (3–5 in) long and greenish yellow in color.

Morou nigra

Family : Moraceae

Genus : *Morus*

Species: *nigra*

Locality: St.Mary's College. Botanical garden

Floristic features:

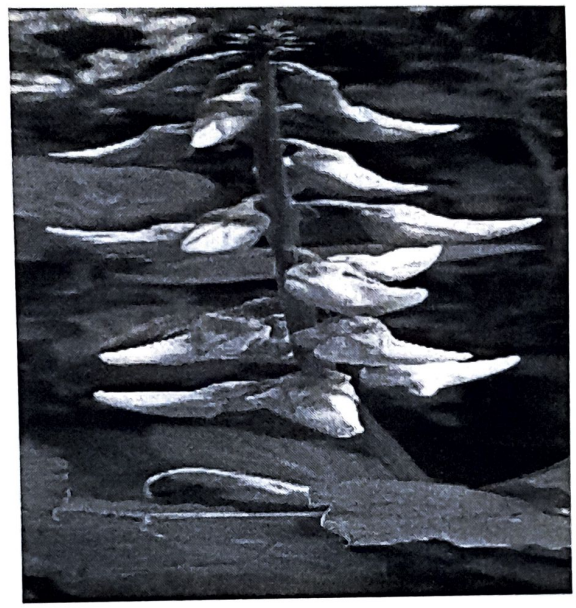
Morus nigra is a deciduous tree growing to 12 metres (39 feet) tall by 15 m (49 ft) broad. The leaves are 10–20 centimetres (4–8 inches) long by 6–10 cm (2–4 in) broad – up to 23 cm (9 in) long on vigorous shoots, downy on the underside, the upper surface rough with very short, stiff hairs. It has 308 (44x ploidy) chromosomes. The fruit is a compound cluster of several small drupes that are dark purple, almost black when ripe, and they are 2.5 cm (1 in) in diameter.Black mulberry is richly flavoured, similar to the red mulberry (*Morus rubra*) rather than the more insipid fruit of the white mulberry (*Morus alba*). Mulberry fruit color derives from anthocyanins.Sometimes other mulberry species are confused with black mulberry, particularly black-fruited individuals of the white mulberry. Black mulberry may be distinguished from the other species by the uniformly hairy lower surface of its leaves.A small to fairly large, slow-growing dioecious tree up to 35 m tall, with spreading crown, picturesque when old.Leaves broadly ovate, 5-16 cm √ó 5-16 cm, deeply cordate at base, shortly and bluntly acuminate at apex, rough above, pubescent below, with a striate, 2-3.5 cm long petiole.Male spikes 1.5-2.5 cm long, female spikes ovoid, 1-2 cm long; syncarp ovoid, 1.5-2.5 cm long, dark purple to black *M. nigra* is cultivated in humid regions, up to 2000 m altitude.

Crotalaria verrucosa

Family : Leguminosae

Genus : *Crotalaria*

Species: *verrucosa*



Locality: St.Mary's College. Botanical garden

Floristic features:

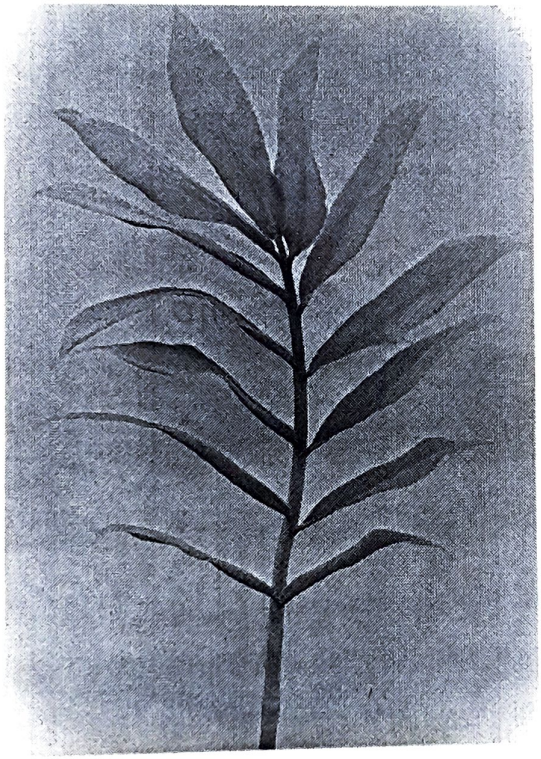
Crotalaria verrucosa, the blue rattle pod, is a species of flowering plant in the legume family, Fabaceae. This shrub belongs to the subfamily Faboideae. The herb found in Bangladesh, China, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Vietnam; Australasia and Africa and Americas regions . Annual, subwoody herb, 0.5-1 m tall, with many quadrangular, velvety hairy, yellow branches. Leaves simple, ovate to elliptical, 5-14 cm × 4-9 cm, pubescent; petiole 4-8 mm long; stipules sickle-shaped, 5-20 mm × 4-14 mm, auricled, persistent. Inflorescence a lax raceme, 5-25 cm long, leaf-opposed, with up to 24 flowers; pedicel filiform, up to 5 mm long; bracts linear-acuminate, 4 mm long. Calyx campanulate, 7-11 mm long, hairy, with subequal triangular-acuminate lobes; corolla blue; standard elliptical to suborbicular, 14 mm in diameter; wings ovate-oblong, 13 mm × 6 mm; keel 12 mm × 6 mm, incurved in the middle. Pod oblongoid, 3-5 cm × 0.8-1.2 cm, short stalked, about 16-seeded.Seed heart-shaped, 3 mm in diameter, blackish.C. verrucosa is found in fallow fields and on marshy ground, along rivers and roads, up to 1200 m altitude. It fixes nitrogen and is self-pollinating.

Lens culinaris

Family : Leguminosae

Genus : Lens

Species : culinaris



Locality : St. Mary's college Thoothukudi

Floristic features :

The plant is a diploid, annual, bushy herb of erect, semi-erect, or spreading and compact growth and normally varies from 30 to 50 centimetres (12 to 20 in) in height. It has many hairy branches and its stem is slender and angular. The rachis bears 10 to 15 leaflets in five to eight pairs. The leaves are alternate, of oblong-linear and obtuse shape and from yellowish green to dark bluish green in colour. In general, the upper leaves are converted into tendrils, whereas the lower leaves are mucronate. If stipules are present, they are small. The flowers, one to four in number, are small, white, pink, purple, pale purple, or pale blue in colour. They arise from the axils of the leaves, on a slender footstalk almost as long as the leaves. The pods are oblong, slightly inflated, and about 1.5 centimetres (⅝ in) long. Normally, each of them contains two seeds, about 0.5 centimetres (¼ in) in diameter, in the characteristic lens shape. The seeds can also be mottled and speckled. The several cultivated varieties of lentil differ in size, hairiness, and colour of the leaves, flowers, and seeds.

Senna siamea

Family: Leguminosae

Genus: *Senna*

Species: *siamea*

Locality: St.Mary's College. Botanical garden

Floristic features:

Tree, 6-12(-30) m tall, with spreading branches forming a dense rounded crown. Bark almost smooth, grey, young shoots ribbed. Leaves simply paripinnate, oblong-elliptical in outline, 10-35 cm long; stipules subulate, 1 mm long, very early caducous; petiole terete but with a shallow ventral groove, 1.5-3.5 cm long, glandless; rachis 4.5-25 cm long, glandless; petiole 2-4 mm long; leaflets in 4-16 pairs, sub-coriaceous, oblong to ovate-oblong, 3-8 cm × 1-2.5 cm, 2-4 times as long as wide, base unequal-sided rounded to cuneate, apex rounded to re use or blunt, often mucronate, glossy and glabrous above, dull and rough to delicately puberulous below. Inflorescence an erect, terminal, 10-60-flowered, leafy panicle, 15-60 cm long, composed of numerous dense corymbs up to 10 cm × 5-6 cm; peduncle robust, 5-7 cm long; bracts obovate in lower half, suddenly narrowing into a linear acute top 3—6 mm long, puberulous, early caducous; bracteoles absent; pedicel 2-3.5 cm long.Sepals 5, unequal, rounded-ovate, 4—9 mm long, thick, puberulous, repanding-reflexed, long persistent; petals 5, orbicular-obovate, 1-2 cm long, yellow, glabrous, standard with 1-2 mm long claw; stamens 10, 3 lower ones with 6 mm long filaments and 5 mm long anthers, 3 upper ones staminodial, 4 meridian ones with 3-4 mm long filaments and 5 mm long anthers; ovary shortly tomentellous, style 4-5 mm long, stigma punctiform.Pod flattened, 20—30-seeded, 15-30 cm × 12-16 mm, alternately bulging and depressed in the centre, rim thick, glabrescent, dull, finally dehiscent.Seed very flat ovoid, 6.5-8 mm × 6 mm, light brown, glossy; areole oblong-elliptical, 3-4.5 mm × 1 mm.

Rosa abietina

Family : Rosaceae

Genus : *Rosa*

Species: *abietina*

Locality: St.Mary's College. Botanical garden

Floristic features:

A rose is a woody perennial flowering plant of the genus Rosa, in the family Rosaceae, or the flower it bears. There are over three hundred species and tens of thousands of cultivars.[citation needed] They form a group of plants that can be erect shrubs, climbing, or trailing, with stems that are often armed with sharp prickles.[citation needed] Their flowers vary in size and shape and are usually large and showy, in colours ranging from white through yellows and reds.

Most species are native to Asia, with smaller numbers native to Europe, North America, and northwestern Africa.[citation needed] Species, cultivars and hybrids are all widely grown for their beauty and often are fragrant. Roses have acquired cultural significance in many societies.

Psidium guajava

Family: Myrtaceae

Genus: *Psidium*

Species: guajava

Locality: St.Mary's College, Botanical garden

Floristic features:

Shallow-rooted shrub or small tree, up to 10 m tall, branching from the base and often producing suckers. Bark smooth, green to red-brown, peeling off in thin flakes. Young twigs 4-angled and ridged, pubescent. Leaves opposite, glandular; petiole 3-10 mm long; blade elliptical to oblong, 5-15 cm × 3-7 cm, glabrous above, finely pubescent beneath, veins prominent below. Flowers solitary or in 2-3-flowered cymes, axillary, ca. 3 cm in diameter; calyx lobes 4-6, 1-1.5 cm long, irregular, persistent; petals 4-5, white, 1-2 cm long; stamens numerous, 1-2 cm long; ovary 4-5-locular; style 1.5-2 cm long, stigma capitate. Fruit a berry, globose, ovoid or pyriform, 4-12 cm long, surmounted by the calyx lobes; exocarp green to yellow; mesocarp fleshy, white, yellow, pink or red, with stone cells, sour to sweet and aromatic. Seeds usually numerous, embedded in pulp, yellowish, bony, reniform, 3-5 mm long.

Punicagranatum

Family: Lythraceae

Genus: *Punica*

Species: *granatum*

Locality: St.Mary's College, Botanical garden

Floristic features:

A shrub or small tree growing 5 to 10 m (16 to 33 ft) high, the pomegranate has multiple spiny branches and is extremely long-lived, with some specimens in France surviving for 200 years. *P. granatum* leaves are opposite or sub opposite, glossy, narrow oblong, entire, 3–7 cm (1+1⁄4–2+3⁄4 in) long and 2 cm (3⁄4 in) broad. The flowers are bright red and 3 cm (1+1⁄4 in) in diameter, with three to seven petals. Some fruitless varieties are grown for the flowers alone.

The Pomegranate is a favorite tree for the art of bonsai due to its small leaves, interesting deadwood and potential long life.

Lagerstroemia indica

Family : Lythraceae

Genus : *Lagerstroemia*

Species: *indica*

Locality: St.Mary's College. Botanical garden

Floristic features:

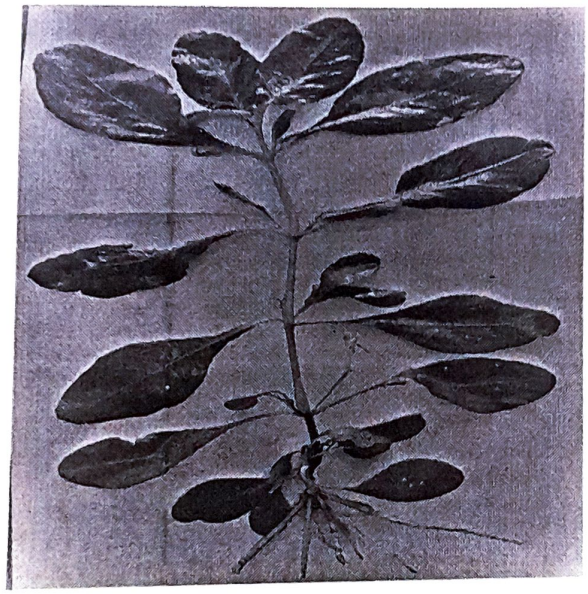
The crepe myrtle is a favorite of many southern gardeners. (Crepe myrtle is the preferred common name in the south). The draw for this plant is that it blooms at a time when most trees are not blooming. If the plant is healthy, it will be covered with blooms that will last for months during the hottest part of the summer. Crepe myrtles are deciduous, have a rapid growth rate, and are often seen in their multi-stemmed form. They will grow in almost any kind of soil: sand, loam or clay, although it prefers moist, well-drained soil and full sunlight. This plant is easily transplanted and is drought and alkaline tolerant, but it does have pest and disease problems. It is even possible to grow them in containers if they are watered and fertilized properly. They will grow in partial shade; however, the best flowering will occur on plants that receive more than 6 hours of direct sun. This plant blooms from June until fall, and to promote flower bloom, it is best to trim off seed pods. Fall color is not very impressive because the tree is tropical and loses it's leaves early. This plant can be grown as a street tree with ground cover underneath, as an espalier, or as a specimen. Crepe myrtle breeding and cultivation have produced several different colors of flowers, ranging from white to purple to every shade of red.

Ludwigia palustris

Family : Onagraceae

Genus : Ludwigia

Species : palustriis (L)



Locality : St. Mar'ys college botanical garden

Floristic Features:

The stem is up to half a meter (20 in) long and spreads to form mats on the mud, rooting at nodes in contact with the substrate, or floats ascending in the water. The leaves are oppositely arranged and green to red or purple in color. Solitary flowers appear in leaf axils. They are made up of tiny green sepals and no petals. They yield small capsular fruits containing many minute seeds. *Ludwigia palustris* is a species of flowering plant in the evening primrose family known by the common names marsh seed box, Hampshire-purslane and water purslane. This is an aquatic or semiaquatic perennial herb which grows in moist to wet to flooded areas. It is sometimes a weed. The species epithet *palustris* is Latin for "of the marsh" and indicates its common habitat.

Mammillaria prolifera

Family: Cactaceae

Genus: *Mammillaria*

Species: *prolifera*

Locality: St.Mary's College. Botanical garden

Floristic features:-

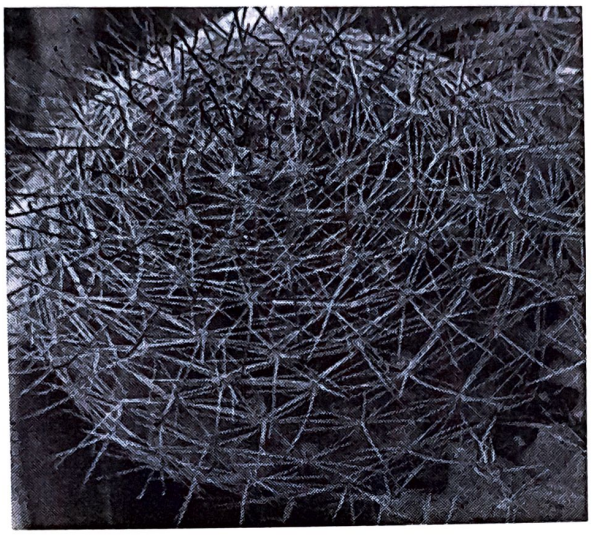
The species occurs very widely from Mexico (Coahuila, Nuevo León, Querétaro, San Luis Potosí and Tamaulipas), the USA (Texas), Cuba, the Dominican Republic, and Haiti. The typical subspecies prolifera occurs throughout much of the Caribbean. The species grows in submontane scrub. In Texas, it occurs in grasslands at low elevations and in Cuba, the plant grows in low, dry thicket. *Mammillaria prolifera* is quite inconspicuous but has a very large extent of occurrence and is abundant. Although there are threats in places (habitat destruction through logging), they are not sufficient to warrant any concern. *Mammillaria prolifera* is a low growing cactus, commonly branching to form colonies often 6 dm in diameter. The individual stems dark green, globose, cylindric or club shaped to 9 cm high, 3 to 7 cm in diameter, of soft texture. Tubercles were cylindrical to conic, about 8 mm long, spreading, without latex, Axils of tubercles with several long, hair-like bristles. Radial spines are 25-40, hair-like, often intergrading with the centrals, straight or twisted, white to yellow to brown, 3-12 mm long. Central spines are 5 to 12, needle-like, puberulent, 4-9 mm long, much stouter than the radials, straight, white to yellow to reddish, with darker tips. Flowers were 10-18 mm long, borne in old axils but toward top of plant, small, yellowish white, cream or pinkish yellow.

Mammillaria wrightii

Family: Cactaceae

Genus: *Mammillaria*

Species: *wrightii*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Mammillaria wrightii grows in gravelly or sandy hills or plains, desert grasslands to pinyon-juniper and surrounding areas. This species is often found among grasses in association with other plant species, including *Echinocereus polyacanthus* and *Mammillaria saboae* . *Mammillaria wrightii* is superficially similar to *Mammillaria grahamii* and the two species overlaps in the Franklin Mountains in New Mexico. *Mammillaria wrightii* is tiny soft-bodied cactus species with short, white dense spines growing parallel to the stem, partially hiding it. Its central spines are few, long, dark and hooked. Wondrous flowers, large and red-purple to magenta, appear in late summer followed by large palatable, grape-shaped fruits. The stem of this jewel is simple usually unbranched. Depressed-globose, to short cylindrical, dark green, 3-8 cm in diameter and 4-8 cm tall aboveground when expanded during moist periods, but during drought periods the stems become flat-topped and shrink to ground level, in part the result of a prolonged underground base.

Mammillaria winterae

Family: Cactaceae

Genus: *Mammillaria*

Species: *winterae*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Mammillaria winterae grows mainly in the bushes in rocky habitats in the Sierra Madre Oriental. Near Monterrey it grows on low hills, with lots of limestone in the soil and subsoil rocks. *Mammillaria winterae* is a spherical cactus species that grows flatted to the ground with a solitary body attaining a large size (up to 30 cm in diameter) and is one of the largest Mammillaria. It is a very distinctive plant with large, angled tubercles decorated by rings of pale greenish-yellow blooms. Plants usually solitary, sometimes forming clumps.Hemispherical to depressed globose, light green to blue green, 20-30 cm in diameter. Sap with latex. *Mammillaria winterae* is an easy to grow species, just as a classic cactus, don't requires any special treatment, that seems to enjoy, in cultivation, good root space, and well drained compost, though it is not a quick grower. It is an excellent plant for container growing. It always looks good and stays small. It look fine in a cold greenhouse and frame. The white, hooked spines of this spherical cactus were used as fish hooks in its native Mexico. It may be attractive to a variety of insects, but plants in good condition should be nearly pest-free, particularly if they are grown in a mineral potting-mix, with good exposure and ventilation.

Opuntia polyacantha

Family: Cactaceae

Genus: *Opuntia*

Species: *polyacantha*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Opuntia polyacantha grows up to 10–30 centimetres (4–12 in) tall. It forms low mats of pads which may be 2–3 m (6+1⁄2–9+7⁄8 ft) wide. Its succulent green pads are oval or circular and reach 27 by 18 cm (10+5⁄8 by 7+1⁄8 in) wide. Its areoles are tipped with woolly brown fibers and glochids. Many of the areoles have spines which are quite variable in size and shape. They may be 0.4 to 18.5 cm (1⁄8 to 7+1⁄4 in) in length, stout or thin, straight or curling, and any of a variety of colors. Flowers grow from spine-covered stem segments which are shaped like semi-flattened pears. The flowers are 2.5 to 4 cm (1 to 1+5⁄8 in) long and may be yellow, magenta, or red in color (tending to turn pink or orange with age). The fruit is cylindrical, brownish, dry and spiny. The cactus reproduces by seed, by layering, and by resprouting from detached segments. In its natural range it survives throughout an immense range of temperatures, ranging from −46 °C (−50 °F) in the Yukon Territory, Canada, to well above 38 °C (100 °F) in places like Chihuahua, Mexico. There are many expressions of *O. polyacantha* and variation is common. Multiple varieties have been proposed. Some are accepted by modern authorities and some require further study.

Acanthocereus tetragonus

Family: Cactaceae

Genus: *Acanthocereus*

Species: *tetragonus*

Locality: St.Mary's College. Botanical garden

Floristic features:-

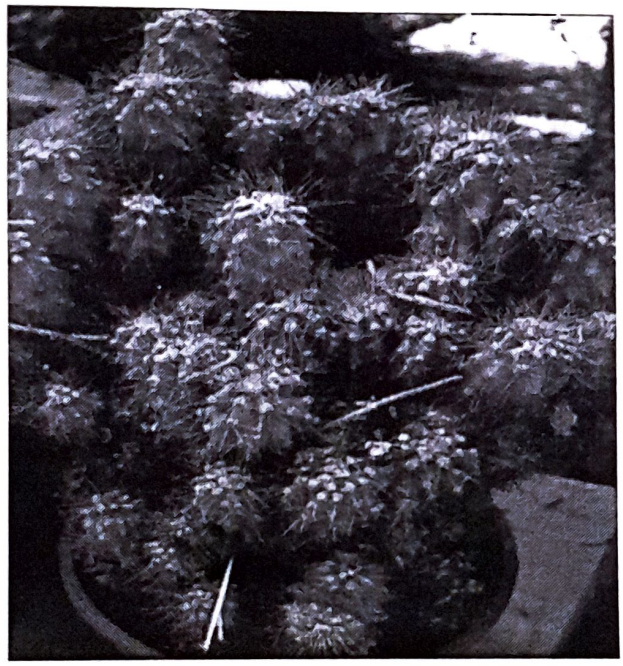
Acanthocereus tetragonus, is a species of cactus that is native to Florida and the Lower Rio Grande Valley of Texas in the United States, Mexico, Central America, the Caribbean, and northern South America. The species is invasive in New Caledonia. Common names include night-blooming cereus, barbed-wire cactus, sword-pear, dildo cactus, triangle cactus, and Organo-alado de pitaya (Spanish). The miniature cultivar is known as fairy castle cactus. It was originally described by Carl Linnaeus in 1753 as Cactus tetragonus but was moved to the genus Acanthocereus in 1938 by Pieter Wagenaar Hummelinck.

Cereus repandus

Family : Cactaceae

Genus: *Cereus*

Species: *repandus*



Locality: St.Mary's College. Botanical garden

Floristic features:-

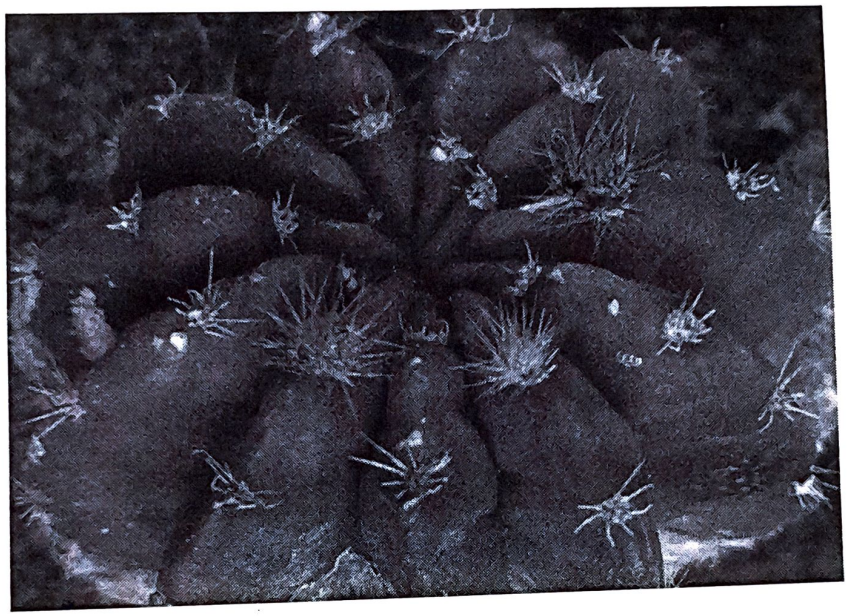
Cereus repandus (syn. *Cereus peruvianus*), the Peruvian apple cactus, is a large, erect, thorny columnar cactus found in South America. It is also known as giant club cactus, hedge cactus, cadushi (in Papiamento and Wayuunaiki), and kayush. With an often tree-like appearance, its cylindrical gray-green to blue stems can reach 10 metres (33 feet) in height and 10–20 cm in diameter as a self-supporting plant. However, if supported by a scaffold, *C. repandus* has grown to a height of 110 feet (34 meters) at the SDM College of Dental Sciences at Dharwad, Karnataka, India, technically making this the tallest cactus plant in the world, although no cactus under natural conditions exceeds eighty-two feet (25 meters) in height in the case of Cereus stenogonus. There are nine to ten rounded ribs that are up to 1 centimeter high. The small areoles on it are far apart. The gray, needle-like thorns are very variable. They are often numerous, but can also be missing entirely. The longest thorns are up to 5 centimeters long.

Echinopsis eyriesii

Family : Cactaceae

Genus: *Echinopsis*

Species: *eyriesii*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Echinopsis eyriesii is a species of cacti of the genus Echinopsis. *E. eyriesii* is a very popular large caespitose cactus, widely grown for the huge nocturnal flowers. It is the best known and most commonly grown globular cactus.In favorable conditions and after a long time, it may exceptionally forms large mounds up to 1,5 m tall and 2–3 m wide.It is globular, later elongated and almost cylindrical, 15–30 cm high, 12 to 15 cm thick, dark green and heavily ribbed (9 to 18). On the ribs, there are circular areoles with very short spines (10 to 18).The flower is very spectacular. The floral stem grows slowly (one month) up to 20 cm long. Then, on a spring or summer evening, the flower (white or light pink) opens with 10–12 cm in diameter. There is a light and delicious smell. It fades the next day, but in cold, rainy weather it may last two, rarely three days. There is a variety without spines : *Echinopsis eyriesii* var. *inermis*, sometimes named *Echinopsis inermis*.

Schefflera actinophylla

Family : Araliaceae

Genus : *Schefflera*

Species : *actinophylla*

Locality: St.Mary's College. Botanical garden

Floristic features:

Heptapleurum actinophyllum is an evergreen tree growing to 15 m (49 ft) tall. It has palmately compound medium green leaves in groups of seven leaflets. It is usually multi-trunked, and the flowers develop at the top of the tree. It often grows as a hemiepiphyte on other rainforest trees. It produces racemes up to 2 m (6.5 ft) long containing up to 1,000 small red flowers. Flowering begins in early summer and typically continues for several months

Hamelia patens

Family: Rubiaceae

Genus: *Hamelia*

Species: *Hamelia patens*

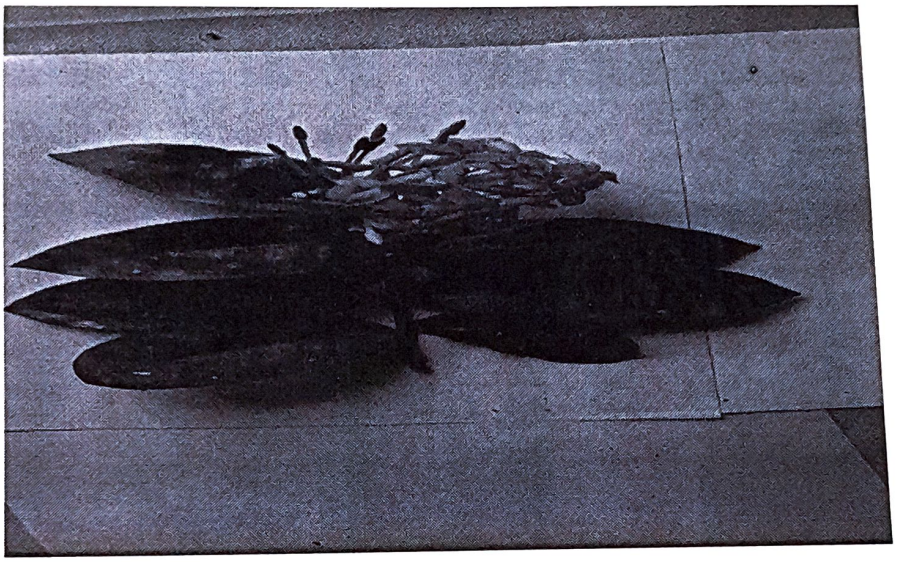
Locality: St.Mary's College. Botanical garden

Floristic features:

Hamelia patens is a large perennial shrub or small tree in the family Rubiaceae, that is native to the American subtropics and tropics. Its range extends from Florida in the southern United States to as far south as Argentina. Common names include firebush, hummingbird bush, scarlet bush, and redhead. In Belize, this plant's Mayan name is Ix Canaan and is also known as "Guardian of the Forest".Firebush has orangish-red tubular flowers, which recruit hummingbirds and butterflies for pollination. The corollas vary greatly in length, making them attractive to a wide range of pollinators.The fruit is a small dark red berry, turning black at maturity.Despite its somewhat scraggy appearance, this is a valuable garden tree in warmer climates and even in temperate ones, as long as the soil remains above freezing.

Ixora coccinea

Family: Rubiaceae
Genus: *ixora*
Species: *coccinea*



Locality : St. Mary's college botanical garden

Floristic features:

Ixora coccinea (also known as jungle geranium, flame of the woods or jungle flame or pendkuli) is a species of flowering plant in the family Rubiaceae. It is a common flowering shrub native to Southern India, Bangladesh, and Sri Lanka. It has become one of the most popular flowering shrubs in South Florida gardens and landscapes. It is the national flower of Suriname.
Ixora coccinea is a dense, multi-branched evergreen shrub, commonly 4–6 ft (1.2–1.8 m) in height, but capable of reaching up to 12 ft (3.7 m) high. It has a rounded form, with a spread that may exceed its height. The glossy, leathery, oblong leaves are about 4 in (10 cm) long, with entire margins, and are carried in opposite pairs or whorled on the stems. Small tubular, scarlet flowers in dense rounded clusters 2–5 in (5.1–12.7 cm) across are produced almost all year long.

Senecioangulatus

Family: Compositae

Genus: *Senecio*

Species: *angulatus*

Locality : St. Mary's college botanical garden

Floristic features:

Its form is a dense tangled shrub 2 metres (6.6 ft) tall or a climber that can reach 6metres (20 ft) high, if suitable support is available.The leaves are rhombic to ovate, 3 centimetres (1.2 in) to 5 centimetres (2.0 in) long and 1 centimetre (0.39 in) to 5 centimetres (2.0 in) wide and occur in 1-4 pairs. They are thick, glossy, fleshy and coarsely toothed, with one to three teeth each side and bluntly lobed,with upper leaves becoming smaller with fewer teeth or none at all. They have a frosted look from a powdery coating on the lower side.Leaf stalks are 1 centimetre (0.39 in) to 4 centimetres (1.6 in) long.The stems are succulent, pale green, and are often variegated with pale yellow green and purple. They are slightly angular (not upright) and usually sparingly branched. Neither stems nor leaves are hairy. Inflorescence of *Senecioangulatus* produces numerous flowers in open clusters at the end of its branches or stems.The honey-scented flowers are on an elongated stem and open in succession from the base up as the stem continues to grow. The flower clusters are more flat at the top than pyramid-like, and are 4 centimetres (1.6 in) to 8 centimetres (3.1 in) in diameter.Often the cluster droops with the flower heads at the end of the cluster turning upwards.Flower stalks are mostly hairless or with some short hairs, 6.5 millimetres (0.26 in) to 10.5 millimetres (0.41 in) long.

Maranta arundinacea

Family : Marantaceae

Genus : *Maranta*

Species : *arundinacea*

Locality : St.Mary's college Botanical garden

Floristic features :

Arrowroot is a perennial plant growing to a height of between 0.3 m (1 ft) and 1.5 m (5 ft). Its leaves are lanceolate. The edible part of the plant is the rhizome. Twin clusters of small white flowers bloom about 90 days after planting. The plant rarely produces seed and reproduction is typically by planting part of a rhizome with a bud. Rhizomes are ready for harvesting 10–12 months after planting as leaves of the plant begin to wilt and die. The rhizomes are fleshy, cylindrical, and grow from 20 cm (8 in) to 45 cm (18 in) long.The arrowroot plant probably originated in the Amazon rainforest of north-western Brazil and neighboring countries. It grows best between temperatures of 23 °C (73 °F) and 29 °C (84 °F) with annual precipitation between 150 cm (59 in) and 200 cm (79 in). The dormant rhizomes can withstand temperatures as low as 5 °C (41 °F). In the continental United States, arrowroot is cultivated as an outside plant only in southern Florida.

Iberis umbellata

Family : Brassiaceae
Genus : *Iberis*
Species : *umbellata*

Locality : St. Mary's college botanical garden

Floristic features:

The biological form of *Iberis umbellata* is hemicryptophyte scapose, as its overwintering buds are situated just below the soil surface and the floral axis is more or less erect with a few leaves.
The stem is twisted at the base while the flowering branches are erect and leafy. This plant reaches a height of 30–50 centimetres (12–20 in). The leaves are green and linear-lanceolate, 15–25 millimetres (0.59–0.98 in) long. The flowers are in umbel-shaped corymbs. The calyx is violet and the corolla is composed of four white, pink or purple petals. The petals are rounded at the apex, with the peripheral ones forming a large vexillum 8–10 millimetres (0.31–0.39 in) long. The flowering period extends from May through June. The flowers are hermaphroditic and pollinated by bees and butterflies. The fruit is a silique 7–10 millimetres (0.28–0.39 in) long.

Mimusops elengi L.

Family: Sapotaceae

Genus: *mimusops*

Species: *elengi L.*

Locality: St.Mary's College. Botanical garden

Floristic features:

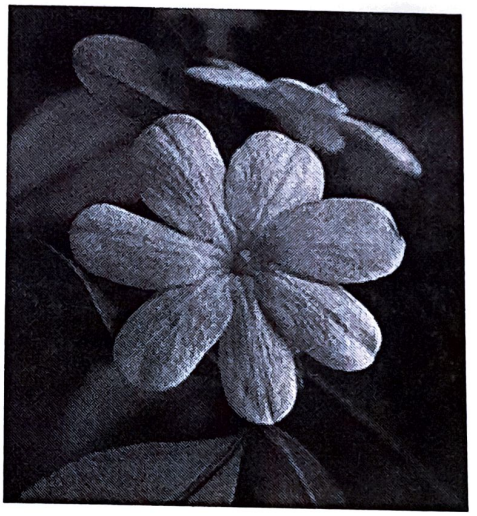
Mimusops elengi is a medium-sized evergreen tree found in tropical forests in South Asia, Southeast Asia and northern Australia. English common names include Spanish cherry,[2] medlar,[2] and bullet wood.[3] Its timber is valuable, the fruit is edible, and it is used in traditional medicine. As the trees give thick shade and flowers emit fragrance, it is a prized collection of gardens.Its flower is the provincial flower of Yala Province, Thailand, as well as the city flower of Ampang Jaya, Selangor.Bullet wood is an evergreen tree reaching a height of about 16 m (52 ft). It flowers in April, and fruiting occurs between June and October. The leaves are glossy, dark green, oval-shaped, 5–14 cm (2.0–5.5 in) long, and 2.5–6 cm (0.98–2.36 in) wide. The flowers are cream, hairy, and scented. The fruits are fleshy, range in color between yellow and brown, and contain a large brown seed. The pulp has a yellow color and it is edible. The bark of the tree is thick and appears dark brownish black or grayish black in colour, with striations and a few cracks on the surface. The tree may reach up to a height of 9–18 m (30–60 ft) with about 1 m (3 ft 3 in) in circumference.

Jasminum mesnyi

Family : Oleacae

Genus : *Jasminum*

Species : *mesnyi*



Locality : St. Mary's college botanical garden.

Floristic features :

It as an evergreen shrub growing to 3m tall and 2m wide. The stems are angular and deep green in colour .Leaves are opposite, petiolate, trifoliate or simple at base of branchlets, leaflets, sessile or sub-sessile .Flowers are usually solitary, axillary or rarely terminal and fragrant in nature. Calyx contains 5-8 lobes and green in colour. Corolla contains 6-8 lobes and yellow in colour. Fruit is ellipsoid berry and grown upto cm in long and 6mm in diameter.

Flowering and fruiting period – November to February.

Asplenium scolopendrium

Family : Aspleniaceae

Genus : *Asplenium*

Species : *scolopendrium* .

Locality: St.Mary's College. Botanical garden

Floristic features:

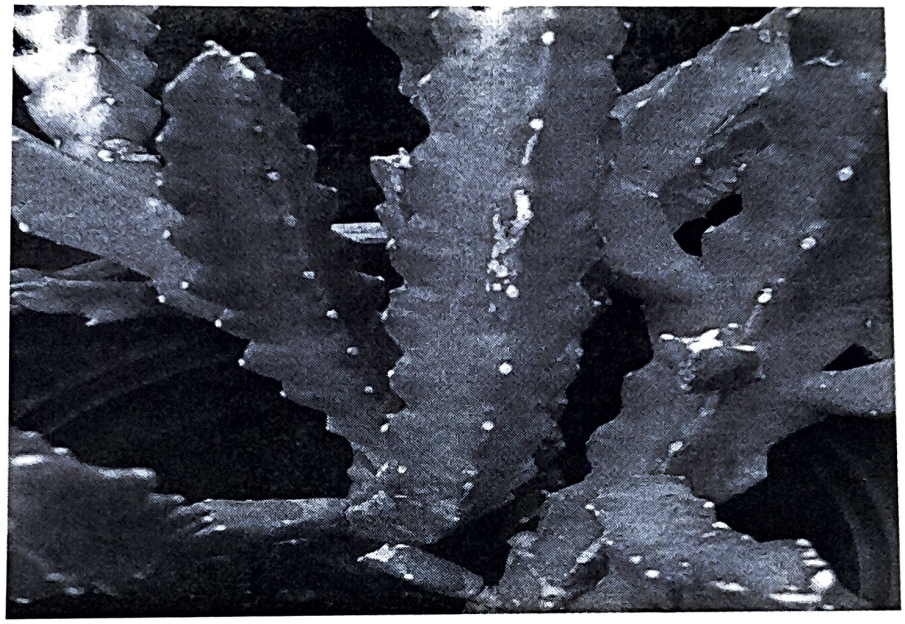
The most striking and unusual feature of the fern is its simple, undivided fronds. The leaves' supposed resemblance to the tongue of a hart (an archaic term for a male red deer) gave rise to the common name "hart's-tongue fern". The Latin specific epithet scolopendrium is derived from the Greek skolopendra, meaning a centipede or millipede; this is due to the sori pattern being reminiscent of a myriapod's legs. The leaves are 10–60 cm long and 3–6 cm broad, with sori arranged in rows perpendicular to the rachis

Stapelia grandiflora

Family: Apocynaceae

Genus: *Stapelia*

Species: *grandiflora*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Stapelia grandiflora is a species of flowering plant in the genus Stapelia of the family Apocynaceae. It is commonly referred to as the carrion plant, starfish flower, giant toad plant, or starfish cactus, although it is not related to cacti at all. This "carrion plant" nickname can also refer to similar *Stapelia* species as well as members of related genera, including *Stapelia gigantea* and *Orbea variegata*. *Stapelia grandiflora* sometimes also goes by the name of *Stapelia flavirostris*. The plant is native to South Africa, including the Northern Cape, Eastern Cape, and Free State. This plant is a very variable species, with many hybrids. The stems can be either erect or ascending 9-10 (-30) cm long and up to 3 cm in diameter (usually less than 2 cm).The flowers are velvety and smaller in size than those of *Stapelia gigantea*, they come in various shapes and colors. Flowers are intermittently produced in the late summer and fall seasons. The name "carrion plant" is due to the odor emitted by the flowers as a technique of attracting flies in areas where other pollinating insects are scarce.

Asparagus densiflorus

Family : Asperagaceae

Genius : *Asparagus*

Species : *densiflorus*

Locality: St.Mary's College. Botanical garden

Floristic features:

Densiflorus is a spiny perennial plant, commonly found in savanna thickets in its native environment in eastern Africa and South Africa. It has been widely introduced globally as an ornamental and has subsequently naturalised and become a problem in a number of countries, including the USA and Australia.

Asparagus Fern is an erect, perennial herb. It is somewhat woody, and branches gracefully with an arching and fern-like habit. Its leaves and alternate and scale-like. The terminal branchlets are very narrow, flat, and needle-like, and form in clusters of 3. Its yellow-green flowers are axillary, drooping, 6-parted, and bell-shaped. The fruit is a bright red berry. Asparagus densiflorus is moderately salt tolerant.

Chorophytum comosum

Family : Asparagaceae

Genus : *Chorophytum*

Species : *omosum*

Locality: St.Mary's College. Botanical garden

Floristic features:

Flowers are produced in a long, branched inflorescence, which can reach a length of up to 75 cm (30 in) and eventually bends downward to meet the earth. Flowers initially occur in clusters of 1–6 at intervals along the stem (scape) of the inflorescence. Each cluster is at the base of a bract, which ranges from 2–8 cm (0.8–3.1 in) in length, becoming smaller toward the end of the inflorescence. Most of the flowers that are produced initially die off, so that relatively, the inflorescences are sparsely flowered.

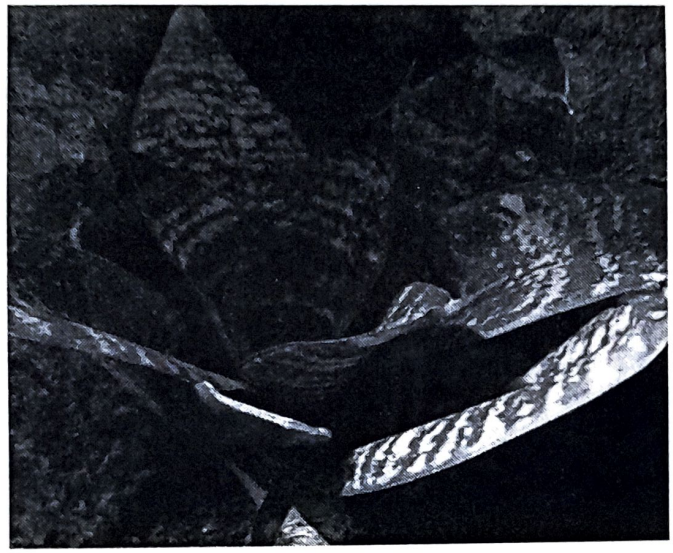
Individual flowers are greenish-white, borne on stalks (pedicels) some 4–8 mm (0.2–0.3 in) long. Each flower has six triply veined tepals that are 6–9 mm (0.2–0.4 in) long and slightly hooded or boat-shaped at their tips. The stamens consist of a pollen-producing anther about 3.5 mm (0.1 in) long with a filament of similar length or slightly longer. The central style is 3–8 mm (0.1–0.3 in) long. Seeds are produced in a capsule, 3–8 mm (0.1–0.3 in) long, on stalks (pedicels) that lengthen to up to 12 mm (0.5)

Sansevieria trifasciata

Family: Asparagaceae

Genus: *Sansevieria*

Species: *trifasciata*



Locality: St.Mary's College. Botanical garden

Floristic features:-

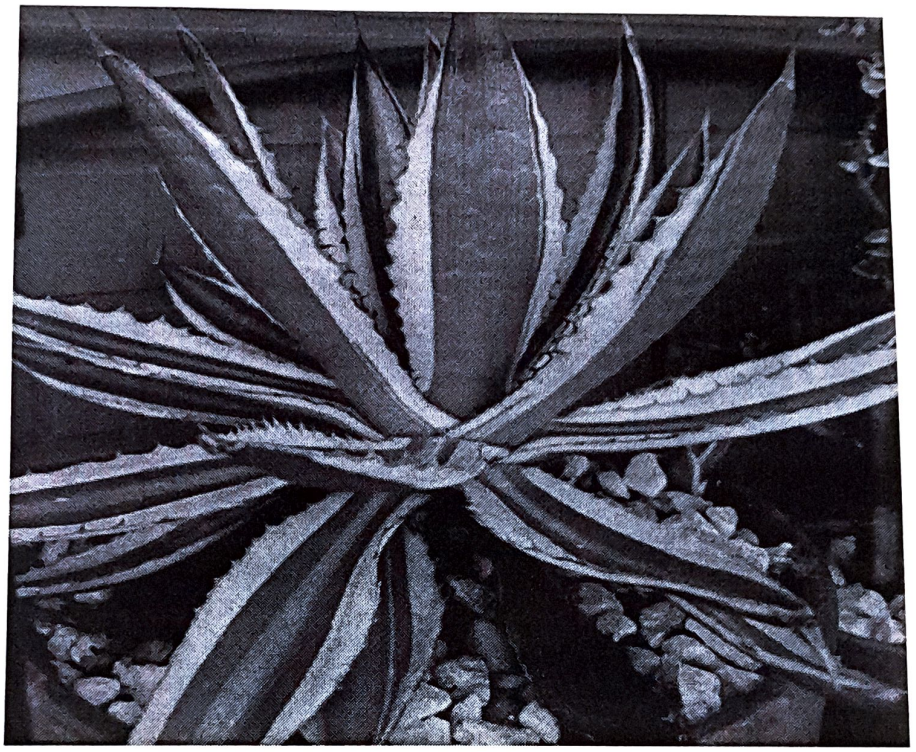
Sansevieria trifasciata is a species of flowering plant in the family Asparagaceae, native to tropical West Africa from Nigeria east to the Congo. It is most commonly known as the snake plant, Saint George's sword, mother-in-law's tongue, and viper's bowstring hemp, among other names. Until 2017, it was known under the synonym *Sansevieria trifasciata*. It is an evergreen perennial plant forming dense stands, spreading by way of its creeping rhizome, which is sometimes above ground, sometimes underground. Its stiff leaves grow vertically from a basal rosette. Mature leaves are dark green with light gray-green cross-banding and usually range from 70–90 centimetres (2.3–3.0 ft) long and 5–6 centimetres (2.0–2.4 in) wide, though it can reach heights above 2 m (6 ft) in optimal conditions.[3]The specific epithet trifasciata means "three bundles". The plant exchanges oxygen and carbon dioxide using the crassulacean acid metabolism process, which allows them to withstand drought. The microscopic pores on the plant's leaves, called the stomata and used to exchange gases, are opened only at night to prevent water from escaping via evaporation in the hot sun. It is a weed in some parts of northern Australia. To get this plant to go into bloom outside of its natural environment is difficult. Replicating its natural environment is possible. Its flowers vary from greenish white to cream-colored, are not fragrant, and have a sticky texture.

Agave univittata

Family: Asparagaceae

Genus: *Agave*

Species: *univittata*



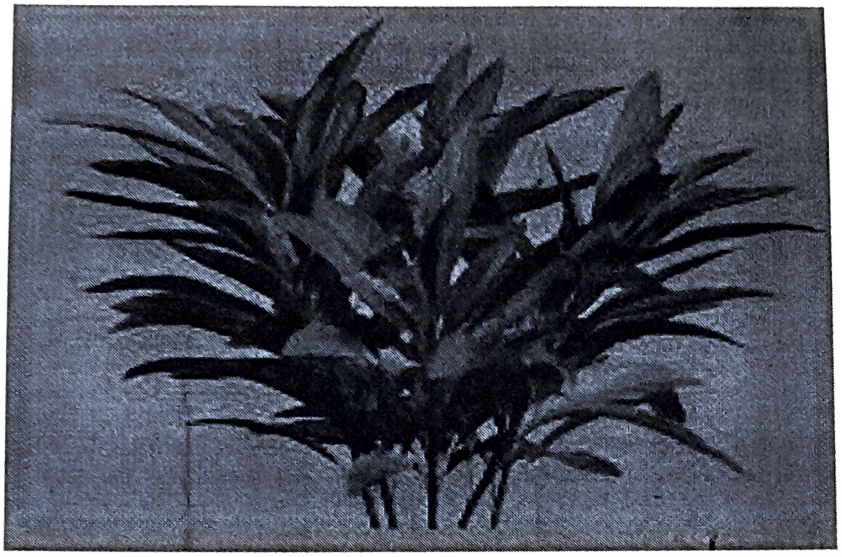
Locality: St.Mary's College. Botanical garden

Floristic features:-

Agave univittata, the thorn-crested century plant or thorn-crested agave, is a plant species native to coastal areas of southern Texas and northeastern Mexico, at elevations less than 100 m (300 feet). It has been widely named *Agave lophantha* by botanists including Howard Scott Gentry,but the name *A. univittata* is older and therefore more in accord with nomenclatural rules of botany.*Agave univittata* has thick, fleshy leaves that are stiff and undulate (wavy) along the margins. It has sharp and prominent spines on the edges and tips of the leaves. Flowering stalk is up to 5 m (16 feet) tall, bearing greenish-white to yellowish green flowers.It is cultivated as an ornamental plant, and in the UK the cultivar 'Quadricolor' has won the Royal Horticultural Society's Award of Garden Merit.Because the species is widespread and the overall population is stable, it is not considered by the IUCN to be threatened

Cordyline fruticose

Family : Asparagaceae
Genus : *Cordyline*
Species : *fruticose*



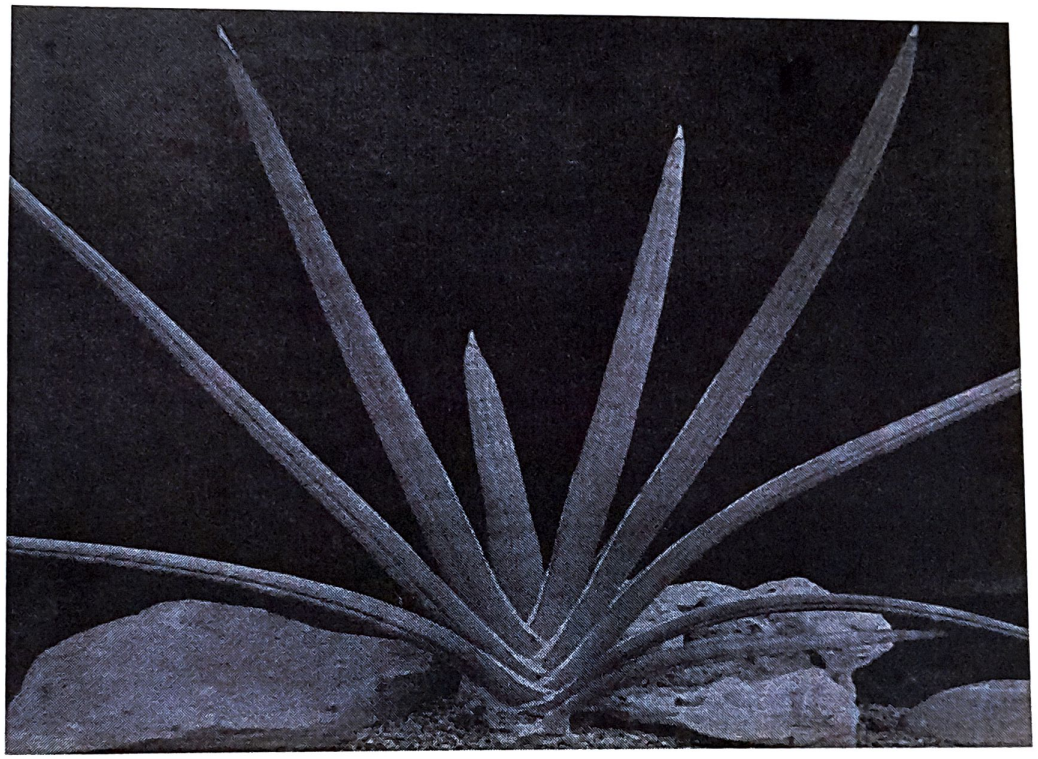
Locality : St.Mary's college botanical garden

Floristic feature:

A shrub or small tree to 15 ft (4.6 m) high with light grey smoothish trunk to 3 inches (7.5 cm) in diameter, becoming warty and slightly cracked, with horizontal rings, not divided into bark and wood. Within the thin brown outer layer, the trunk is whitish, soft, and bitter.Leaves are alternate but very crowded in a spiral at end of erect stout hairless branch, with stout grooved greenish leafstalk of 2–4 inches (5–10 cm), hairless. Blades narrowly oblong, 7–18 inches (18–45 cm) long and 2–4 inches (5–10 cm) wide, broadest near middle and gradually narrowed to long-pointed ends, not toothed on edges, thin and flexible, with many long fine parallel veins, shiny green on both surfaces, leaving a ring scar.Flower clusters (panicles) large, arising from center of cluster of leaves, 12–15 inches (30–38 cm) long, curved and branched. Flowers many, stalkless on slender drooping branches, from narrow whitish buds 0.5 inches (13 mm) long, tinged with purple, composed of narrow calyx whitish tube with six pointed lobes curled back, six yellow spreading stamens inserted in throat, and white pistil with three-celled ovary and slender style.Fruits (berries) rarely formed, about 0.4 inches (6 mm) in diameter, yellow, turning to bright red. Seeds few, shiny black.

Sansevieria personii

Family : Asparagaceae
Genus : *Sansevieria*
Species *: personiii*



Locality : St.Mary's college botanical garden

Floristic feature:

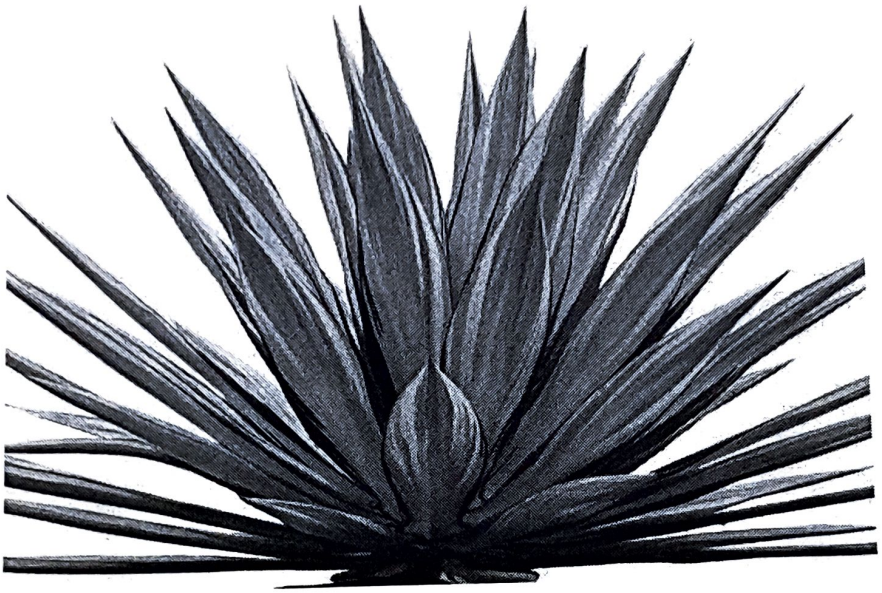
Sansevieria pearsonii is a is a stemless evergreen perennial plant, producing succulent, erect, rigid leaves from 45 - 120cm long and 45 - 60mm wide from a spreading, rhizomatous rootstock The plant is harvested from the wild for local use as a source of a strong fibre.

Furcraea foetida

Family : Asparagaceae

Genus : *Furcraea*

Species : *foetida*



Locality : St.Mary's college botanical garden

Floristic features:

Furcraea foetida is an evergreen perennial subshrub, stemless or with a short stem up to 1 m tall. The leaves are sword-shaped, 1-1.8 m long and 10–15 cm broad at their widest point, narrowing to 6–7 cm broad at the leaf base, and to a sharp spine tip at the apex; the margins are entire or with a few hooked spines. The flowers are greenish to creamy white, 4 cm long, and strongly scented; they are produced on a large inflorescence up to 7.5 m tall.

Agave attenuata

Family : Asparagaceae
Genus : *Agave*
Species : *attenuate*

Locality : St.Mary's college botanical garden

Floristic features :

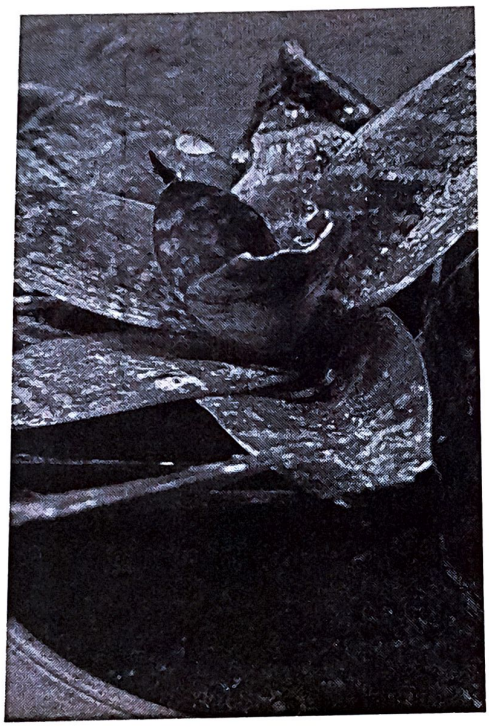
Although the plant can appear acaulescent, stems often reach 50 to 150 cm (20–60 in) in length, and old leaves fall off, leaving the stems visible. The leaves are ovate-accuminate, 50–70 cm (20–28 in) long and 12–16 cm (5–6 in) wide, pale in color, ranging from a light gray to a light yellowish green. There are no teeth, nor terminal spines, although the leaves taper to soft points that fray with age. The numerous, broad, succulent, tapering leaves are slightly less rigid than most Agave species' leave;, they are a bright glaucous gray to light yellowish-green and stingless.
The inflorescence is a dense raceme 2.5 to 3 meters (8 to 10 ft) high (usually curved), with greenish-yellow flowers, growing after many years. As with other *Agave* species, the plant dies following seed development, but numerous suckers consequently sprout, both from the base of the plant and from the flower raceme.

Sanseviera trifasciata

Family : Asparagaceae

Genus : *Sanseviera*

Species : *trifasciata*



Locality : St. Mary's college botanical garden

Floristic features :

It is an evergreen perennial forming dense stands, spreading by way of its creeping rhizome which is sometimes above ground, sometimes underground. Its stiff leaves grow vertically from a basal rosette. Mature leaves are dark green with light grey-green cross-banding and usually range from 70–90 centimetres (2.3–3.0 ft) long and 5–6 centimetres (2.0–2.4 in) wide, though it can reach heights above 2 m (6 ft) in optimal conditions. It is now used predominantly as an ornamental plant, outdoors in warmer climates, and indoors as a houseplant in cooler climates. It is popular as a houseplant because it is tolerant of low light levels and irregular watering; during winter, it needs only one watering every couple of months. The plant contains saponins which are mildly toxic to dogs and cats and can lead to gastrointestinal upset if consumed.

Meremmia dissecta

Family : Convolulaceae
Genus : *Meremmia*
Species : *dissecta*

Locality : St.Mary's college botanical garden

Floristic feature:
Merremia dissecta is a perennial climbing plant with slender twining stems growing from a long, thin taproot. The stems can be 3 - 6 metres long and up to 2cm in diameter, scrambling over the ground and twining into nearby vegetation for support.

Solanum americanum

Family : Solanaceae

Genius : *Solanum*

Species : *americanum*

Locality: St.Mary's College. Botanical garden

Floristic features:

Solanum americanum grows up to 1–1.5 metres (39–59 in) tall and is an annual or short-lived perennial. The leaves are alternate on the branch, and vary greatly in size, up to 10 centimetres (3.9 in) long and 7 centimetres (2.8 in) broad, with a 4-centimetre (1.6 in) petiole and a coarsely wavy or toothed margin. The flowers are about 1 cm diameter, white or occasionally light purple, with yellow stamens. The fruit is a shiny black berry 5–10 millimetres (0.20–0.39 in) diameter, containing numerous small seeds.

Datura innoxia

Family: Solanaceae

Genus: *Datura*

Species: innoxia

Locality: St.Mary's College, Botanical garden

Floristic features:

Daturainnoxia is a tuberous-rooted, subshrub that typically reaches a height of 0.6 to 1.5 metres. Its stems and leaves are covered with short and soft grayish hairs, giving the whole plant a grayish appearance. It has elliptic smooth-edged leaves with pinnate venation. All parts of the plant emit a foul odor similar to rancid peanut butter when crushed or bruised, although most people find the fragrance of the flowers to be quite pleasant when they bloom at night.

The flowers are white, trumpet-shaped, 12–19 cm (4.5–7.5 in) long. They first grow upright, and later incline downward. It flowers from early summer until late fall. The fruit is an egg-shaped spiny capsule, about 5 cm in diameter. Like those of other species belonging to section Dutra of the genus *Datura*, it splits open irregularly when ripe to disperse its seeds. Another means of dispersal may also occur, in which the spiny fruit becomes entangled in the fur of animals, who then carry the fruit far from the mother plant. The seeds are long-lived, having the ability to lie dormant in the soil for many years.

Ruellia simplex

Family: Acanthaceae

Genus: *Ruellia*

Species: *simplex*

Locality: St.Mary's College. Botanical garden

Floristic features:

Ruellia simplex is an evergreen perennial growing 3 ft (0.91 m) tall, forming colonies of stalks with lance-shaped leaves that are 6 to 12 in (15 to 30 cm) and .5 to .75 in (1.3 to 1.9 cm) wide. Trumpet shaped flowers are metallic blue to purple, with five petals, and 3 in (7.6 cm) wide. There is a dwarf variety that is only 1 ft (0.30 m) tall. Ruellia simplex, the Mexican petunia, Mexican bluebell or Britton's wild petunia, is a species of flowering plant in the family Acanthaceae. It is a native of Mexico, the Caribbean, and South America. It has become a widespread invasive plant in Florida, where it was likely introduced as an ornamental before 1933. Growth Form Short, herbaceous plant with erect growth habit. Foliage leaves are narrow and linear with distinct purplish veins. The venation is pinnate or feather-like; the veins curve to the tip and run nearly parallel to the leaf edges. Tubular flowers are light purple and 5-lobed. Petals are approximately round to obovate with irregular edges and a longitudinal groove in the middle. Fruits are light brown fruits are long, cylindrical seed pods with pointed tips.

Hygrophila auriculata

Family: Acanthaceae

Genus: *Hygrophila*

Species: *auriculata*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Hygrophila auriculata is a herbaceous, medicinal plant in the acanthus family that grows in marshy places and is native to tropical Asia and Africa. In India it is commonly known as kokilaksha or gokulakanta, in Sri Lanka as neeramulli. In Kerala it is called vayalchulli . In Tamil it is called Neermulli .Hygrophilla or marsh barbel (English) It is commonly called in Tamil as a niramuli. An annual herbal plant growing up to 60 cm in height. The stem of the plant is tetragonal, hairy and stiff at the nodes. The bark is dark brown, although the leaves are elliptic-lanceolate and herpid. The flowers are purple and to a lesser extent violet blue. The fruit resembles a four-sided shape, linear, glabrous and about 1 cm long with seeds that are hairy and brown in colour. In ayurveda, its seeds, roots and panchang (pancha = five and ang = parts, i.e. root, flowers, stem, fruits and leaves as ash burnt together) are used as a medication.

Crossandra infundibuliformis (L)

Family : Acanthaceae

Genus : Crossandra

Species : infundibuliformis

Locality : St. Mary's college botanical garden

Floristic features:

It is an erect evergreen subshrub growing to 1 m with glossy, wavy-margined leaves and fan-shaped flowers, which may appear at any time throughout the year. The flowers are unusually shaped with 3 to 5 asymmetrical petals. They grow from four-sided stalked spikes, and have a tube-like 2 cm stalk. Flower colours range from the common orange to salmon-orange or apricot, coral to red, yellow and even turquoise. The tiny flowers are often strung together into strands, sometimes along with white jasmine flowers and therefore in great demand for making garlands which are offered to temple deities or used to embellish women's hair.

Tradescantia spathacea

Family : Commelinaceae

Genus : *Tradescantia*

Species : *spathacea*.

Locality: St.Mary's College. Botanical garden

Floristic features:

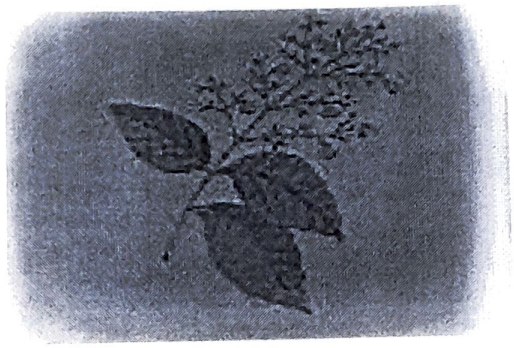
Tradescantia spathacea has fleshy rhizomes and rosettes of waxy lance-shaped leaves. Leaves are dark to metallic green above, with glossy purple underneath. These will reach up to 0.30 m (1 ft) long by 76 mm (3 in) wide. They are foliage plants that reach a height of around 0.30 m (1 ft). They are hardy in USDA zones 9-12 and are also grown as ornamental houseplants.

Tectona grandis

Family : Verbenaceae

Genus : *Tectona*

Species : *grandis*



Locality : St. Mary's college botanical garden

Floristc features :

Tectona grandis is a large, deciduous tree reaching over 30 m in height in favourable conditions. Crown open with many small branches; the bole is often buttressed and may be fluted, up to 15 m long below the 1st branches, up to 1 m dbh. Bark is brown, distinctly fibrous with shallow, longitudinal fissures. The root system is superficial, often no deeper than 50 cm, but roots may extend laterally up to 15 m from the stem.The very large, 4-sided leaves are shed for 3-4 months during the later half of the dry season, leaving the branchlets bare. Shiny above, hairy below, vein network clear, about 30 x 20 cm but young leaves up to 1 m long.Flowers small, about 8 mm across, mauve to white and arranged in large, flowering heads, about 45 cm long; found on the topmost branches in the unshaded part of the crown.Fruit is a drupe with 4 chambers; round, hard and woody, enclosed in an inflated, bladder-like covering; pale green at first, then brown at maturity. Each fruit may contain 0 to 4 seeds. There are 1 000-3500 fruits/kg.

Rhapis excelsa

Family : Arecaceae

Genus : *Rhapis*

Species : *excelsa*

Locality: St.Mary's College. Botanical garden

Floristic features:

Rhapis excelsa grows up to 4 m in height and 30 mm in diameter in multi-stemmed clumps with glossy, palmate evergreen leaves divided into broad, ribbed segments. Leaf segments are single or few in young plants and increase to a dozen or more in mature plants, segments are divided to the petiole. Leaf-ends are saw-toothed unlike most other palms, occurring on slender petioles ranging from 20 to 60 cm in length. New foliage emerges from a fibrous sheath which remains attached to the base. As the plants age, the sheaths fall, revealing the bamboo-like trunks. This usually dioecious palm species produces a small inflorescence at the top of the plant with spirally-arranged, fleshy yellow flowers containing three petals fused at the base. Ripe fruit are fleshy and white, though *R. excelsa* more readily propagates via underground rhizome off shoots.

Chamaedorea elegans

Family : Arecaceae

Genus : *Chamaedorea*

Species : *elegans*

Locality: St.Mary's College. Botanical garden

Floristic features:

A woody, rhizomatous plant with a slender green trunk, it is found in tropical areas and grows to 2–3 m (6 ft 7 in – 9 ft 10 in) tall (rarely to 4–5 m (13–16 ft). It has 1.2 centimetres (1⁄2 in)-long ringed stigma, punctured crescent-shaped leaves, erect buds, and flexible tubular stems without spines with generally pinnate foliage. The crown carries 3-10 long-leaf pinnate leaves (more when mature). A remarkable feature of this species is the early age of the onset of flowering, with some plants blooming with a height of only 30 centimetres (12 in).

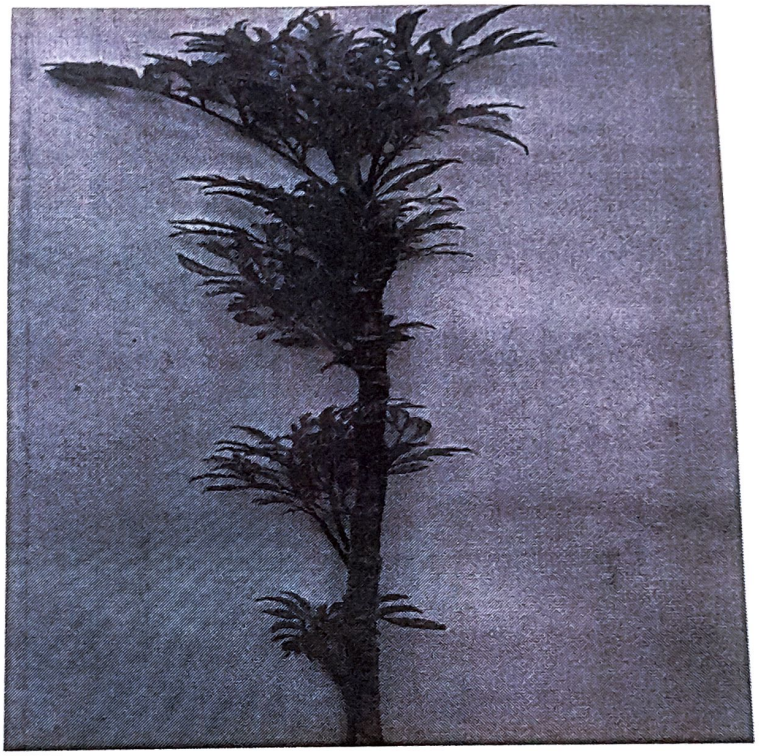
The small, light yellow, yellow, or orange-red odorous flowers appear on irregularly branched petioles that grow below or among the leaf. They emerge from the trunk as lateral buds and open in the form of clusters of small balls without petals. These have a certain resemblance to those of the mimosa. Occasionally, pea-sized berries develop after flowering, which are 6mm in diameter, and dark, collected in paniculate inflorescences.

Polyscias fruticosa

Family : Arecaceae

Genus : *Polyscias*

Species : *fruticosa*



Locality: St.Mary's College. Botanical garden

Floristic features:

Polyscias fruticosa, or Ming aralia, is a perennial plant, dicot evergreen shrub or dwarf tree native to India. The plant grows fairly slowly but can reach up to 1 to 2 meters in height. The leaves are of a dark green pigment, glossy in texture, and are tripinnate and appear divided. Individual leaves vary from narrowly ovate to lanceolate and are about 10 cm long.

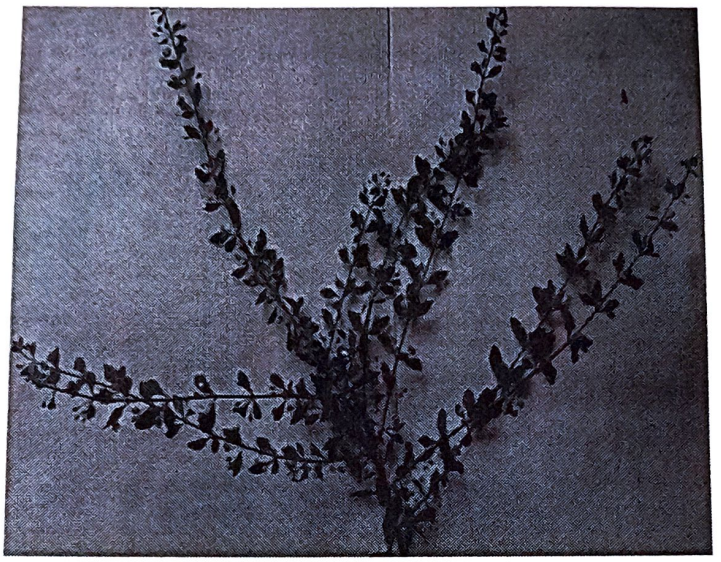
The Ming aralia is widely cultivated in several countries of Southeast Asia and the tropical islands of the Pacific region. It was originally located in Polynesia and thrives in environments medium humidity, with temperatures varying from 16–29 °C (60–85 °F).

Scoparia dulcis L.

Family: Plantaginaceae

Genus: *Scoparia*

Species: *dulcis L.*



Locality: St.Mary's College. Botanical garden

Floristic features:

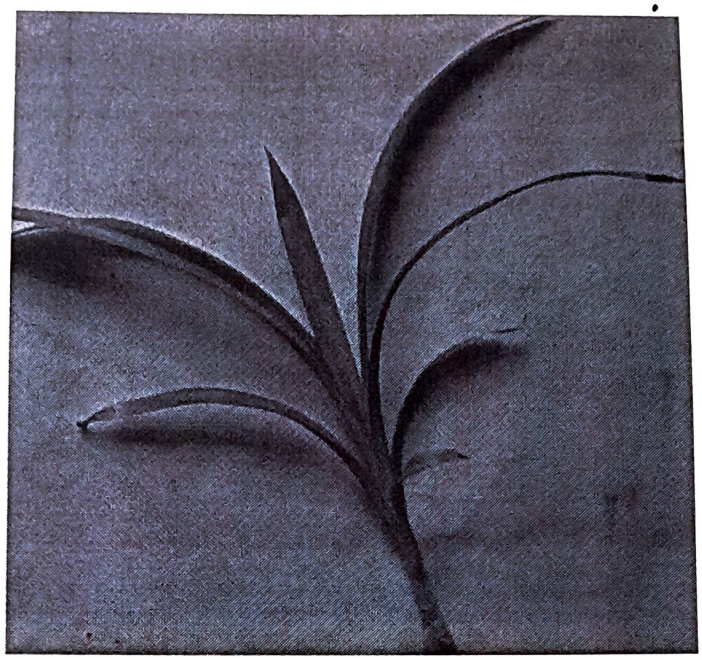
An annual to perennial, erect, much branched herb, 20-75 cm tall, glabrous, 4-6-striate. Leaves opposite or 3-4-whorled, oblanceolate to oblong-obovate, 0.5-3.5 cm × 0.2-1.5 cm, base attenuate, apex acute, margin coarsely acute-serrate, glabrous above, gland-dotted beneath; petiole 0.5-1 cm long; stipules absent. Flowers axillary, bisexual, actinomorphic, 1-4-fascicled, regular, pedicel 3-7 mm long; calyx deeply 4-lobed, 2 mm long, accrescent in fruit to 2.5 mm, lobes oblong-ovate, subacute; corolla rotate, white or very pale-purple, sometimes with darker centre, deeply 4-lobed, lobes oblong, 3 mm long, apex obtuse, throat on inside with dense, long white hairs; stamens 4, subequal, erect, filaments filiform, 2 mm long; ovary superior, 2-celled, glabrous, style filiform, 1.5 mm long, stigma capitate. Fruit a subglobose capsule, 2.5-3 mm long, 4-valved, thin-walled, yellowish-brown, seeds numerous. Seed oblong-globose to ovoid, 0.5-0.6 mm × 0.3-0.4 mm, angular, covered with thin, reticulate testa, brown. Seedling with epigeal germination; cotyledons rhomboid, up to 2.2 mm long, glabrous, petiolate, epicotyl 1.5-4 mm long, 4-angular; first leaves 2, ovate, up to 6 mm long, glandular dotted, margin with rounded teeth, midvein present, petiolate.

Pandanus amaryllifolius

Family : Pandanaceae

Genus : *Pandanus*

Species : *amaryllifolius*



Locality: St.Mary's College. Botanical garden

Floristic features

Perpetuated sucker shoots. Stem slender, 1-1.6 m tall, 2-5 cm in diameter, decumbent and ascending, emitting aerial roots throughout its length. Leaves oblong, 25-75 cm × 2-5 cm, rather pale green, somewhat thin and flaccid, more or less glaucous and keeled beneath, the apex with rather distinct twin lateral pleats, the margins entire, unarmed except a few minute prickles less than 1 mm long near the apex. Flowers and fruits unknown. Eventually producing an erect stem, 2-4.5 m tall, 15 cm in diameter, unbranched or sparsely branched, bearing large aerial prop roots. Leaves oblong, 150-220 cm × 7-9 cm, apex acute, rather dark green above, glaucous and keeled beneath, the twin lateral pleats above somewhat prominent, margins entire, unarmed except near leaf apex with small antrorse prickles about 1 mm long and very rarely with 1-3 small stout prickles near the base. Female inflorescence unknown. Male inflorescence (evidently exceedingly rare), probably pendent, up to 60 cm long, the spathes 90 cm long, white or the lower ones with green foliaceous tips, bearing several oblong spikes to 35 cm long or more, several cm wide; upper ones much shorter, about 9-10 cm long, 2 cm wide, composed of many crowded staminal phalanges; staminal phalange with column 4-9 mm × 1.5-2.5 mm, compressed to flat, containing 3-6 stamens with very short filaments, 0.5-1.5 mm × 0.4-0.6 mm and oblong anthers, 2.5 mm long.

Alternanthera brasiliana

Family : Amaranthaceae

Genus : *Alternanthera*

Species: *brasiliana*

Locality: St.Mary's College. Botanical garden

Floristic features:

It is an erect, sprawling, herbaceous plant that may grow up to 3 metres tall, though it is usually less than 1 metre as a cultivated plant. The plant's stems, which range between red, green and purple, are delicately hirsute when juvenile, though they'd become glabrescent as they get older. Its opposite leaves, which are 1–10 cm long and 0.7–5 cm wide, are usually coloured purple-specked or luminous reddish-purple.It may lose some of its leaves in winter, making it partially "deciduous" in places that have slightly cool winters.Its vanilla-coloured, pom-pom flowers are ordered in compact clusters (7–20 mm long) in the top leaf branching and are small in shape. These clusters are rounded to slightly lengthened in shape and are foaled on stalks which are normally 3–10 cm long. It can flower any time of the year, but in temperate and cooler subtropical climates it flowers more often in winter.Its very small brown fruit (1.5–2 mm long) contains one seed that's generally hidden within the older flower parts.A perennial herb up to 3 m tall, decumbent at base, ascending-erect or clambering higher up, often widely branched, stems solid; hairs minutely dentate.Leaves ovate-lanceolate. Flowering heads are stalked. Fruit is ellipsoidal.

Alternanthera brasiliana

Family : Amaranthaceae

Genus : *Alternanthera*

Species : *brasiliana*

Locality: St.Mary's College. Botanical garden

Floristic features:

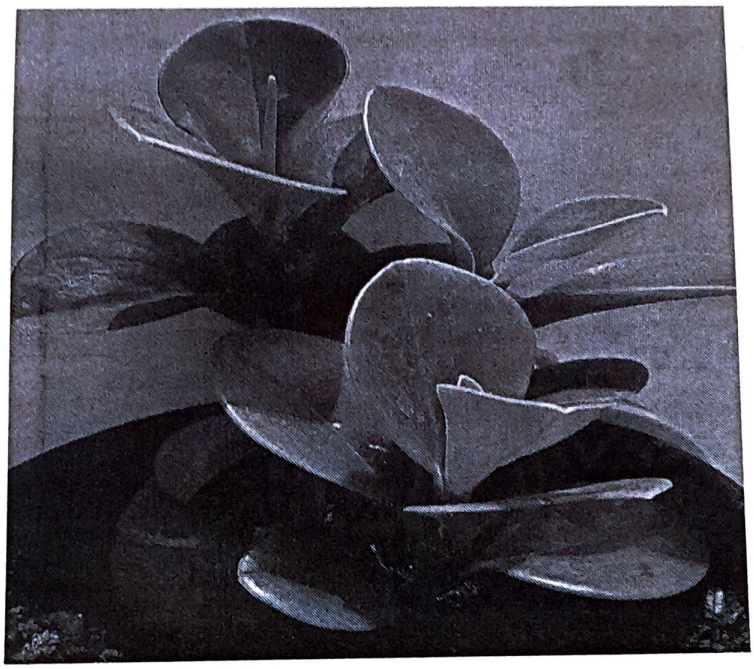
It is an erect, sprawling, herbaceous plant that may grow up to 3 m tall, though it is usually less than 1metre as a cultivated plant. The plant's stems, which range between red, green and purple, are delicately hirsute when juvenile, though they'd become glabrescent as they get older. Its opposite leaves, which are 1–10 cm long and 0.7–5 cm wide, are usually colored purple-specked or luminous reddish-purple. It may lose some of its leaves in winter, making it partially "deciduous" in places that have slightly cool winters. Its vanilla-colored, pom-pom flowers are ordered in compact clusters (7–20 mm long) in the top leaf branching and are small in shape. These clusters are rounded to slightly lengthened in shape and are foaled on stalks which are normally 3–10 cm long. It can flower any time of the year, but in temperate and cooler subtropical climates it flowers more often in winter. Its very small brown fruit (1.5–2 mm long) contains one seed that's generally hidden within the older flower parts.

Peperomia mangoliifolia

Family : Piperaceae

Genus : *Peperomia*

Species : *mangoliifolia*



Locality: St.Mary's College. Botanical garden

Floristic features:

Peperomia is one of the two large genera of the family Piperaceae. It is estimated that there are at least over 1,000 species, occurring in all tropical and subtropical regions of the world, though concentrated in Central America and northern South America. A limited number of species (around 17) are found in Africa. The exact number is difficult to tell as some plants have been recorded several times with different names (c. 3,000 names have been used in publications) and new species continue to be discovered.

Peperomias have adapted to many different environments and their appearance varies greatly. Some are epiphytes (growing on other plants) or lithophytes (growing in rock crevices), and many are xerophytes (drought-tolerant) either with thick succulent structures or with underground tubers (geophytes). Most species are compact perennial shrubs or vines.

Piper betle

Family : Piperaceae
Genus : *Piper*
Species : *betle*

Locality : St. Mary's college botanical garden

Floristic features:

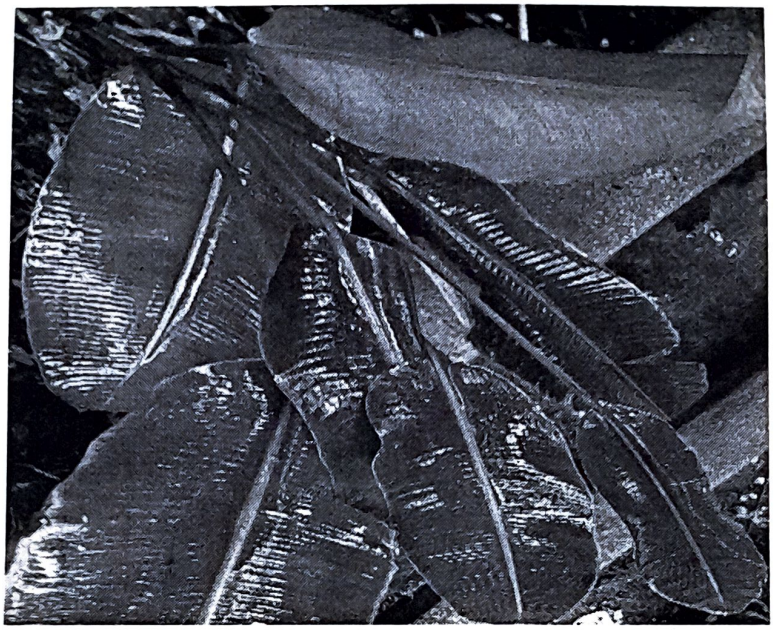
A semi-woody branching vine with a sprawling or climbing growth habit. Light green to bright green leaves are glossy, deeply veined and hairless. They are heart-shaped with entire leaf margin. The petiole (leaf stalk) is reddish like the stem. Round stems are orangish to reddish. White catkins developed on the nodes, erect or pendulous. Flowers are small, without sepal and petal. Fleshy, globose to ellipsoidal shaped.

Heliconia latispatha

Family : Heliconiaceae

Genus : *Heliconia*

Species : *latispatha*



Locality: St.Mary's College. Botanical garden

Floristic features :

Plants 2-4 m, pseudo stem well-developed. Leaves are green, Inflorescences erect, to 45 cm; cincinnal bracts spirally arranged, middle bract with outer surface orange and/or red and glabrous, 5—7 cm wide at base. Flowers are green, yellow, or orange with dark green sepal margins, sometimes sparsely puberulent along fused sepal margins, 3.5—4.6 cm, 7—8 mm wide at gibbous base, nearly straight; sepal margins, fused, dark green, glabrous, sometimes sparsely puberulous. Flowering early fall at September.

Daphne laureola

Family : Thymelaeceae
Genus : *Daphne*
Species : *laureola*

Locality : St. Mary's college botanical garden

Floristic features :

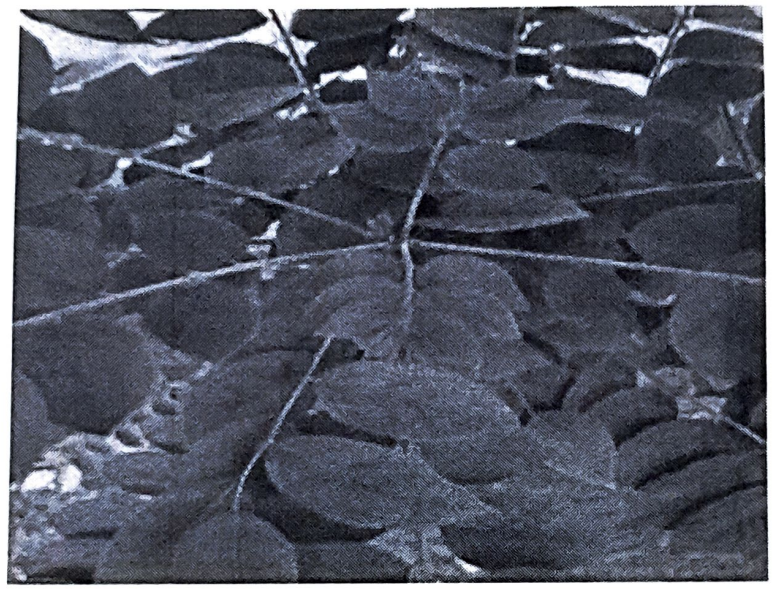
Daphne laureola reaches a height between 0.5–1.5 m (1.6–4.9 ft). The habit of this shrub can be upright or decumbent (arched at the base then spreading upward). The bark is thin and yellow-grey when mature, while immature stems are green. The cambium is malodorous with a scent reminiscent of herb robert.The alternate leaves usually form dense whorls at the shoot tips, but may clothe entire branches. The leaves are oblanceolate to obovate-oblanceolate, 2–13 cm long and 1–3 cm wide. They are glabrous (smooth), dark green and glossy on the upper surface and lighter in colour beneath.The inconspicuous yellow-green axial flowers, usually hidden among the leaf bases, may be strongly fragrant, or may exhibit no scent at all.

Phyllanthusacidus

Family : Phyllanthaceae

Genus:*Phyllanthus*

Species:*acidus*



Locality : St. Mary's college botanical garden

Floristic features:

*Phyllanthusacidus*is an intermediary between a shrub and tree, reaching 2 to 9 m (6½ to 30 ft) high. The tree's dense and bushy crown is composed of thickish, tough main branches, at the end of which are clusters of deciduous, greenish, 15-to-30-cm long branchlets. The branchlets bear alternate leaves that are ovate or lanceolate in form, with short petioles and pointed ends. The leaves are 2–7.5 cm .

Euphorbia palustris

Family: Euphorbiaceae

Genus: *Euphorbia*

Species: *palustris*

Locality: St.Mary's College. Botanical garden

Floristic features:

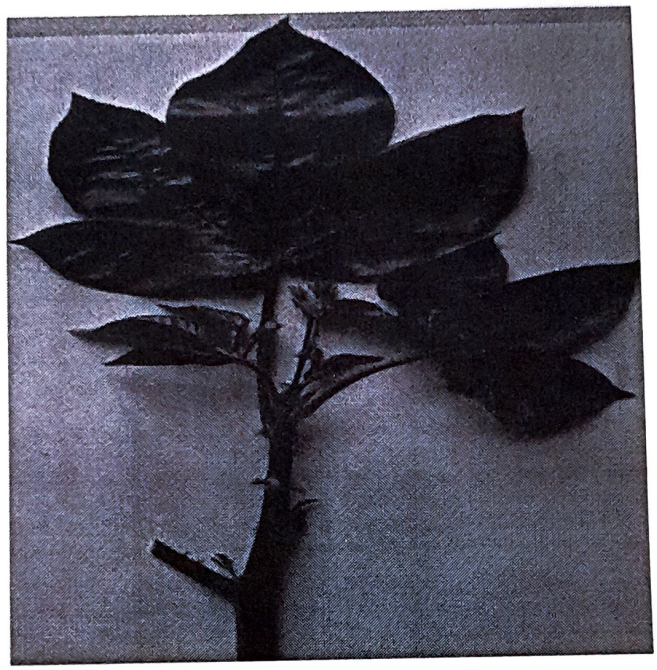
It was published and first described by Carl Linnaeus in his book, Species Plantarum on page 462 in 1753.It prefers permanently moist conditions in full sun, hence the common name "marsh spurge" and the Latin specific epithet palustris, "of marshland. It is thought to be an ideal plant for gardening because it has a different colour for almost all of the seasons.Euphorbia palustris has gained the Royal Horticultural Society's Award of Garden Merit.Like all euphorbias, all parts of the plant are toxic if ingested, and cut stems produce an irritant sticky sap.Euphorbia palustris, commonly called marsh euphorbia or spurge, is an upright, clump-forming, herbaceous perennial that typically grows to 2-3' (infrequently to 5') tall. It is native to marshland in Europe and western Asia. Inconspicuous, greenish true flowers (lack both petals and sepals) bloom in late spring to early summer (June-July). Although these true flowers (borne in large 6" wide clusters known as cyathia) are not particularly showy, they are subtended by large, long-lasting, greenish-yellow bracts which are exceptionally showy. Flower color in effect comes from the floral bracts. Elliptic stem leaves (to 2-3" long) and narrower axillary branch leaves are medium green but turn yellow and orange/red in fall.Genus name probably honors Euphorbus, physician to the King of Mauretania.Specific epithet from Latin means marsh-loving.

Jatropha gossypiifolia L.

Family: Euphorbiaceae

Genus: *Jatropha*

Species*: gossypiifolia L.*



Locality: St.Mary's College. Botanical garden

Floristic features:

Jatropha gossypiifolia, commonly known as bellyache bush, black physicnut or cotton-leaf physicnut, is a species of flowering plant in the spurge family, Euphorbiaceae. The species is native to Mexico, Philippines, South America, Gujarat State (India), and the Caribbean islands. It is a declared noxious weed in Puerto Rico and is naturalized in northern Australia, including Queensland where it is listed as a Class 2 declared pest plant.It grows to 2.5–4 m (8.2–13.1 ft) high. The three lobed leaves are purple and sticky when young and become bright green with age. The small red flowers with yellow centres appear in clusters. These are followed by cherry-sized seed pods that are poisonous. Powdery mildew fungal disease was reported.There are many common names for Jatropha gossypiifolia including: bellyache-bush, black physicnut, and cotton-leaf physicnut in English; p inon negro, pinon colorado, and tua-tua in Spanish; medicinier noir and medicinier rouge in French; mamoninha and peao-roxo in Brazil; jarak merah and sibidigua in India.

Jatropha curcas L.

Family: Euphorbiaceae

Genus: *Jatropha*

Species: *curcas L.*

Locality: St.Mary's College. Botanical garden

Floristic features:

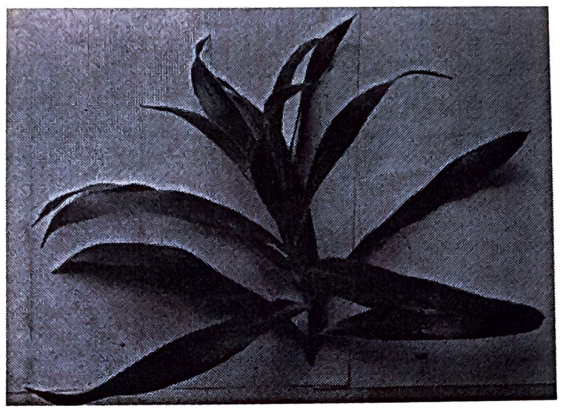
Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiaceae, that is native to the American tropics, most likely Mexico and Central America. It is originally native to the tropical areas of the Americas from Mexico to Argentina, and has been spread throughout the world in tropical and subtropical regions around the world, becoming naturalized or invasive in many areas.The specific epithet, "curcas", was first used by Portuguese doc Garcia de Orta more than 400 years ago.Common names in English include physic nut, Barbados nut, poison nut, bubble bush or purging nut.In parts of Africa and areas in Asia such as India it is often known as "castor oil plant" or "hedge castor oil plant,but it is not the same as the usual castor oil plant, Ricinus communis (they are in the same family but different subfamilies).J. curcas is a semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft) or more.It is resistant to a high degree of aridity, allowing it to grow in deserts.It contains phorbol esters, which are considered toxic.However, edible (non-toxic) varieties native to Mexico also exist, known by the local population as piñón manso, xuta, chuta, aishte, among others.J. curcas also contains compounds such as trypsin inhibitors, phytate, saponins and a type of lectin known as curcin. The seeds contain 27–40% oil[13] (average: 34.4%[14]) that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine.[15] Edible (non-toxic) varieties, as those developed by selection by ethnic Mexican natives in Veracruz can be used for animal feed and foot.

Dracaena Fragrance

Family: Euphorbiaceae

Genus: *Jatropha*

Species: *curcas L.*



Locality: St.Mary's College. Botanical garden

Floristic features :

Dracaena fragrans (cornstalk dracaena), is a flowering plant species that is native throughout tropical Africa, from Sudan south to Mozambique, west to Côte d'Ivoire and southwest to Angola, growing in upland regions at 600–2,250 m (1,970–7,380 ft) altitude.It is also known as striped dracaena, compact dracaena, and corn plant.Dracaena fragrans is a slow growing shrub, usually multistemmed at the base, mature specimens reaching 15 m (49 ft) or more tall with a narrow crown of usually slender erect branches. Stems may reach up to 30 cm (12 in) diameter on old plants; in forest habitats they may become horizontal with erect side branches. Young plants have a single unbranched stem with a rosette of leaves until the growing tip flowers or is damaged, after which it branches, producing two or more new stems; thereafter, branching increases with subsequent flowering episodes. The leaves are glossy green, lanceolate, 20–150 cm (7.9–59.1 in) long and 2–12 cm (0.79–4.72 in) wide; small leaves are erect to spreading, and larger leaves usually drooping under their weight. The flowers are produced in panicles 15–160 cm (5.9–63.0 in) long, the individual flowers are 2.5 cm (0.98 in) diameter, with a six-lobed corolla, pink at first, opening white with a fine red or purple central line on each of the 7–12 mm (0.28–0.47 in) lobes; they are highly fragrant, and popular with pollinating insects. The fruit is an orange-red berry 1–2 cm (0.39–0.79 in) diameter, containing several seeds.

Euphorbia didiereoides

Family: Euphorbiacae

Genus: *Euphorbia*

Species: *didiereoides*

Locality: St.Mary's College. Botanical garden

Floristic features:-

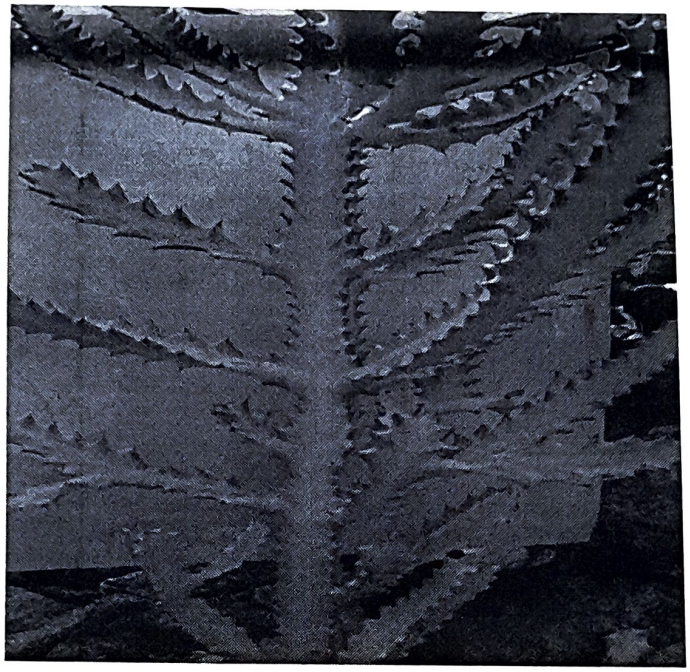
Euphorbia didiereoides is a succulent shrub with erect stem and short branches covered with stout grey spines with reddish tips. It grows upto 8.2 feet tall. Stems are upto 6 inches in diameter. Near the base, tapering to around 1.2 inches at their apex. The branches are upto 8.8 inches long. Leaves are oval, green, tinged with red. Flowers are small, green and appear in dense groups or upto 64. *Euphorbia didiereoides* is a very spiny, succulent perennial plant or shrub with many erect stems developing from the base. The plant can grow up to 2.5 metres tall with stems up to 15cm in diameter near the base, tapering to around 3cm at their apex, with many short branches 1 – 2cm long. The plant is sometimes harvested from the wild for local use as a medicine. It is cultivated as an ornamental pot plant. *Euphorbia didiereoides* has a limited distribution area, and numbers are diminishing due to habitat degradation, fires and collection for horticultural trade. The plant is classified as 'Endangered' in the IUCN Red List of Threatened Species.

Euphorbia lactea

Family: Euphorbiacae

Genus: *Euphorbia*

Order: *lactea*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Euphorbia lactea is a species of spurge native to tropical Asia, mainly in India. It is an erect shrub growing up to 5 metres (16 ft) tall, with succulent branches 3–5 centimetres (1.2–2.0 in) diameter, ridged, with a triangular or rhombic cross-section; the ridges are spiny, with short spines up to 5 millimetres (0.20 in) long. The leaves are minute, and soon deciduous. All parts of the plant contain a poisonous milky latex. Common names include mottled spurge, frilled fan, elkhorn , candelabra spurge, candelabrum tree, candelabra cactus, candelabra plant, dragon bones, false cactus, hatrack cactus, milkstripe euphorbia, mottled candlestick. It is used medicinally in India. It is widely grown as an ornamental plant, both in the tropics, and as a houseplant in temperate regions; a number of cultivars have been selected for ornamental use, notably 'Cristata' with frilled branching

Euphorbia milii

Family: Euphorbiacae

Genus: *Euphorbia*

Species: *milii*

Locality: St.Mary's College. Botanical garden

Floristic features:-

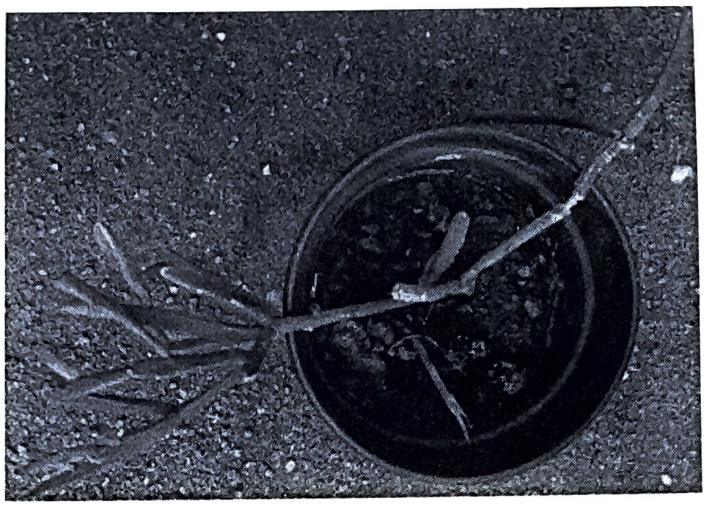
Euphorbia milii, the crown of thorns, Christ plant, or Christ thorn, is a species of flowering plant in the spurge family Euphorbiaceae, native to Madagascar. The species name commemorates Baron Milius, once Governor of Reunion , who introduced the species to France in 1821. It is imagined that the species was introduced to the Middle East in ancient times, and legend associates it with the crown of thorns worn by Christ. It is commonly used as an ornamental houseplant that can be grown in warmer climates. The common name is due to the thorns and deep red bracts referring to the crown thorn Jesus had to wear during his crucifixion and his blood. It is a woody succulent subshrub or shrub growing to 1.8 m (5 ft 11 in) tall, with densely spiny stems. The straight, slender spines, up to 3 cm (1.2 in) long, help it scramble over other plants. The fleshy, green leaves are found mainly on new growth, and are up to 3.5 cm (1.4 in) long and 1.5 cm (0.59 in) broad. The flowers are small, subtended by a pair of conspicuous petal-like bracts, variably red, pink or white, up to 12 mm (0.47 in) broad. Wat Phrik in Thailand claims to be the home of the world's tallest Christ thorn plant. The plant thrives between spring and summer but produces flowers all year round.

Euphorbia tirucalli

Family: Euphorbiacae

Genus: *Euphorbia*

Species: *tirucalli*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Euphorbia tirucalli (commonly known as Indian tree spurge, naked lady, pencil tree, pencil cactus, fire stick, or milk bush) is a tree that grows in semi-arid tropical climates. A hydrocarbon plant, it produces a poisonous latex that can cause temporary blindness.The milky latex from *E. tirucalli* is extremely irritating to the skin and mucosa and is toxic. Exposure to it can cause temporary blindness. Skin contact causes severe irritation, redness and a burning sensation. If ingested, it can cause burns to the mouth, lips and tongue. It is suggested to wear eye protection gear and gloves for handling the plant.

Euphorbia cyathophora

Family : Euphorbiaceae
Genus : Euphorbia
Species : cyathophora

Locality : St. Mary's college botanical garden

Floristic feature :

This plant is a summer annual about 1–2¾' tall, ½–2' across, and more or less flat-topped. It is more branched and wider above than below; the lower stem is usually unbranched. The stems are light green to medium green, more or less terete, and glabrous (or nearly so). The lower to middle leaves are either alternate or opposite, while the upper leaves near the inflorescences are opposite. The lower leaves are often early-deciduous. The leaves are 1-4" long, ¼–1½" wide, and widely spreading; they are variably shaped, either unlobed or lobed, and irregularly dentate to entire (toothless). The leaves of this plant are obovate, oblanceolate, elliptic, or linear-oblong in shape; sometimes these variations in shape can occur even on the same plant. When lateral lobes occur, they are few in number (1-6) with pointed tips and concave sinuses; they are often irregular in size, shape, and position. The upper leaf surface is medium green and glabrous, while the lower leaf surface is light green and glabrous to mostly glabrous; sometimes there are a few hairs along the undersides of major veins. Leaf venation is pinnate.

Codiaeum variegatum (L)

Family : Euhorbiaceae

Genus : *Codiaeum*

Species : *variegatum*

Locality : St. Mary's college botanical garden

Floristic features:

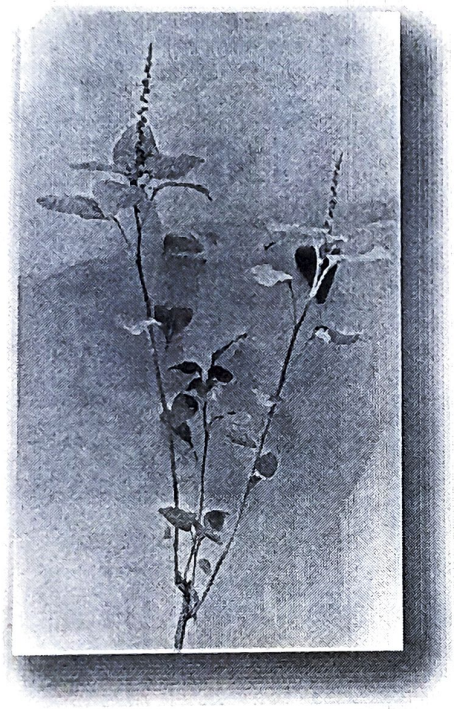
It is a tropical, evergreen, monoecious shrub growing to 3 m (9.8 ft) tall and has large, thick, leathery, shiny evergreen leaves, alternately arranged, 5–30 cm (2.0–11.8 in) long and 0.5–8 cm (0.20–3.15 in) broad. The leaf blades can, for example, be ruler-lanceolate, oblong, elliptic, lanceolate, ovate inverted, ovate spatulate, or violin-shaped and coloured green, yellow, or purple in various patterns, depending on the variety. The petiole has a length of 0.2 to 2.5 cm. The inflorescences are long racemes, 8–30 cm (3.1–11.8 in) long, with male and female flowers on separate inflorescences; the male flowers are white with five small petals and 20–30 stamens, pollens are oval approximately 52x32 microns in size. The female flowers yellowish, with no petals. The flowering period is usually in early autumn. The fruit is a capsule 9 mm (0.35 in) in diameter, containing three seeds that are 6 mm (0.24 in) in diameter. When cut, stems bleed a milky sap like many of the Euphorbiaceae.

Acalpha indica

Family name : Euphorbiaceae

Genus : *Acalypha*

Species : *indica*



Locality : St. Mary's college botanical garden

Floristic features:

An erect annual herb that can be easily distinguished by the cup-shaped involucre that surrounds the small flowers in the catkin-like inflorescence. The leaf base is rounded to shortly attenuate. The leaf margin is basally 5-nerved and is crenate-serrate with an acute or obtuse apex. The petiole is 1.5–5.5 cm in long. The flower spikes are axillary, 2.5–6 cm in long, monoecious, with a rachis terminating in a hood. The tiny male flowers are white-green, located on the upper part of the flower spikes. The green female flowers are located lower on the spikes, and are subtended by 3–7 mm in long suborbicular-cuneiform, many-nerved, toothed bracts that are foliaceous. The stem is striate, longitudinally ribbed and pubescent. The fruit is 1.5–2 mm 3-lobed, tuberculate and pubescent.

Pilea microphylla

Family: Urticaceae

Genus: *Pilea*

Species: *microphylla*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Pilea microphylla also known as angeloweed, artillery plant, joypowder plant or brilhantina is an annual plant native to Florida, Mexico, the West Indies, and tropical Central and Southern America. In the southern part of Mexico, specifically Campeche and Merida, the local name is Frescura.The plant belongs to the family Urticaceae. It has light green, almost succulent, stems and tiny 1/8" leaves which contribute to its other nickname, "Artillery Fern", though it is not related to ferns. It is grown as a ground cover in many areas.

Aechmea fasciata

Family : Bromeliaceae
Genus : *Aechmea*
Species : *fasciata*

Locality : St.Mary's college botanical garden

Floristic features :

The plant grows slowly, reaching 30–90 cm (12–35 in) in height, with a spread of up to 60 cm (24 in). It has elliptic–oval-shaped leaves 45–90 cm (18–35 in) long and arranged in a basal rosette pattern.

Cryptanthus araulis

Family: Bromeliaceae

Genus: *Cryptanthus*

Species: *araulis*

Locality: St.Mary's College. Botanical garden

Floristic features:-

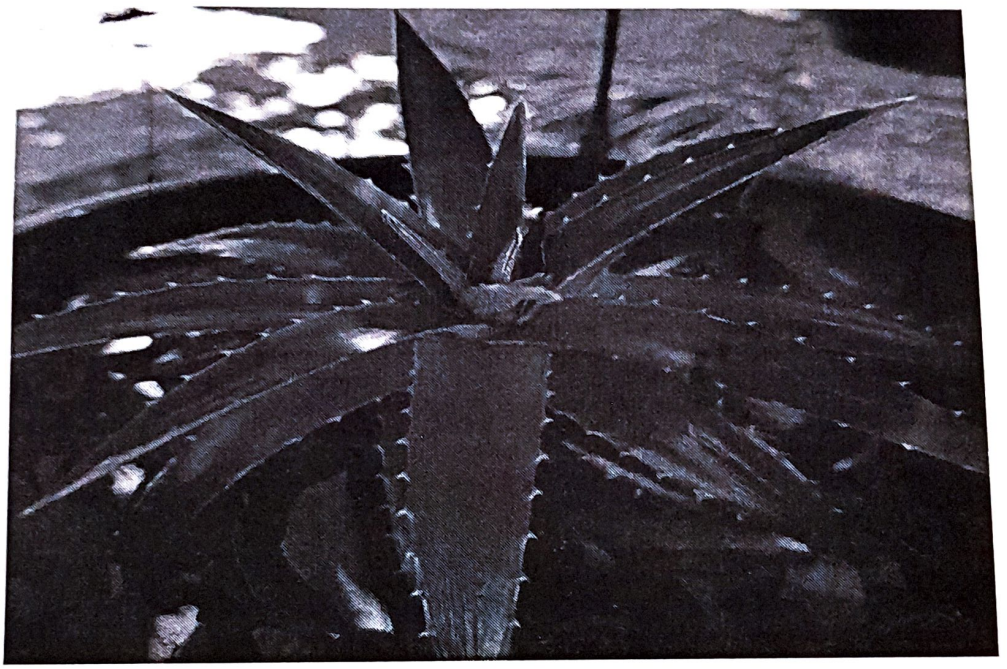
Cryptanthus, genus of epiphytes (plants that are supported by other plants and have aerial roots exposed to humid atmosphere) of the pineapple family (Bromeliaceae), composed of about 10 to 20 South American species. The prickly-edged, stemless leaves grow in a rosette directly from the root. The flowers, usually white, are in a stalkless, dense bunch at the centre of the rosette. A few species, especially *C. acaulis* and *C. zonatus*, are grown indoors for their attractive foliage. Both species have wavy-edged leaves that are silvery or whitish underneath. *C. acaulis* grows to about 15 centimetres (6 inches) and has several to many leaves. The strap-shaped leaves of *C. zonatus* are greenish brown or coppery on top with bands of tan or brown; the plant is about 22 cm tall.

Dyckia brevifolia

Family: Bromeliaceae

Genus: *Dyckia*

Species: *brevifolia*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Dyckia brevifolia, or sawblade, is a species of flowering plant in the Bromeliaceae family. This species is endemic to Brazil. Dyckias have stiff and thorny leaves and prefer rocky and/or sunny areas and have a natural tendency to clump leading to thick, large mats. Some varieties are terrestrial while others are found on rocks in their native habitat. They have remarkable drought tolerance for short periods of time and can even withstand a brief freeze. Thy can be grown outside in summer or year around in warmer regions. Unlike true succulents they cannot store their own water internally. They simply respond to periods of stress, such as dry weather conditions, by going dormant. *Dyckia brevifolia* or Sawblade is a terrestial bromeliad native to central South America, with mid green tapering triangular leaves that have vertical grey- white bands of powdery scales underneath and sharp backward facing. White spines. In spring it bears clusters of little yellow bell flowers atop a central stem.

Costus spiralis

Family : Costaceae
Genus : *Costus*
Species : *spiralis*

Locality : St.Mary's college botanical garden

Floristic features:

Costus spiralis is superficially similar to Costus scaber, with non-appendaged bracts and tubular-type flowers with closed labellums. The colors of the bracts and flowers are variable. It is formally distinguished from other similar species by the coriaceous texture of the bracts and the lengths of the bracteoles and calyx.

Agapanthus praecox

Family : Amaryllidaceae

Genus : *Agapanthus*

Species : *praecox*

Locality: St.Mary's College. Botanical garden

Floristic features :

Agapanthus praecox is a variable species with open-faced flowers. It is a perennial plant that can survive up to 75 years. Its evergreen leaves are 2 cm wide and 50 cm long. Its inflorescence is in umbel. The flowers of the agapanthus are blue, purple or white and bloom from late spring to summer. They give capsules filled with fine black seeds (to be kept cool in sand until sowing). Its stem reaches one meter high. Its roots are very powerful and can break concrete. This subspecies occurs in the Eastern Cape and southern KwaZulu-Natal. Although it is about the same height as subsp. Praecox, it has up to 20 poisonous, strap-like leaves per plant which are arching and are not leathery.[1] These range in length from 20 to 70 cm long and 3 to 5 cm wide.[5] Flower colour ranges from blue to white.[1][3] Shiny black seeds are produced in three-sided capsules.[3] These have perianth segments which are less than 50 mm in length.

Aglaonema commutatum

Family : Araceae

Genus : *Aglaonema*

Species : *commutatum*

Locality: St.Mary's College. Botanical garden

Floristic features :

Aglaonema commutatum, the poison dart plant, is a species of flowering plant in the Chinese evergreen genus Aglaonema, family Araceae. It is native to the Philippines and northeastern Sulawesi, and has been introduced to other tropical locales, including Cuba, Puerto Rico, Trinidad and Tobago, Comoros, the Chagos Archipelago, India, Bangladesh, and the Cook Islands. Its hybrid cultivar 'Silver Queen' has gained the Royal Horticultural Society's Award of Garden Merit as a houseplant. Chinese evergreen is an herbaceous perennial in the Araceae (arum) family, native to the Philippines and northeastern Celebes and commonly grown as a houseplant. This tropical shrub grows to 1.5 feet, is erect and bushy, and resembles dumb cane (Dieffenbachia). The tendency to have only 5-8 main lateral veins distinguishes it from Dieffenbachia. It has elliptic, dark green, lance-shaped leaves that reach 4 to 8 inches long and 2 to 3 inches wide. Attractive silver-gray blotches appear on upright stems. It blooms rarely as a houseplant in the late summer or early fall with a white spadix and greenish-white spathe. Red clusters of berries follow the blooms. The plant does well in diffuse sun or good indirect light and prefers high humidity but will tolerate dryer air.

Epipremnum aureum

Family : Araceae

Genus : *Epipremnum*

Species : *aureum*

Locality: St.Mary's College. Botanical garden

Floristic features:

Epipremnum aureum is an evergreen vine growing to 20 m (66 ft) tall, with stems up to 4 cm (2 in) in diameter, climbing using aerial roots which adhere to surfaces. The leaves are alternate, heart-shaped, entire on juvenile plants, but irregularly pinnatifid on mature plants, up to 100 cm (39 in) long and 45 cm (18 in) broad; juvenile leaves are much smaller, typically under 20 cm (8 in) long.

E. aureum is classified as an angiosperm, which typically produces flowers at some point in their life cycle, it is the only reported species in its family (Araceae) that does not develop a flower. Regardless of where this "shy-flowering" plant is grown or what the conditions are like, it will not flower due to a genetic impairment of the gibberellin (GA) biosynthetic gene, EaGA3ox1. This impairment causes the plant to be unable to develop bioactive Gas, which is what is responsible for the flowering of plants via the floral meristem identity gene EaLFY. In E. aureum, the floral meristem identity gene expression is absent due to the lack of Gas from EaGA3ox1.

Alocasia longiloba

Family : Araceae

Genus : *Alocasia*

Species : *longiloba*

Locality: St.Mary's College. Botanical garden

Floristic features:

Alocasia cucullata is a clumping evergreen herbaceous plant that is grown as a food plant and as an ornamental in areas within and outside its native distribution range of tropical and temperate Asia. This species spreads by seed and vegetatively by root suckers, rhizomes and corm fragments.The Alocasia Cucullata plant is believed to bring good luck. Therefore, it is often grown in Buddhist temples across Thailand. Undoubtedly, its lush green foliage can make any place look like a haven of peace and rest.

Philoendron Xanadu

Family : Araceae

Genus : *Philoendron*

Species : *Xanadu*

Locality: St.Mary's College. Botanical garden

Floristic features :

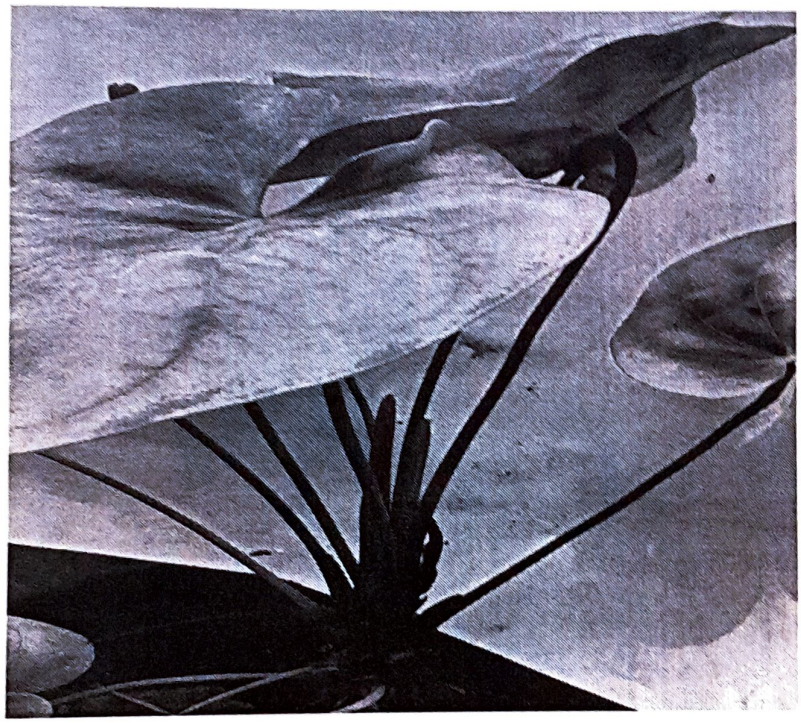
Thaumatophyllum xanadu eventually forms dense clumps up to 1.5 metres (5 ft) tall by 2 metres (7 ft) wide. It has glossy green, deeply dissected, lobed leaves up to 40 cm (16 in) long by 30 cm (12 in) wide. Its flowers have dark red spathes. It may occasionally produce aerial roots. *Thaumatophyllum xanadu* is a species of the genus *Thaumatophyllum*, which previously was the self-heading Meconostigma subgenus of Philodendron. "It differs from all other species of Meconostigma in details of the sexual parts of its spadix, the shape of the leaf scars on the rhizomes, shape of leaf blade, intravaginal squamules, etc".

Alocasia cuculatta

Family : Araceae

Genius : *Alocasia*

Species : *cuculatta*



Locality: St.Mary's College. Botanical garden

Floristic features:

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Syngonium podophyllus

Family: Araceae

Genus: *Syngonium*

Species: *podophyllus*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Syngonium podophyllum is a species of aroid that is a popular houseplant. Common names include: arrowhead plant, arrowhead vine, arrowhead philodendron, goosefoot, nephthytis, African evergreen, and American evergreen. The species is native to a wide region of Latin America from Mexico through Bolivia, and naturalized in the West Indies, Florida, Texas, Hawaii, and other places.It climbs a few meters tall over the trunks of tropical jungle trees, clinging by its roots. The cultivars cultivated indoors reach a height of up to 1.5 m (4.9 ft). During the year, the plant grows about 30 cm (12 in) and produces 6-7 leaves. Its single leaves, usually arrow-shaped, are up to 30 cm (12 in) long. In the wild, the leaves are dark green and without variegation. Cultivated varieties have leaves in various shades of green, often light green and usually with different types of lighter tannins. There are several variegated cultivars, the main differences being in the position and extent of the cream or white markings. Some leaves are almost entirely white, pink or yellow.Its flowers are small, greenish or whitish on spadices within light-yellow through green spathes. However, the plants grown indoors do not bloom, aside from the older, well-cared-for specimens.

Pistia stratiotes

Family: Araceae

Genus: *Pistia*

Species: *stratiotes*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Pistia is a genus of aquatic plants in the arum family, Araceae. It is the sole genus in the tribe Pistieae which reflects its systematic isolation within the family. The single species it comprises, Pistia stratiotes, is often called water cabbage, water lettuce, Nile cabbage, or shellflower. Its native distribution is uncertain but is probably pantropical; it was first discovered from the Nile near Lake Victoria in Africa.It is now present, either naturally or through human introduction, in nearly all tropical and subtropical fresh waterways and is considered an invasive species as well as a mosquito breeding habitat. The genus name is derived from the Greek word (pistos), meaning "water," and refers to the aquatic nature of the plants. The specific epithet is also derived from a Greek word, meaning "soldier," which references the sword-shaped leaves of some plants in the Stratiotes genus.

Homalomena rubescens

Family : Araceae

Genus : *Homalomena*

Species : *rubescens*

Locality : St.Mary's college botanical garden

Floristic features :

The plants of this genus are clump-forming evergreen perennials with mainly heart-shaped or arrow headed shaped leaves. The flowers are tiny and without petals, enclosed in a usually greenish spathe hidden by the leaves

Dieffenbachia seguine

Family : Araceae

Genus : *Dieffenbachia*

Species : *seguine*

Locality : St.Mary's College Botanical garden

Florestic features:

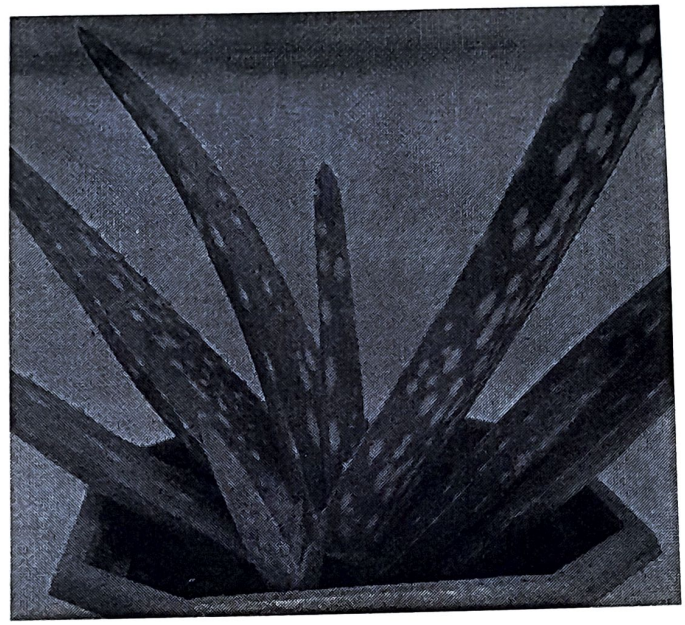
The herbaceous perennial grows 3 feet (0.91 m) to 10 feet (3.0 m) in height and 2 feet (0.61 m) to 3 feet (0.91 m) in width. The plant's leaves are large and green, and often with variegated white patterns. It has showy white flowers.

Aloe succotrina

Family : Xanthorrhoeaceae

Genus : *Aloe*

Species: *succotrina*



Locality: St.Mary's College. Botanical garden

Floristic features:

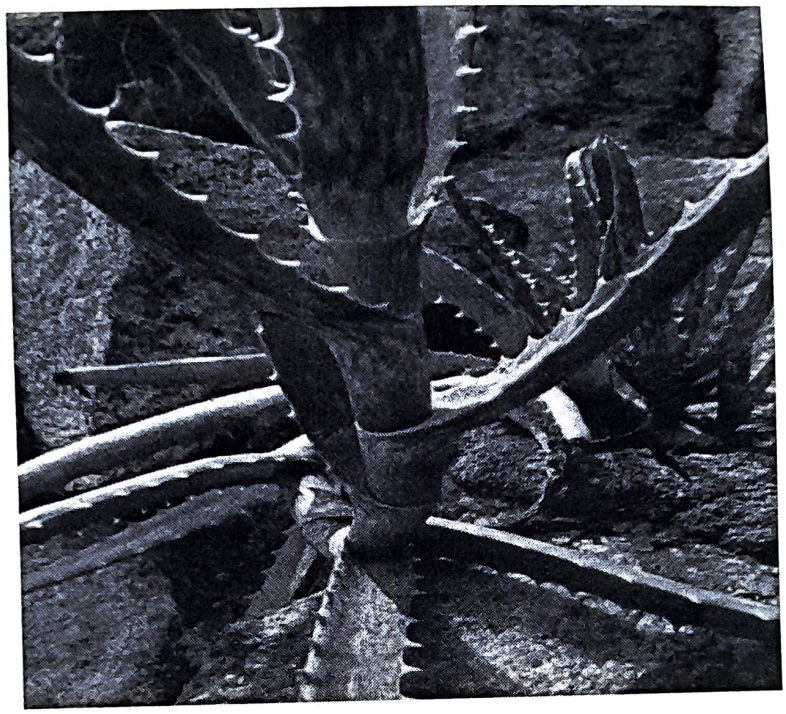
The *Aloe succotrina* plant forms clusters of between 1–2 metres (3.3–6.6 ft) diameter, with its leaves forming dense rosettes. In winter when it flowers (June to September) it produces a tall raceme, bearing shiny red flowers that are pollinated by sunbirds. Taxonomically, it forms part of the Purpurascentes series of very closely related Aloe species, together with *Aloe microstigma, Aloe gariepensis, Aloe khamiesensis* and *Aloe framesii.*

Aloe arborescens

Family: Xanthorrhoeaceae

Genus: *Aloe*

Species: *arborescens*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Aloe arborescens, the krantz aloe or candelabra aloe, is a species of flowering succulent perennial plant that belongs to the genus Aloe, which it shares with the well known and studied Aloe vera. The specific epithet arborescens means "tree-like". *Aloe arborescens* is valued by gardeners for its succulent green leaves, large vibrantly-colored flowers, winter blooming, and attraction for birds, bees, and butterflies. *Aloe arborescens* is a large, multi-headed, sprawling succulent, and its specific name indicates that it sometimes reaches tree size. A typical height for this species is 2–3 metres (6.6–9.8 ft) high. Its leaves are succulent and are green with a slight blue tint. Its leaves have small spikes along its edges and are arranged in rosettes situated at the end of branches. Flowers are arranged in a type of inflorescence called a raceme. The racemes are not branched but two to several can sprout from each rosette. Flowers are cylindrical in shape and are a vibrant red-orange color. Taxonomically, it forms part of the Arborescentes series of very closely related Aloe species, together with *Aloe pluridens* and *Aloe mutabilis*.

Haworthia limifolia

Family: Xanthorrhoeaceae

Genus: *Haworthia*

Species: *limifolia*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Haworthiopsis limifolia, formerly *Haworthia limifolia*, is a species of flowering plant in the genus Haworthiopsis, native to southern Africa and first described in 1910. It is native to southeastern Africa (southern Mozambique, Swaziland, KwaZulu-Natal, and Mpumalanga. It is a relatively widespread species, with several known varieties, including arcana, gigantea, glaucophylla, keithii, striata, ubomboensis. The white-striped "striata" variety, from the Pongola region of northern KwaZulu Natal. The large "gigantea" variety, from Nongoma district, KwaZulu Natal. The light-coloured "keithii" variety, from the Ubombo mountains, Swaziland. The smooth, blue-green glaucophylla variety from Barberton, South Africa. The smooth, slender-leaved "ubomboensis" variety, from the Ubombo mountains, Swaziland.

Aloe humilis

Family: Xanthorrhoeaceae

Genus: *Aloe*

Species: *humilis*

Locality: St.Mary's College. Botanical garden

Floristic features:-

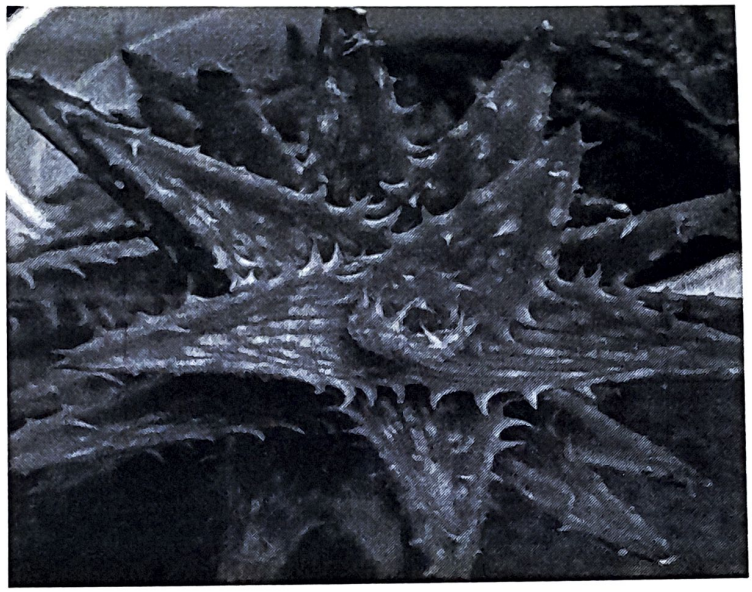
Aloe humilis, also known as spider aloe is a species of succulent plant in the genus Aloe. It is endemic to South Africa's Cape Province, and is a low growing, short stemmed Aloe with small spines and which grows in dense clusters. *Aloe humilis* is a low growing succulent that forms dense clusters of small up to 8 inch (20 cm) wide stemless (or very shortly stemmed) rosettes. The leaves are up to 5 inches (12.5 cm) long and 0.7 inch (1,8 cm) wide, pale blue-green or grey-green, incurved triangular-shaped (20-30 per rosette). They have long soft white marginal spines up to 0.1 inch (3 mm) long and a gray-green waxy surface covered with irregularly spaced bumps. The unbranched up to 1.1 feet (35 cm) tall flower spikes bearing about 20 pendulous up to 2 inches (5 cm) long bright red-orange flowers.

Aloe juvenna

Family: Xanthorrhoeaceae

Genus: *Aloe*

Species: *juvenna*



Locality: St.Mary's College. Botanical garden

Floristic features:-

Aloe juvenna (The "Tiger-tooth Aloe") is a species of plant in the genus Aloe. It is popular in cultivation but extremely rare in its natural habitat in Kenya.It has long been common in cultivation, but its origin was not known. The first recorded cultivated specimens were in South Africa, but although there were rumours that it had come from Kenya, its origins were a mystery even then. It was first believed to be a juvenile Aloe, due to its small size, and it was labelled "juvenna" ("juvenile") for this reason. However that label eventually became its formal name. Later, it was thought to be a hybrid perhaps of *Aloe distans* with *Haworthia coarctata* or a species of *Astroloba*.It underwent genetic testing in the 1970s and, when it was discovered that *Aloe juvenna* had a doubled set of chromosomes (tetraploidy), it came to seem more likely that this Aloe came from East Africa, where most other tetraploid Aloes originate. The name *Aloe juvenna* was finally published as a valid species name in 1979. Finally, in 1982, an expedition in the far south west of Kenya discovered a few plants on a tiny rocky mountainous ridge, high about tropical rainforest. The existence of these plants in such a remote locality confirmed that the species was a natural one. However, how it originally came to be brought into cultivation in the first place, remains a mystery.

Portulacaria afia

Family: Didieceaceae

Genus: *Portulacaria*

Species: *afia*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Portulacaria afia (known as elephant bush, dwarf jade plant, porkbush, purslane tree and spekboom in Afrikaans) is a small-leaved succulent plant found in South Africa. These succulents commonly have a reddish stem and leaves that are green, but also a variegated cultivar is often seen in cultivation. They are simple to care for and make easy houseplants for a sunny location. In frost-free regions they may be used in outdoor landscaping.It is a soft-wooded, semi-evergreen upright shrub or small tree, usually 2.5–4.5 metres (8–15 ft) tall. It is sometimes confused with Crassula ovata (Jade plant), which it is not closely related to. P. afra has smaller and rounder pads and more compact growth (shorter internodal spaces, down to 1.5 millimetres (0.059 in)). It is much hardier, faster growing, more loosely branched, and has more limber tapering branches than Crassula once established. The genus Portulacaria has been shown to be an outlier, relatively unrelated to the other genera in the family, which are all restricted to small ranges in the arid far west of Southern Africa.

Eichhornia crassipes

Family: Pontederiaceae

Genus: *Eichhornia*

Species: *crassipes*

Locality: St.Mary's College. Botanical garden

Floristic features:-

Eichhornia crassipes (formly *Pontederia crassipes*), commonly known as common water hyacinth and by its Bengali name "kochuripana," is an aquatic plant native to South America, naturalized throughout the world, and often invasive outside its native range. It is the sole species of the subgenus Oshunae within the genus Pontederia. Anecdotally, it is known as the "terror of Bengal" due to its invasive growth tendencies. Water hyacinth is a free-floating perennial aquatic plant (or hydrophyte) native to tropical and sub-tropical South America. With broad, thick, glossy, ovate leaves, water hyacinth may rise above the surface of the water as much as 1 meter (3 feet) in height. The leaves are 10–20 cm (4–8 inches) across on a stem which is floating by means of buoyant bulb-like nodules at its base above the water surface. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8–15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals. When not in bloom, water hyacinth may be mistaken for frog's-bit .One of the fastest-growing plants known, water hyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants. Each plant additionally can produce thousands of seeds each year, and these seeds can remain viable for more than 28 years.

Talinum fruticosum

Family : Talinaceae

Genius : *Talinum*

Species : *fruticosum.*

Locality: St.Mary's College. Botanical garden

Floristic features:

The plant is easily propagated from seeds or cuttings. Left in the right environment the plant will spread itself, although I have never seen it reach "invasive" proportions. If you know what you're looking for this is a plant you will find growing out walls, cracks, potholes, vacant lots, throughout the city, along with purslane, a close relative.

The edible leaves are rich in Vitamins A and C as well as iron and calcium. This species is grown in west Africa, south and south east Asia, warmer areas of north america and throughout central and south America. It is reported to be one of the most important leafy vegetables in Nigeria.

Talinum fruticosum

Family: Talinaceae

Genus: *Talinum*

Species: *fruticosum (L.)*

Locality: St.Mary's College. Botanical garden

Florstic features:

The plant grows erect, reaching a height of 30 to 100 cm (12 to 39 in). It bears small, pink flowers and broad, fleshy leaves.Talinum fruticosum is a herbaceous perennial plant that is native to Mexico, the Caribbean, West Africa, Central America, and much of South America. Common names include Ceylon spinach,[2] waterleaf, cariru, Gbure, Surinam purslane, Philippine spinach, Florida spinach, potherb fameflower, Lagos bologi, sweetheart, and Kutu bataw in Ghana from the Akan language[1] It is widely grown in tropical regions as a leaf vegetable.

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AQUATIC MEDICINAL PLANTS OF THOOTHUKUDI DISTRICT

A Short – Term Field Project Submitted to

ST. MARY'S COLLEGE (Autonomous),

THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN BOTANY

By

I. JERMINA 21APBO03

R. JOTHI KANI 21APBO04

E. PONMARI 21APBO12

DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI – 628001

2021-2022

CERTIFICATE

It is certified that this short-term field project work entitled "**AQUATIC MEDICINAL PLANTS OF THOOTHUKUDI DISTRICT**" submitted to **St. Mary's college (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfillment of the requirements for the degree of **Master of Science in Botany** and is a record of work done in the Department of Botany, **St. Mary 's College (Autonomous)**, Thoothukudi during the year 2021- 2022 by the following students.

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GUIDE

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EXAMINER

26/5/22

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INTRODUCTION

Aquatic plants are defined as "those species which normally stand in water and must grow for at least a part of their life cycle in water either completely submerged or emerged". Growth of vegetation occurs in "surface water". Surface water includes waterfalls, streams, rivers, canals, ponds, natural lakes etc. Low land tanks, ponds and flooded areas are rich in dissolved salts, consequently these stagnant or nearly stagnant shallow reservoirs of water with clay beds nourish a large variety of aquatics, semiaquatic and amphibious vegetation. Deeper lakes with dissolved salts and with beds of insoluble rocks do not generally sustain luxuriant vegetation growth. Vegetation in surface water is dependent on various factors such as the chemical and physical nature of the water, the nature of the bottoms whether muddy or silt- laden, the climatic environment including meteorological conditions of a locality, edaphic and geological nature of the substratum and last not the least on the biological nature of the water.

India is known for variety of medicinal plants from Himalayas to Cape Comerin. They are widely spread, some are restricted to hills and some are to plains and waste lands. They are used in traditional medicines such as Siddha, Ayurved and Yunani. About 1900 medicinal plants are commonly used in the preparation of medicines prescribed for various ailments.

The habitat of medicinal plants varies from the sea coastto alpine region. Some are also aquatic especially freshwater origin. Certain plants are observed to flourish on moist swampy ground and along the margin of water; Some other plants under certain conditions spread over the surface of water, while others remain suspended in the water. Some are rooted to the bottom of the pond and entirely submerged. There are others which are rooted to the bottom with leaves floating or submerged and flower above the surface of water. In addition to these, there are also a number of minute plants, many of which are microscopic. These float freely in water and thus live a life of free unattached existence.

Since diseases, decay and death have always co existed with life, the study of disease and their treatment must also have been contemporaneous with the dawn of the human intellect. The primitive man must have used as therapeutical agents and remedial measures those things which he was able to procure most easily. There is no authentic record of medicines used by the primitive man.

The " doctrine of signature" would also account for the use of several plants as medicinal agents. This doctrine is based on the resemblance in shape or colour of some organ in animal economy. In the ignorance of anatomical or physiological data to work upon the primitive man thinks that these articles possess some action on those organs which the resemble in shape, size or colour. Again another reason for the extensive use of vegetable drugs may be the fact that plants are everywhere at hand, their number is very great and their forms are distinct and peculiar and thus are procured without trouble.

It is greatly to the credit of the people of India that they were acquainted with a far large number of medicinal plants than the natives of any other country on the face of Earth. The Botanical Survey v of India generally survey the plants of India in total. The survey of medicinal plant is functioning under the ministry of health and family welfare. They also survey the plants with special reference to the medicinal value.

Flora of Tamil Nadu exhibits a diverse kind of medicinal plants. Many of them derive from hilly source, some are from freshwater plants and some are of coastal region. Studies have been done in Tamil Nadu with reference to general account of medicinal plants of the state. No study has been exclusively done for the aquatic medicinal plants.

The importance of primitive attempts at medicine were based on speculation and superstitions but in the present age importance of medicinal plants is increasing day by day due to the fact that many new and important alkaloids are being isolated more and more from the plants

as a result of better techniques of chemical analysis

A pond is either a natural or an artificial body of water that is enclosed. Ponds can occur naturally in the world or they can be human made (such as a garden pond). A pond ecosystem is a fresh water ecosystem that can either be temporary or permanent and consists of a wide variety of aquatic plants and animals interacting with each other and the surrounding aquatic conditions.

According to the National Geographic Society, an ecosystem is a "a geographic area where plants, animals, and other organisms, as well as weather and landscape, work together to form a bubble of life". Ecosystem ponds are small geographic areas that contain living organisms such as plants, animals and microbes as well as non-living parts such as rocks, gravel, water, soil and air. Ecosystem ponds can create a complete, low maintenance system that works together with nature to provide food, shelter and safety to the wildlife and plants that lives in and around it.

The water in the pond ecosystem is stagnant. Either natural or artificial boundaries surround the pond ecosystem. The pond ecosystem exhibits three distinct zones, the littoral zone, limnetic zone, profundal zone and benthic zone. The biotic components of the pond ecosystem occupy different levels in the pond ecosystem, therefore, avoid the competition for survival. Scavengers and decomposers occupy the bottom level, and fish occupy the middle level. The plants enclose the pond's boundaries and provide shelter to small animals and insects. Pond ecosystem show a wide range of variety in their size.

Some aquatic plants help to improve the water quality by absorbing pollutants and heavy metals. The shoreline plants absorb nitrogen and phosphorus and therefore prevent the algal bloom and maintain the oxygen level in the pond. Moreover, aquatic plants absorb animal wastes to reduce the nutrient availability for plants and therefore prevent the growth of algae. The pond ecosystem is one of the sites for the conservation of biodiversity as different types of plants and consumers occupy different strata in the pond and live together by interactingwith each other.

Ponds in mountain regions conserve the endangered species. The pond ecosystem also serves as a source of water for the species that do not live in the pond. Pond ecosystem contribute to the beauty of nature as they accommodate a variety of ornamental flowering plants. Stratification in the pond ecosystem determines the distribution of animals species in the pond. It reduces the competition among the species to some extent.

The present study is intended to make a preliminary survey of the availability of aquatic and semiaquatic plants nearby Thoothukudi city because of their medicinal importance. The area under study has been chosen because many plants thrive throughout the year due to perennial water source and also these water bodies are surrounded by paddy fields.

REVIEW OF LITERATURE

Most aquatic plants inhabit the shallow water or littoral zone of lakes and streams. Aquatic plants growing along a lake's edge are both a protective and nourishing component of the lake ecosystem. From a human viewpoint, aquatic plants are often seen as a hindrance to human recreation, but many people also recognize the importance of macrophytes for healthy lakes. The aquatic plant community is a critical habitat and nursery for fish, a source of oxygen for all organisms, a refuge for prey as well as a foraging area for predators, a buffer against erosion and sediment resuspension from both waves and shoreline inputs, and can significantly contribute to overall lake primary productivity (Knight et.al.2009).

Aquatic plants are still an untapped reservoir of antimicrobial and functional compounds that have potential as food ingredients toward the development of novel functional food and nutraceutical products.

Plants which have helpful pharmacological and restorative impacts on human wellbeing are considered as medicinal plants. These plants are rich in bioactive mixes like alkaloids, sterols, saponins, flavonoids, unpredictable oils and so on and are being used since old times. Folk or conventional medicine systems are used and preferred all over the world depending on choice, religious conviction, superstitions, social elements like socio-economic reasons and family-based therapeutic systems since time immemorial. They make use of a number of terrestrial, aquatic and semiaquatic plants and plant parts or their extracts for treatments of various disorders and diseases. Aquatic and semiaquatic plants are used for a number of purposes including their ornamental uses, biomonitoring and phytoremediation of water ecosystems and as medicinal plants. More than 5000–8000 plants are used as herbal medicine all over the world as synergic,

supportive or preventive medicine by providing raw material for healing and treatments. Although people all over the world are interested to know about the role of aquatic plants in herbal medicinal systems, rare studies are found on the subject. (Aasim et.al .2019).

Aquatic/semi aquatic plants unquestionably play momentous ecological roles as the dominant primary producer component of swallow water ecosystems. They are also referred to as hydrophytes or macrophytes and offers great economic importance to mankind (Bornette and Puijalon, 2011)

Aquatic flora directly serves as the major source of energy for greater diversity of biota besides conserving the aquatic habitat. In India, however, aquatic plants have been extensively used for a diversity of purposes since historical times, and are used (often cultivated) even today particularly for food, fodder, fibre and medicine (Krishnasamy et.al. 2014).

Aquatic plants have many unique biological features and are potential for their agricultural, horticultural, nutraceutical, ornamental and medicinal importance. Many plant species under aquatic origin were reported to have valuable folklore utilization in traditional medicine and used in phytoremediation. (Saravanakumar and Prabhakaran, 2013).

The quantitative and qualitative floristic survey, constant monitoring and protection of aquatic and semi-aquatic bodies are the need of the hour in order to save the aquatic flora and to maintain the wild progenitors as well as to explore the richness of aquatic flora in the field of drug discovery (Manohari et. al. 2019)

Aquatic ecosystems are important one which provide livelihoods for the millions of people who live around them. Man depends ponds for most of his needs like fishing, agriculture, irrigation, and other domestic purposes. Ponds are playing a very good role in rain harvesting, storage of water and regulation of groundwater level. So in order to maintain the ground water level we must conserve ponds and pond habitat. In earlier days aquatic plants are used as food, fodder, medicine etc. but with the advancement in life styles the uses of aquatic plants are foregone and are treated as mere weeds which are making the ponds useless (Bhagyaleena and Gopalan 2012).

Materials and Methods

A number of ponds are present in Thoothukudi city and are receiving water from the channels of the river Tamirabarani as well as from monsoon rains. The ponds are usually surrounded by the cultivar lands. The farmers also sometimes get water from such ponds. The vegetative in these areas are disturbed because they are quite often used by farm animals for grazing. The ponds and other marshy places nearer to Thoothukudi city are chosen for the present study to collect aquatic and semiaquatic medicinal plants.

Area of collection: Ponds in Korampallam and Peikulam. These spots are chosen because they are the perennial sources of water. Various aquatic medicinal plants are collected and photographed. The vegetative, floral characteristics and medicinal uses of these plants are collected from the sources listed in the bibliography. The plants are arranged in alphabetical order.

ALTERNANTHERA PHILOXEROIDES (MART.) GRISEB.

AMARANTHACEAE

Herbs perennial. Stem ascending from a creeping base, 55-120 cm, branched; young stem and leaf axil white hairy; old ones glabrous. Petiole 3-10 mm, glabrous or slightly hairy; leaf blade oblong, oblong-obovate, or ovate-lanceolate, 2.5-5 × 0.7-2 cm, glabrous or ciliate, adaxially muricate, base attenuate, margin entire, apex acute or obtuse, with a mucro. Heads with a peduncle, solitary at leaf axil, globose, 0.8-1.5 cm in diam. Bracts and bracteoles white, 1-veined, apex acuminate; bracts ovate, 2-2.5 mm; bracteoles lanceolate, ca. 2 mm. Tepals white, shiny, oblong, 5-6 mm, glabrous, apex acute. Filaments 2.5-3 mm, connate into a cup at base; pseudostaminodes oblong-linear, ca. as long as stamens. Ovary obovoid, compressed, with short stalk.

Uses:

It has been used extensively as a traditional remedy for various viral diseases (e.g., measles, influenza, and haemorrhagic fever. This plant has traditionally been used in India as a remedy for anaemia, for the treatment of diarrhoea and dysentery in Bangladesh, and to treat certain blood conditions, fever, post-natal depression, wounds and to stimulate milk secretion.

EICHHORNIA CRASSIPES SOLMS

PONTEDERIACEAE

Water plant, rooting in mud or free floating. Leaves in a rosette, spoon or paddle - shaped, apex rounded; petioles usually turbinately swollen to form floates, upto 10 in - long - Flowers in simple, rarely paniculate, sub – spicate racemes from the sheath of the leaf. Perianth funnel – shaped, tube short, often 2 lipped. Stamens 6, declinate, irregularly inserted. Ovary sessile, 3 celled; stigma slightly swollen. Capsule ovoid - oblong or linear

MEDICINAL USES:

Flowers are highly esteemed as alternatives and tonics useful as blood purifiers.

HYDROLEA ZEYLANICA VAHL.

HYDROPHYLLACEAE

An annual herb, rooting at the nodes. Leaves alternate, entire. Flowers blue, in short terminal racemes or cymes. Calyx 5 partite, lobes lanceolate. Corolla rotate or campanulate. Stamens 5, filaments filiform. Ovary 2 - celled with fieshy placentas adnate to the dissepiments; styles 2; stigmas capitates. Fruit a globose or septicidal capsule, thin and transparent. Seeds minute, irregularly wrinkled.

MEDICINAL USES:

The leaves beaten into a pulp and applies as a poultice are considered to have a cleaning and healing effect on neglected and callous formed ulcers. They apparantely possess some antiseptic properties. The leaves are considered to possess cleansing and antiseptic properties. Paste of whole plant with coconut oil is applied in minor cuts, wounds and boils as antiseptic for quick healing

HYGROPHILA AURICULATA SCHUM

OR

ASTERACANTHA LONGIFILIA NEES

ACANTHACEAE

A stout herb of wet places; stems, numerous, with long sharp thorns at the nodes. Leaves narrow lanceolate, lineolate, in whorls of 6, the two outer leaves of each whorl the larger. Flowers in sessile auxiliary whorls, surrounded by the slightly recurved spines; bracts leafy; bracteoles linear. Calyx deeply 4 - partite, the upper lobe the largest. Corolla 2 - lipped, the upper lip - 2 the lower 3 lobed with 2 crested folds on the palate. Stamens 4, didynamous. Ovary 2 - celled. Fruit a linear – oblong capsule. Seeds 4 – B on hard retinacula, flat and white hairy when wet.

MEDICINAL USES:

The leaves are sweet, sour, tasty; oleaginous, tonic, aphrodisiac, useful in diarrhoeas and dysenteries, thirst, urinary calcuit, urinary discharge, inflammations, biliousness, diseases of the eye, pains, ascites and abdominal troubles, anaemias, constipation, anuria.

The seeds are cooling, tasty, acrid, bitter, aphrodisiac, tonic, sedative to the gravid uterus; constipating; useful in diseases of the blood and biliousness (Ayurveda).

The leaves are good for cough, applied for gleet and in lumbago and pains in the joints. The seeds are tasteless, fattening, aphrodisiac, tonic; improves the blood (Yunani). The seeds are given for gonorrhea and with milk sugar in spermatorrhoea. The decoction of the root was used in 1 - ounce doses as a diuretic in dropsy of chronic Bright's disease and found to be fair diuretic. The seeds together with the fruits of Tribulus terrestris. L. are powdered and given to cure impotency.

IPOMOEA AQUATICA FORSK

CONVOLVULACEAE

An annual pretty water plant with hollow stems. Leaves alternate, entire, hastate or cordate at base, long petioled. Flowers axillary, in cymes. Sepals glabrous, sometimes slightly pubescent, subequal, smooth, ovate, obtuse. Corolla funnel shaped, pink corolla darker in the throat. Stamens 5, usually included; filaments filiform. Ovary 2 celled; ovules 4; style filiform. Fruit a 4 vaived capsule. Seeds minutely silky puberulous.

MEDICINAL USES:

Bitter variety : Anthelmintic; useful in leucoderma and leprosy.

Sweet variety : cooling; useful in biliousness and fever; increases " kapha" and "vata"

It is carminative, lessens inflammation, useful in fever, jaundice, biliousness, bronchitis, liver complaints. The juice when dried is nearly equal to scammony in purgative properties. It is considered very wholesome for females who suffer from nervous and general debility. The leafy juice with salt is used in stomach disorders. Plant juice in antitode arsenic and opium poisoning. Filtrate of crushed flowers is used as eye drops.

Dried juice has purgative properties. Leaves and stems are said to be cooling. The buds are used in the treatment of ring worm. In Assam, the plant is given for nervous and general debility. Fried leaves are taken to cure head reeling. Leaf juice along with cow ghee is given to cure gonorrhea; is a purgative and acts as blood purifier

IPOMAEA CARNEA JACQ.

CONVOLVULACEAE

A large diffuse shrub with milky juice. Leaves ovate, entire, acuminate. Flowers in dichotomous. Axi;;ary cymes. Pale rose, large, campanulate. Sepals ovate. Patals 5, connate in a funnal shaped corolla. Staments 5, included. Ovary 2 celled; style filiform; stigma capitates. Capsules 0.5 in.long, glabrous. Seeds silky.

MEDICINAL USES:

When administered intravenously the water soluble toxin present in the leaves causes haemolysis and reduces the blood pressure. When given orally the either soluble toxin acts as a mild purgative.

JUSTICIA OVATA (WALTER) LINDAU

ACANTHACEAE

It is a perennial from a rhizome, sometimes forming extensive clones. The stems are erect, 4-angled, green in color, and glabrous. Leaves are opposite, sessile or with short petioles, lanceolate to elliptic or ovate in outline, glabrous or with a few scattered hairs, with entire or crenate margins. The flowers are produced solitary or in pairs in axillary and terminal spikes. The calyx has 5 linear green lobes. The corolla is 2-lipped and white to purple in color. The lower lip of the corolla is marked with dark purple. The fruit is a capsule.

Uses:

It has been used in treating gastrointestinal and cardiac diseases, anemia, and diabetes. It has also been used as an antipyretic and an antibiotic. It has been also used to treat dysmenorrhea, syphilis, and acne.

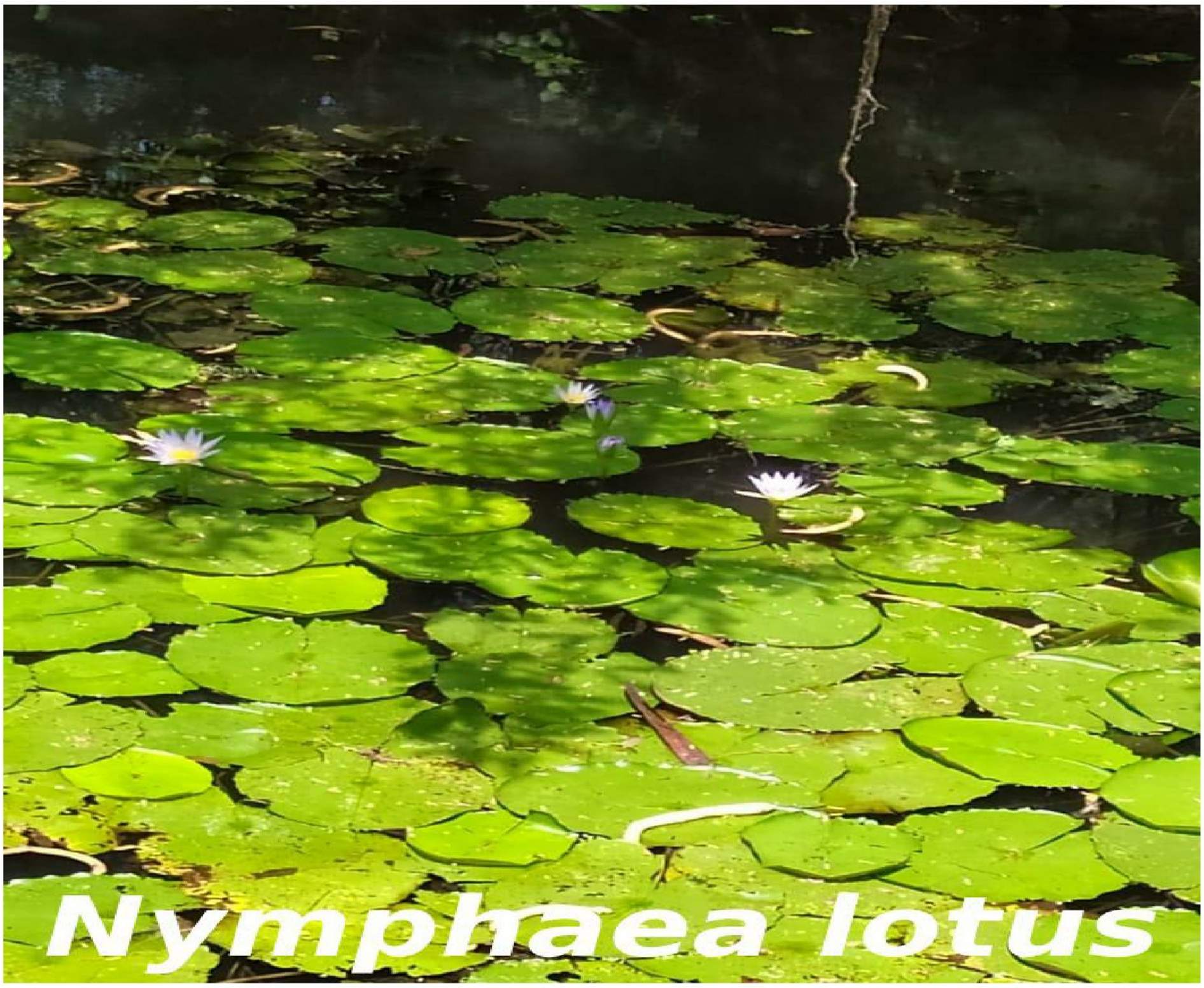
MARSILEA MINUTA L.

MARSILEACEAE

Rhizome long creeping, branched, subterranean bout 30c.m. long, upto 2mm.thick, green, aquatic plants. Stipes scattered, usually green, glabrous. Leaves 4, sessile arranged at the tip of the stipe in clover leaf model, obovate, lateral margin entire; Sporocarps borne at the nodes in clusters alternately, five per cluster, sporocarp bean - shaped adnate to the peduncle laterally.

MEDICINAL USES:

Plants are used in cough, spastic condition of leg muscles and also in insomnia. With milk fruits are used in nervous and general debility, seminal weakness and Leucorrhoea. The juice expressed from the stem is used in eye - diseases. Raw leaf paste is applied on forehead to cure headache and for head cooling. Leaves fried in cow ghee are taken regularly as curry to cure biliousness. Leaf juice along with root extract of Asparagus racemosus and sugar candy powder is taken orally or leaf juice with ginger juice and honey is also taken to increase sperm formation. Warm root paste with black pepper is applied around boils as suppurate.



NYMPHAEA LOTUS L.

NYMPHACEAE

White lotus is a perennial plant growing to a height of 45 cm. The flowers are white in color sometimes, with a pink tinge. The leaves vary from green to red-brown, with a number of purple spots robust aquatic herb with tuberous rhizome. Leaves 10–32 x 11–28 cm., coriaceous, orbicular or suborbicular, incised-cordate, somewhat peltate, lobes nearly closed or slightly overlapping, margin ± repand, dentate-mucronate, teeth formed by the convergence at the margin of (2) 3 nerves, upper surface smooth, under surface prominently nerved to the edge, primary lateral nerves 7–9 on each side of the midrib, forking dichotomously 3–4 times. Flowers white or cream, 10–18 cm. in diam., peduncle stout, glabrous. Fruit 4–6 cm. in diam., depressed-globose. Seeds 1.2 mm. long, ellipsoid, with longitudinal lines of hairs.

Uses:

White lotus was used in ancient Egypt, as a key to good health, sex and re-birth. The plant is an aphrodisiac for both men and women and a general remedy for all kind of illnesses. Continued use of tiger lotus enhances sexual vigor and general good health. It is a tonic richer than ginseng, pain reliever richer than arnica, circulation stimulant richer than ginkgo biloba and sexual stimulant richer than Viagra. The flowers of white lotus are used for preparing tea that creates a warm, euphoric glow.

The dried flowers are smoked by themselves or mixed with other herbs to add flavor to smoking mixtures. The effects of tiger lotus are enhanced when soaked in wine or other alcohol.

The plant is effectively used to increase memory and create a feeling of euphoria and ecstasy, without the use of narcotics. Its rhizomes are cooling, sweet, bitter and tonic and used in diarrhea, dysentery, dyspepsia and general debility. White lotus is used internally in treatment of gastrointestinal disorders and jaundice. The leaves are used in cutaneous, subcutaneous parasitic infection, eye treatments and pregnancy. The seeds are used in sauces, condiments, spices and flavorings.

NYMPHAEA TETRAGONA GEORGI

NYMPHAEACEAE

Nymphaea tetragona is an aquatic perennial with floating leaves and white flowers growing from unbranched rhizomes. The leaves have entire margins and maybe tinted purple or sometimes mottled reddish brown or purple. The receptacle is four-angled. Plants produce a single floating flower that is 1.5 to 3 inches wide, with up to 15 petals; each flower has 30 to 45 yellow stamens. The sepals and out petals are produced in whorls of four, the sepals are green in color. The seeds are rounded in shape and 2-3 × 1.5-2 mm long, being 1.3-1.5 times as long as broad;

Uses

The rhizome is used locally to cure acute diarrhea and dysentery by tribal herbal practitioners and it can also used to join bone-fractures. The rhizome (underground horizontal stem) and petiole (leaf-stalk) are consumed as a vegetable. It is applied to the body for vaginal conditions, diseases of the throat and mouth, and for burns and boils. A decoction prepared from the flower is administered as a uterine injection can cure uterine cancer completely and also the flower has the effect of reducing sexual desire when its chemicals are extracted with drinking alcohol.

PANICUM REPENS L.

POACEAE

Annual erect herb. Leaves distichous, involute, rigid, glabrous. Inflorescence open panicles. Spikelets lanceolate, elliptic. Glumes herbaceous; Lower glume suborbicular. Lemmas dissimilar. Lodicules 2. Stamens 3. Styles 2, free. Grain tightly enclosed in the hardened lemma and palea.

MEDICINAL USES:

The seeds and roots are reported to be slightly cyanogenetic. In Java the rhizome is reported to be used for irregular mensuration.

PERSICARIA AMPHIBIA L.

POLYGONACEAE

The herbaceous perennial plant has both terrestrial form as well as aquatic form.

In form of terrestrial, it is 1 to 3 feet tall, more or less erect and either branched or sparingly branched. Leaves are alternate that occurs along entire length of terrestrial stems. Leaves are 2½-8 inches long and ½-3 inches across, narrowly lanceolate to lanceolate. Margins are entire or toothless. Upper surface of leaf is medium to dark green, glabrous to sparsely short pubescent. The lower surface is light green, glabrous and fine hairs occur along midvein and toward the leaf base. Leaf bases are wedge shaped or narrowly rounded and leaf tips are acute and slender. In aquatic form, stems of the plant are 6 feet long. The upper leaves float on the water surface. The leaf shape is lanceolate to oblong or elliptic to oblong having obtuse tips and rounded bases. The upper surface and lower surface of the leaves are glabrous. The terrestrial plants have light green or yellowish green stems which are glabrous to pubescent, relatively stout and terete. Flowers are 4-6 mm long and each flower contains 5 pink to rosy pink sepals, five stamens with white filaments and pink anthers. Flowers are perfect and sometimes unisexual. Sepals are oval spreading outward slightly when fully open. Seeds are broadly ellipsoid or ovoid, somewhat flattened and brown measuring 2 to 3.5 mm long.

Uses

Infusion made with leaves and stems are used for treating stomach pains and diarrhea in children. Roots are consumed raw or infusion made with dried roots is used for treating chest colds. Apply the poultice of fresh roots to the mouth for treating blisters.

PISTIA STRATIOTES L.

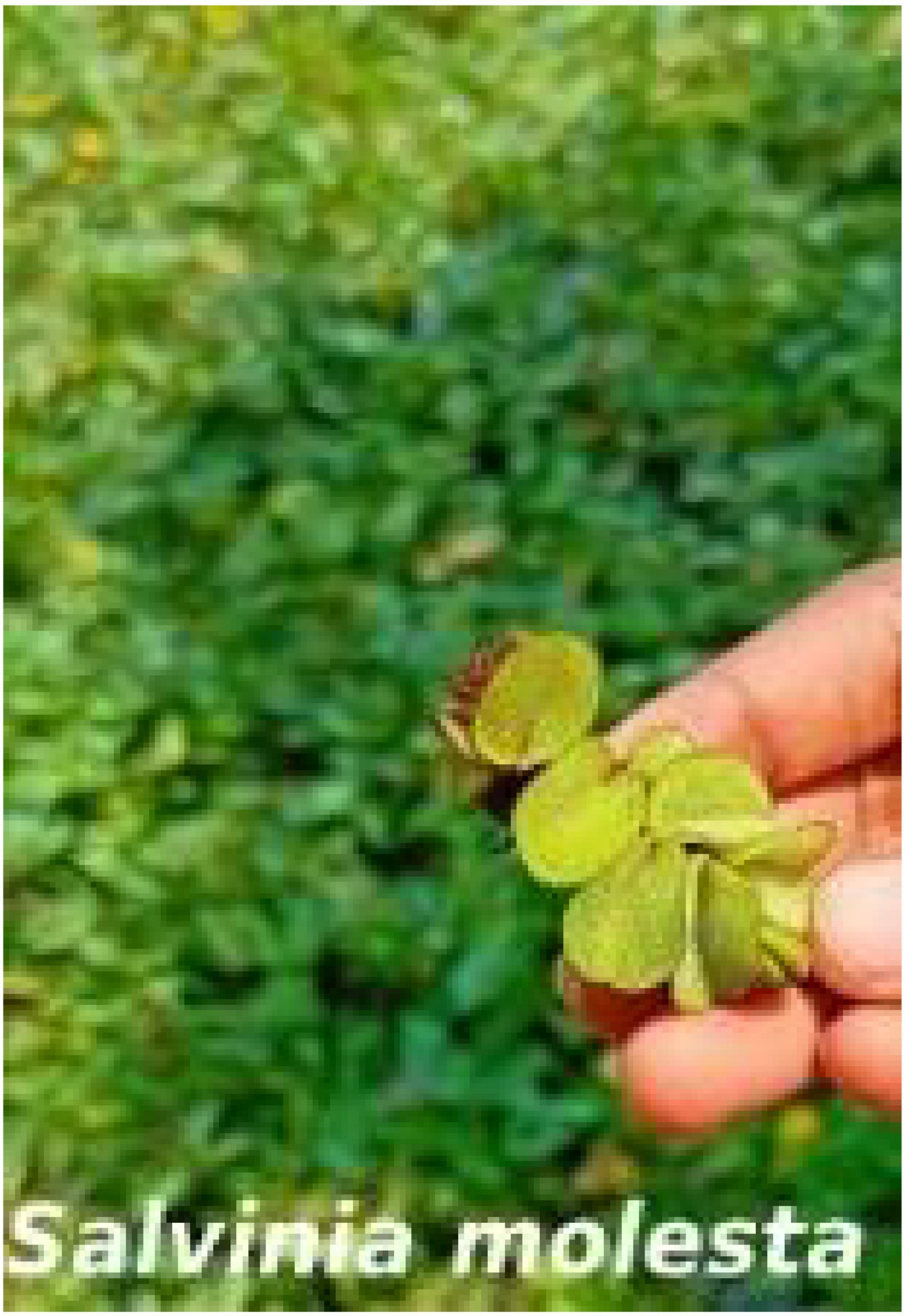
ARACEAE

A small, floating, gregarious, stoloniferous herb; with roots of tufted fibres. Leavessesile in a close spirel together rorming a cup. Spathe small, shortly peduncled, shortly tubular below opening out into an ovate, concave limp. Spadix adnate to the back of the tube of the spathe, free above. Male flowers in a whorl of a few connate stamens bebeath the apex of the spadix. Neuters few, minute, confluent in a ring below the male. Female flowers solitary. Perianth O.Ovary one - celled; concial style; stigma discold; Ovules many. Berry ovoid. Seeds many oblong.

MEDICINAL USES:

The plant is useful in "tridosha", fevers, diseases of the blood tubercular glands. The root is bitter, diuretic; good for wounds, information, burns. The palnt is cooling and demulcent, and is given in dysuria.

The leaves are made into poultices and applied to haemorrhoids. Mixed with rice and coconut milk, they are given in dysentery and with rose - water and sugar in cough and asthma. The ash is applied to ringworm of the scalp. An oil is prepared by boiling the juice of the leaves in coconut oil and used externally in chronic skin diseases.



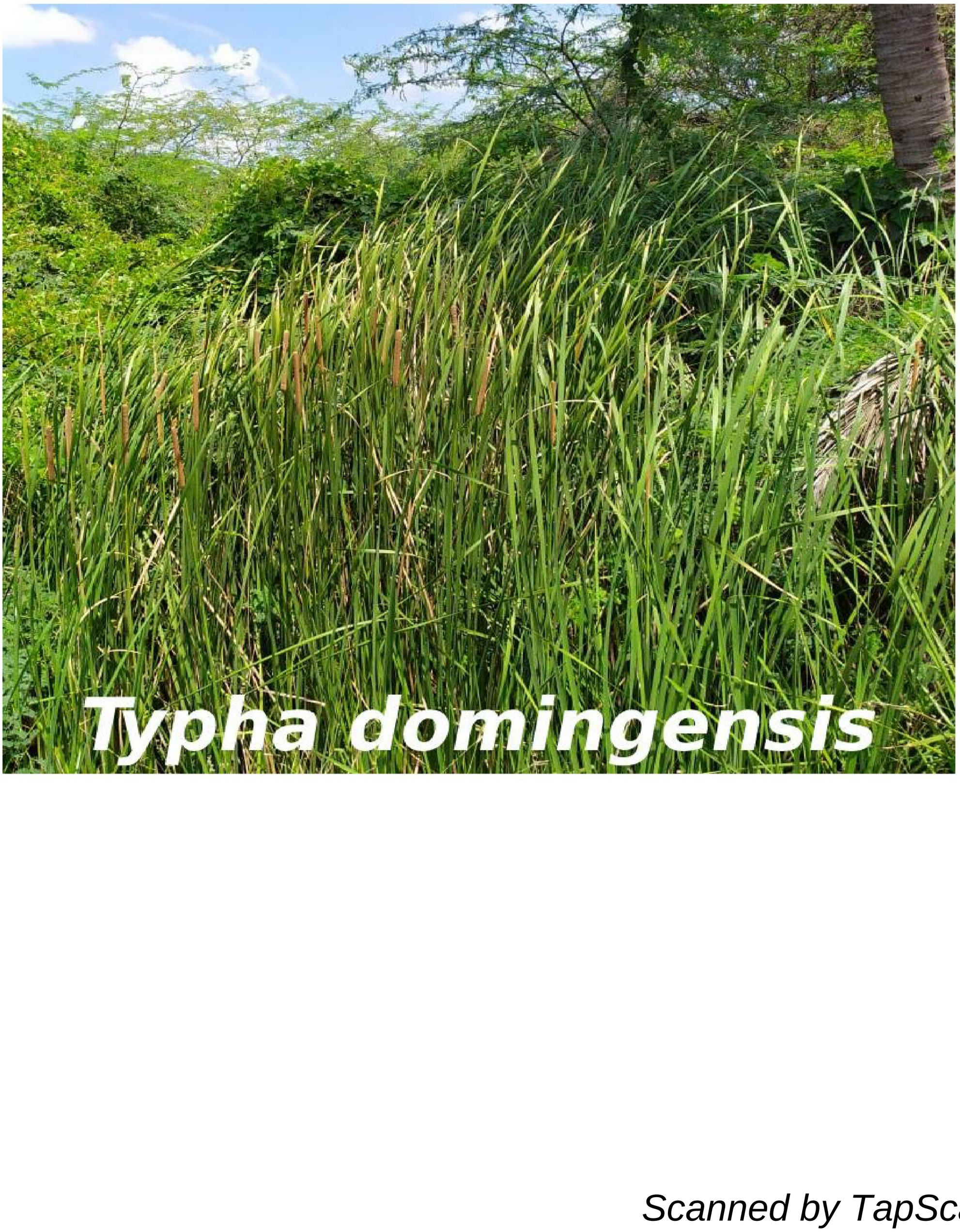
SALVINIA MOLESTA D.S.MITCH.

SALVINIACEAE

Free-floating, green-brown, freshwater fern with branching horizontal stems and has submerged feathery 'roots'.Plants produce slender, branching runners and form mats of vegetation very quickly. These slender stems (1-2 mm thick) are much-branched and grow up to 30 cm long (usually only 6-25 cm long) before separating to form new plants. Leaves borne in threes; appear 2-ranked, but with 3rd leaf finely dissected and dangling, resembling roots; rounded to somewhat broadly elliptical, to 2 cm long, with cordate base, upper surface with 4-pronged hairs joined at the tips (resembling an egg beater), lower surface hairy.

Uses:

S. molesta has been used to extract nutrients and pollutants from the water. When this plant is dried out, it is used as satisfactory mulch. Favored environmental conditions Salvinia molesta prefers to grow in slow-moving waters such as those found in lakes, ponds, billabongs (oxbows), streams, ditches, marshes, and rivers.



TYPHA DOMINGENSIS (PERS.) STEUD.

TYPHACEAE

Typha domingensis is a very vigorous, herbaceous perennial plant. Growing from a fast-spreading rhizomatous rootstock, it forms a colony of unbranched leafy stems 150 - 400cm tall. Rhizomes with stolons; cylindrical, erect shoots, 3-4 mm wide near the inflorescence. Leaves alternate; shape linear; margin entire; surface glabrous, brownish glands visible from base of inner (adaxial) leaf surface Flowers monoecious, terminal, spiked inflorescence with female spike below the male spike on the stem; usually a 1-8 cm bare stem separates the two spikes; female spike is medium brown in color. Fruits follicle

Uses

The leaves are analgesic, antioxidant, diuretic. The leaves have shown significant nootropic activity and have potential for use in the treatment of Alzheimer's Disease. The pollen is astringent, desiccant, diuretic, haemostatic and vulnerary.It is used in the treatment of nose bleeds, haematemesis, haematuria, uterine bleeding, dysmenorrhoea, postpartum abdominal pain and gastralgia, scrofula and abscesses.It is contraindicated for pregnant women.The seed down is haemostatic .The rootstock is analgesic, antioxidant, astringent, cytotoxic and diuretic.

Conclusion

Each and every plant in the world is useful in some way or another. Earlier, the plants are utilized based on the "Doctrine of Signature" that is, God marked or signed each plant in some way or the other to indicate its medicinal property. The present work describes the edible and medicinal properties of aquatic plant species in ponds of Thoothukudi district, Tamil Nādu. The present study reveals that the plants in ponds which is becoming serious weeds in the water bodies of the country, can be effectively utilized for their food and medicine attributes, which will change the status of the plants from worst weed to important medicines or food which are useful for mankind. The present work emphasizes the usefulness of the aquatic plants wealth which in turn may form other criteria to conserve the delicate ecosystems considering the services they provide to the mankind.

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