A STUDY ON THE MORPHOLOGY AND SEED COAT ANATOMY OF SELECTED ECONOMICALLY IMPORTANT SEEDS OF BRASSICACEAE FAMILY

A Short Term Project Work Submitted to St. Mary's college (Autonomous) affiliated to Manonmaniam Sundaranar University in Partial Fulfillment for the Degree of

BACHELOR OF SCIENCE IN BOTANY

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CERTIFICATE

It is certified that this short term project work entitled **"A Study on the Morphology and Seed Coat Anatomy of Selected Economically Important Seeds of Brassicaceae Family "** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfillment of the requirements for the degree of **Bachelor 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 2020 - 2021 by the following students.

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INTRODUCTION

Plants are very important for the living world and one of the most invaluable natural resources for mankind. The importance of plants to the living world hardly needs any elaboration. Human beings from the time immemorial directly and indirectly in many ways related with plants (Benedict et al., 2016). During the history of civilization man has been continuously exploiting the nature for the best possible life. In the past, the needs of man were limited, the nature provided him with what he needed, and the two lived in complete harmony. With the development of civilization the needs of the man increased and he without knowing the consequences, began to over exploit the nature (Adeel et al., 2012).

Even today, when the scientific worth of environmental factors and natural resources and their interrelationships are better understood, the process of destruction and damage to the environment in continuing under the pressure of population explosion. The increased per capita needs of man has inevitably led to the denudation of forest to meet the demands for food crops, fodder crops, cash crops like jute and cotton and plantation crops like rubber, coffee,' tea mulberry etc. along with roads made for hydroelectric works, townships, railways, road ways, canals, mines etc. causing many ecological damages and depriving many of their traditional live hood (Barclay, 2015).

Seeds are probably more valuable than any other plant part for men as well as for plant itself also. Form the time immemorial; in every SOCiety the three fundamental and basic needs of the people are: food, shelter and clothing; among these food is the most important and basic need for the existence of human being. The

chief source of human food is cereals, legumes, nuts, vegetables and fruits. Committed over a one million years to a nomadic life, man settled down about 10,000 years ago when he learn to satisfy his hunger by growing food, specially seed foods. Seeds, the great staple food, feed more people than does any other type of food in the most part of the universe and are valued for their chemical composition and nutrition. The endosperm with their rich food reserves for the embryo and seedling offer man and other animal a highly nutrious food that can be easily stored (Bobrov et al., 2004)

According to (Fukuhara and Lidén, 1995), seed is the seat of partial development of new embryo and embryo is a connecting link between two generations of a plant, provides a continuity of genetic material and constitute a slender threshold of life for plant. Biochemically seeds are consisting of starch, carbohydrates, proteins, oil content etc.

As per the seed definition, it mainly consists of three parts: Embryo, endosperm/cotyledons and seed coat/testa. These three parts of a seed offer an interesting field of seed study. An embryo is a future plan for establishment of new generations attracted towards a very complex and the most important phenomenon - seed germination that is one of the important index of seed quality. Endosperm/cotyledons with its nutritive reserve food provide wide and important field of the study about biochemical make-up of seeds. This study must be resulted in to some nutritive information about balanced diet in relation to population explosion. Many seeds contain edible/non-edible oil content in more/less percentage. In case of non-edible oil it will be useful to various industries in the production of oil based products viz. soap, cosmetics, toiletries, lubricant, paints, varnishes etc. In the last few decades, oil seed sp. of forest origin has attracted the attention of researchers. Seed

coat/testa - protective layer of seeds with its different color, thickness, surfaces, etc. provide field for the study of external seed morphology. It also includes various seed shapes, size, weight, and hilum shape etc. these seed characters are very useful and important tool for the correct identification of plant (Gabr, 2014).

Brassicaceae are commonly named as the ‘‘mustard’’(from the Latin mustum ardens) plant family due to the sharp, potent flavour attributable to their main metabolites, the glucosinolates (GLSs), which contain sulphur.The members of the mustard are the earliest cultivated plants in the Brassicaceae family (Rahman et al., 2018). Among 338 genera, more than 3,700 species belong to the wide range of family Brassicaceae which is also known to be crucifers. Since 1500 BC oldest cultivated plants known to humans are said to be Brassica plants. For the discovery of the new drugs, medicinal plants provide a good source either as a pure compound or as an extract (Paudel and Heo, 2018). A person consumes Brassica vegetables of about 6.3 kg person annum. Cruciferous are the vegetables that belong to the family Brassicaceae which are commonly referred to as crucifera. Broccoli, brussels sprouts, kale, mustard, cabbage, turnips, cauliflower, boy Choy and Chinese cabbage are some of the commonly consumed vegetables of Cruciferae which has high phytochemical constituents and a rich source of vitamin C. They are also grown and used all over the world by various cultures due to its great environmental adaptation. Numerous species used in traditional medicine and culinary belongs to the family Brassicaceae and these are also recognized as the functional food (Shah et al., 2018).

Brassica vegetables exhibit biological activities like antibacterial, anticancer activity, antiviral and for the innate immune response system these vegetables act as a potent modulator. In the traditional systems of medicines like Chinese and Unani, the

crude extracts of medicinal plants are widely used at a domestic level in rustic areas (Moon, 2014). From ancient times, most of the cruciferous plants are cultured. Mediterranean basin is the native for these plants and there has high consumption in local markets. All over the world, many areas consume Brassicaceae vegetables as stable food and it considered to be a good source of amino acids, minerals, carbohydrates, vitamins, different groups of phytochemicals. To improve the phytochemicals with good health benefits and to tolerate herbicides, insects, soil pests and diseases, these plants are incorporated in extensive breeding programs as a source of value-added traits of agronomic interest (Devi et al., 2017).

Keeping the above things in mind, three economically important seeds like *Raphanus sativus, Brassica juncea* and *Brassica oleracea.*

SCOPE AND OBJECTIVES

Brassicaceae, a mustard family includes about 338 genera and more than 3,700 species that are most commonly consumed a group of plants all over the world. Regular consumption of Brassicaceae vegetables provides a good source of bioactive compounds and different levels of nutrients in the everyday diet. It consists of many numbers of mineral, fiber, vitamin and phytochemical content thereby it is considered to be the staple food in various parts of the world. After soybean and palm, Brassica oilseed crops serves as the third most significant source with 14% of the world's edible vegetable oil. Not only the edible oil, the phytochemicals from the different parts of these plants provide a great source for medicinal and agronomic purposes.

The main objectives of the present study is to study the

1. Macroscopic characters of selected seeds.
2. Organoleptic characters of selected seeds
3. Microscopic analysis of selected seeds
4. Powder microscopy of the selected seeds
5. Histochemical analysis of selected seeds.

REVIEW OF LITERATURE

The spermatophytes, comprised of the gymnosperms and angiosperms, are plants that produce seeds that contain the next generation as the embryo. Seeds can be produced sexually or asexually; the former mode guarantees genetic diversity of a population, whereas the latter (apomictic or vegetative reproduction) results in clones of genetic uniformity. Sexually produced seeds are the result of fertilization, and the embryo develops containing, or is surrounded by, a food store and a protective cover. Seeds of different species have evolved to vary enormously in their structural and anatomical complexity and size (the weight of a seed varies from 0.003 mg for orchids to over 20 kg for the double coconut palm (*Lodoicea maldvica*)). **(Black *et al.,* 2006).**

Brassicaceae, a mustard family includes about 338 genera and more than 3,700 species that are most commonly consumed a group of plants all over the world. It consists of many numbers of mineral, fiber, vitamin and phytochemical content. The phytochemicals from the different parts of these plants provide a great source for medicinal and agronomic purposes. Around worldwide it is estimated that most frequently consuming vegetables include cauliflower, cabbage, turnip, broccoli and kohlrabi because of the presence of several useful dietary health attributes and also presence of several antioxidant phytochemical like carotenoids, ascorbic acid, and phenolic compounds, it involves in controlling various diseases related to cancer, heart and degenerative diseases **(Saranya *et al,* 2019).** Aroma, taste and health benefits of the vegetables belongs to Brassicaceae attracts the people to towards them.

USES OF BRASSICACEAE FAMILY

Brassicaceae comprise a diverse family of plants and provide one of the most extensive and varied range of end products used by man from a single plant genus. Mustards are members of the Brassicaceae family, and are among the earliest cultivated plants. Their seeds are one of the oldest recorded spices with use and cultivation dating back over 5000 years (**Watson and Preedy 2010).**

Literature suggests that, within the history of human settlement in Australia and New Zealand, different types of Brassicaceae mustards, namely *Alliaria petiolata, Brassica alba, B. carinata, B. juncea, B. napus, B. nigra, B. rapa, Calepina irregularis, Erysimum repandum, Neslia paniculata, Sisymbrium officinale, S. orientale* and *S. erysimoides* have been naturalized and adapted for use as food, incorporated into traditional medicine and play an important role in the agriculture of the two countries **(Rahman *et al.,* 2018)**

Mustards have been consumed for centuries as vegetables, and their products used as condiments and as edible and industrial oils (Raymer, 2002). The oil is commonly used for cooking and to add a hot and spicy flavor to food (Duke, 2002). As a crop, they are also one of the highest oil yielding and high protein containing oilseed species. Economically important members of this family include vegetables like broccoli, cabbage, Chinese cabbage, turnip, and cauliflower, and the seed oil crop canola **(Collett, 2014; Spragg 2016).**

MORPHOLOGY AND ANATOMY OF SEED

Seed morphology is one of the key factor in determining the growth and development of a plant. Morphological parameters varies according to species too species even it differs significantly among the varieties also. The seed coat (testa) is a maternal tissue that surrounds the embryo, endosperm and perisperm (if present). It protects the internal structures from biotic stresses and against desiccation and mechanical injury, assists in gas exchange and water uptake, provides a conduit for nutrients for the embryo and endosperm during their development, and may be a means for seed dispersal. Its structure influences seeds' viability, longevity and germinability/dormancy. Sometimes the seed coat can serve as a useful characteristic for taxonomy **(Black *et al.,* 2006).**

During seed development the integuments, which are an initial seed coat tissue, undergo differentiation into layers containing specialized cells. Some cell layers in the seed coats may accumulate large quantities of compounds, such as mucilage, phenolics or pigments **(Moise *et al.,* 2005).** In the Brassicaceae and Fabaceae, some cell layers do not undergo any significant differentiation and remain parenchymatous (they are often crushed at maturity), while others undergo a slight thickening of the cell wall and become collenchymatous. Some cell layers undergo extensive secondary thickening of parts of the cell walls and become sclerified (palisade layers).

Morphological variation in seed Characters includes difference in Seed size and shape. Seed Shape is an important trait in plant identification and classification. In addition it has agronomic importance because it reflects genetic, physiological, and ecological Components and affects yield , quality, and market price. The use of digital technologies, together with development of quantification and modeling

methods, allows a better description of seed shape . Image processing system are used in the automatic determination of Seed size and shape, becoming a basic tool in the study of diversity. The Comparison of the seed images to a geometrical figure (circle, Cardiod, ellipse, ellipsoid, etc) provides a precise quantification of shape .The methods of shape quantification based on these models are useful for an accurate based on these models are useful for an accurate description allowing to complete between genotypes or along developmental phases as well as to establish the level of variation in different set of Seeds **(Emilio *et al.,* 2016).**

Seeds vary greatly in shape between species. They can be round, round-oblate, oval, ellipsoid, oblong, flattened, reniform, spindly, triangular or curved; the longitudinal axis of the seed may be straight (anatropous or hemianatropous) or bent (campylotropous). The surface texture of a seed depends on the testa or pericarp structure and can be smooth, shiny, matt, grooved or concave.

Seed size varies over ten orders of magnitude. In general, larger plants produce larger seeds. However, certain habitats and plant life history patterns are associated with the production of larger or smaller seeds. They tend to be larger when produced by plants growing in harsher habitats. Shady and/or dry habitats favour larger-, and high altitudes smaller seeded species **(Lundgren, 2009).**

Mariana and Carlos (2000) described the distinctive feature of cactus such as colour, from, and size. Seed physiological characters such as germination and dormancy. Seeds of Vitaceae can be easily recognized by their unique features. To facilitate identification of genera based on seed morphology, an experiment of 252 seeds representing 15 genera of Vitaceae was studied with attention to morphological characters and seed coat anatomy. Shape and position of Ventral infolds and Chalaza,

shape of Ventral- infold cavities, and testa anatomy are characters that can genetically differentiate Vitaceous Seed. Typically, Seeds of *Leea, Cissus, Cyphostemma, Tetrastigma , Rhoicissus* and *Cayratia* have a long or linear Chalaza, whereas those of the rest of the family usually have an oval Chalaza. Seed morphology implies, in some cases, a closer relationship among these genera, but in other cases it probably reflects convergent or parallel evolution **(Iju and Steven, 2011).**

In the Fabaceae the outermost layer of the seed coat is a waxy cuticle of variable thickness **(Moise et al., 2005**). The next layer is the epidermis consisting of a single layer of palisade cells (macrosclereids) that are elongated perpendicular to the surface of the seed. Inside the palisade layer is an hourglass cell layer, which is composed of thick-walled osteosclereids. The innermost multicellular layer is composed of partially flattened parenchyma.

Dalia and Gbar (2014) studied the seed shape , dimensions, surface texture and sculpture, hilum shape and sculpture, and position were recorded for seven species of each of the Apocynaceae and Asclepiadaceae by using light microscope (LM) and scanning electron microscope (SEM). Seven patterns were recognized based on surface sculpturing pattern: reticulate (with five subtypes), striate, ruminate, papillate, colliculate, aculeate and rugose. Anatomical investigation using light microscope showed that the hypodermis is present in the outer integument of two species and absent in the rest. The inner integument is two types.

Pericarp, trichome, and seed coat anatomy display great features of taxonomic value in the Calycanthaceae. The unicellular trichome morphology is common in all species in Calycanthaceae. Density of trichome is highest in *Calycanthus*

occidentalis. Different variation of seed coat and pericarp layers are characteristics of potential phylogenetic significance in the family **(Niroji and Kweon ,2018).**

Seeds of 41 taxa corresponding to 13 genera of the family were investigated by **Balkrishna *et al.,*(2017).** Seeds were minute and less than or slightly larger than 1 millimeter in length except for *Melampyrum* and *Pedicularis* species. The seed shape ranged from elliptical to broad elliptical and ovoid. In the studied species the surface sculpture was predominantly reticulate-striate, regular reticulate, sometimes colliculate, and rugose, or - rarely - ribbed, as in *Lindernia procumbens* and *Paulownia coreana*. Seed coats comprised the epidermis and the endothelium. In all Melampyrum and some Veronica species the seed coat was very poorly represented and only formed by a papery layer of epidermis.

Alexey *et al.,* (2015) reported seed anatomy, morphology and germination capacity analysis of some species in the subfamily Sedoideae cultivated in Siberian Botanical Garden of Tomsk State University. The research revealed the identify the morphological characteristics of seeds in 21 species and 2 subspecies.

Macro and micromorphalogical seed characters of five species belonging to genus Corchorus was investigated. The results showed that seed shape varied from angular, oblong, ellipsoid rhomboidal, and triangular cuneiform. The seed coat pattern varied from rugoselystriate, ruminate, ruminate and reticulate **(Fawzi, 2018).**

The microscopic study showed outermost layer of testa ,linea, Lucida, columella, endosperm layer with aleurone grains and mucilage respectively in methika seeds. **(Santosh *et al.,* 2019).** The findings of **Teema and Thara (2019**) reported detailed microscopic profile of lentil seeds and seed coat using bright field microscope.

Forty eight species of Astragalus from Turkey were studied in order to describe and investigate their seed morphologies and to evaluate the diagnostic value of this character using a stereoscopic microscope **(Cem *et al.,* 2008)**. Calycanthaceae seeds were collected and the density of the trichomes were observed by **(Niroj and Kweon, 2018).** Density of trichome is highest in Calycanthus occidentalis. Different variation of seed coat and pericarp layers are characteristics of potential phylogenetic significance in the family.

Seeds from 14 species belongs to Euphobiaceae family were studied. Significant features like seed size, seed shape, shape of caruncle and seed coat were studied. Three kinds of seed surface, four major shapes as well as seven types of seed coat ornamentation (reticulate-areolate, falsifoveate, puniculate and smooth) were observed. **(Ilker and Sukran, 2018).**

Kasem *et al.,* (2011) studied the seed exomorphic characters of 32 taxa of Brassicaceae.The diagnostic characters like seed shape, dimensions, colour, epidermal cells, and seed coat surface and aspect of anticlinal and periclinal walls were observed. Seed shape among the studied taxa showed wide range of variations. Most of the studied seeds vary from globose to oblong-ellipsoid or elongate. Most of the seeds have no wings except *Farsetia aegyptia*.

POWDER MICROSCOPY ANALYSIS

The organoleptic features and powder analysis is a promising test to determining the authenticity and for identifying the adulterant s in the plant sample. The excessive exploitation of medicinal plants during recent years raises the issues regarding their quality, safety and efficiency **(Tabassum *et al.,* 2017).**

Sharma *et al.,* (2019) observed the presence of oil globule, osteosclerids with tannin content and oil globules, stone cells, prismatic crystal, simple fiber, lignified annular vessel, fragments of endosperm cells and the fragment of palisade cells in the seed powder of *Adansonia digitata*.

From the last decade the interest towards molecular studies increased dramatically. As a result interest towards taxonomy research related studies declined and researches on this topic are often neglected and under- estimated. Comparative morphology as integrative discipline still assumes a pivotal role in modern sciences, remaining fundamentally relevant to nearly all fields of plant biology, such as systematics, evolutionary biology, ecology, physiology, genetics, molecular biology, As like other recent trends it is open to constant innovations involving both concepts and methods. **(Salmeri, 2019).**

MATERIALS AND METHODS

Materials:

Selection of Seeds:

Brassicaceae family was selected for the present study. This family included lots of seeds with great economic importance. So based on the economic importance and availability, seeds of *Raphanus sativus*, *Brassica oleracea* and *Brassica juncea* were selected.

Collection of seeds:

Once the seeds were selected, it was collected from the Panchamuga Ura kadai, Thoothukudi. Seeds were separated, shade dried, pulverized, sieved and preserved in an air tight glass bottle for further study.

Methods:

Seed measurement

Seed dimension (length, width and thickness) was measured with the help of standard measures. Seed length (L) was the first measurement of seed, measured parallel to hilum. The second measurement was width (W) measured from the center of the hilum to opposite side of seed and right angle to the plane of hilum. Third measurement was seed size (T), measured at right angle to hilum and right angle to plane of hilum.

Organoleptic characters:

Organoleptic evaluation can be done by means of sense which includes the parameters like shape, colour, odour, taste and texture and there by define some specific characteristics of the material which can be considered as a first step towards the establishment of identification.

Macroscopic and microscopic evaluation

Thin free-hand transverse sections (TSs) of fresh seed were taken by following standard methods. To identify various cellular constituents, the sections were treated with phloroglucinol and hydrochloric acid. Photomicrographs were taken by using a Trinocular microscope LV50U.

Powder microscopy

A small amount of powder is taken and boiled with chloral hydrate to remove chlorophyll, then stained with phloroglucinol solution for few minutes and followed by concentrated hydrochloric acid (1:1) in watch glass. It is mixed and allowed to stand for about 3 min. It is then mounted in glycerine (50%) and observed under microscope.

Histochemical evaluation

Thick sections were subjected to histochemical tests to find out the presence of starch, tannin, calcium, lignified cells, calcium oxalate crystals, oil globule, and calcium carbonate by treating with various reagents such as phloroglucinol + concentrated HCL, iodine, HCL respectively.

Imbibition percentage:

Weigh 1 gram of different types of dry seeds and put them separately into a 250 ml beaker containing 100 ml distilled water. Leave the beakers as such for 1 hour. Take out the seeds from each beaker and blot them dry. Weigh the seeds and note the change in weight in each case. Calculate the percentage of water imbibed by unit time in each case by using the following formula

$$\text{Percentage of water imbibed} = \frac{\text{increase in the weight of the seed in unit time}}{\text{initial weight of the seed}} \times 100$$

RESULT AND DISCUSSION

Economic importance of the seeds:

a) ***Raphanus sativus*** **seeds (Plate 1-A)**

It is commonly called as radish. It is an edible root vegetable. Radishes are grown and consumed throughout the world, being mostly eaten raw as a crunchy salad vegetable with a pungent flavour. There are numerous varieties, varying in size, flavour, colour, and length of time they take to mature. Radishes owe their sharp flavour to the various chemical compounds produced by the plants, including glucosinolate, myrosinase, and isothiocyanate.

Uses:

- It is an economically important crop for the United Kingdom, as 5800 tons of radish are grown annually.
- Predominately grown for its swollen tap root, it has multiple uses as a leafy vegetable, oil crop, root vegetable, and cover annual plant.
    - As a cover crop, it is used to minimize nitrogen loss, improve soil fertility, and mitigate soil erosion

b) ***Brassica juncea*** **(Plate 1-B)**

It is commonly called as mustard seeds. Mustard seed is used as a spice. Grinding and mixing the seeds with water, vinegar, or other liquids creates the yellow condiment known as prepared mustard.

Uses

Seed is used as condiment in the preparation of vegetable and curries.

Plate 1

Seeds Selected from Brassicaceae Family for the Present Study

Plate 1-A *Raphanus sativus* Seeds

Plate 1-B *Brassica juncea* Seeds

Plate 1-C *Brassica oleracea* Seeds

- Split seed (Mohari dal) and oil is used for pickling.
- The leaves of the young plants are used as vegetable.
- Oil cake is fed to cattle.

*c) **Brassica oleracea*** **(Plate 1-C)**

It is commonly called as cabbage. Most cabbages have thick, alternating leaves, with margins that range from wavy or lobed to highly dissected; some varieties have a waxy bloom on the leaves. Plants have root systems that are fibrous and shallow

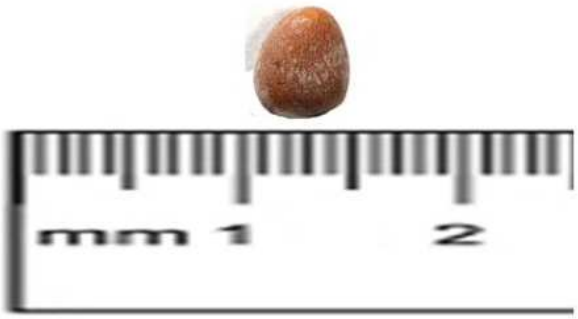
Uses:

- Cabbage is mostly used as cooked or fried vegetable, sometimes pickled or preserved by steaming and drying.
- It is also eaten fresh in mixed salads, it contains good nutritional contents.
- Apart from being used as vegetable, the leaves are cooling and stomachic, and good for biliousness and skin diseases.
- Leaf juice is very effective in chronic coughs and bronchial asthma.

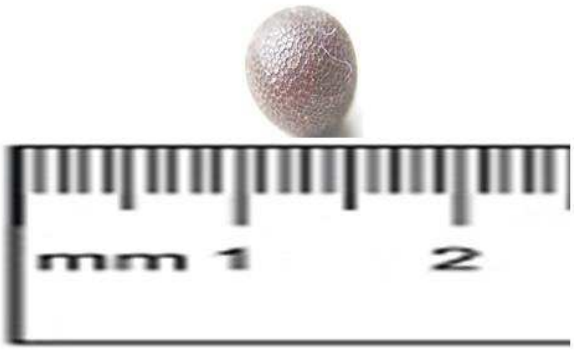
Seed measurement:

Seeds are very small and appeared to be in the same size until measured. The width and length of the three seeds are shown in Table 1 and Figure 1. Seeds of *Raphanus sativus* showed the length of 0.27mm and the width of 0.87 mm whereas the seeds of *Brassica juncea* and *Brassica oleracea* showed the same length of 0.21mm and width of 0.6mm respectively. This is also been shown in Plate 2. The size of the seeds are also measured using classic method. The size of *Raphanus sativus* seeds is measured as 7.29± 1.7mm^2, the seeds of *Brassica juncea* is 5.14 ± **Plate 2**

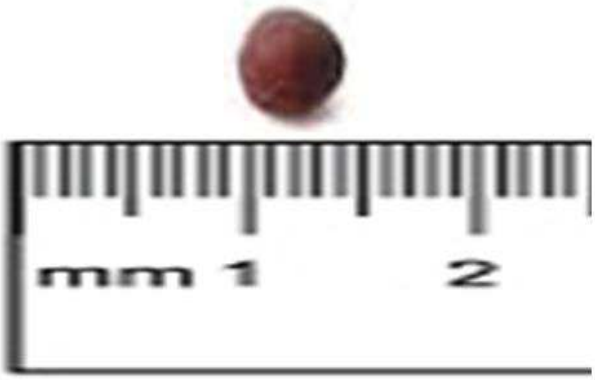
Measurement of seeds selected from Brassicaceae Family



2-A *Raphanus sativus* Seed



2-B *Brassica juncea* Seed



2-C *Brassica oleracea* Seeds

1.1mm^2 and *Brassica oleracea seeds showed* 3 ± 0.81mm^2 of size. This is shown in Table 2 and Figure 2.

Brassicaceae members ususally have small seeds which are economically important. They are mostly millimetre in length and show varied length and width sizes when measured. This has been elaborated in the work done by Ramiez *et al*., in 2020.

Organoleptic characters:

The various characteristics of the three seeds are shown in Table 3. The seeds of *Raphanus sativus* are round in shape, reddish brown in colour, pungent in odour, bitter in taste and soft and glittering in texture. The seeds of *Brassica juncea* are round in shape, black in colour, pungent in odour, oily in taste and soft and smooth in texture. The seeds of *Brassica oleracea* are round in shape, brown in colour, pungent in odour, bitter in taste and rough in texture.

The organoleptic characters of different seeds plays an important role in identifying their use in field of food preparation and drug manufacturing. The bitter taste and pungent smell make them a perfect product for utilization of drug preparation. The oily nature of mustard seeds prove them to be secreting oils (Hushmukh *et al*., 2016).

Microscopic findings:

The T.S. of the *Raphanus sativus* seeds are oval in outline, showed outer thin testa, centrally located, and linearly arranged thin cotyledon encircled by endosperm. This is shown in Plate 3-A. Detailed T.S. shows an outer layer of epidermis, and 2-3

Table 1: Seed Size Measurement from of Selected Seeds from Brassicaceae Family

S. No	Seed	Width (mm)	Length (mm)
1.	*Raphanus sativus*	0.87 ± 0.22	0.27 ± 0.13
2.	*Brassica juncea*	0.6 ± 0.1	0.21 ± 0.1
3.	*Brassica oleracea*	0.6 ± 0.05	0.21 ± 0.03

Figure 1: Width and Length of Selected Seeds from Brassicaceae Family

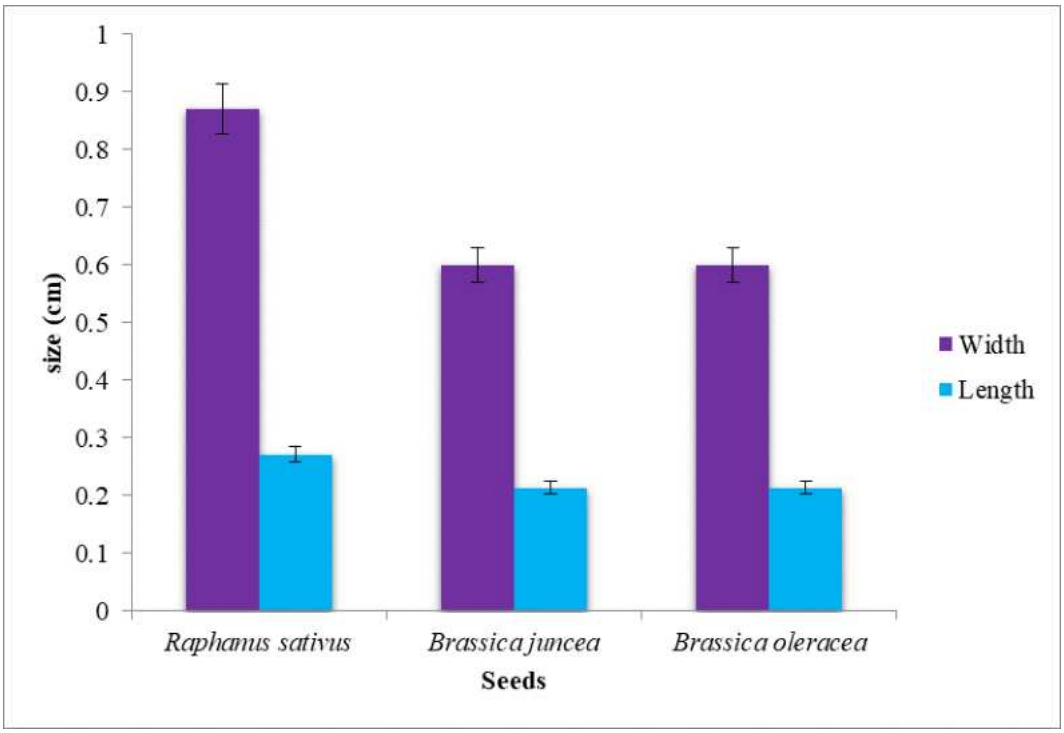


Table 2: Seed Size of Selected Seeds from Brassicaceae Family

S. No	Seed	Size of the seed (mm^2)
1.	*Raphanus sativus*	7.29 ± 1.7
2.	*Brassica juncea*	5.14 ± 1.1
3.	*Brassica oleracea*	3 ± 0.81

Figure 2: Seed Size of Selected Seeds from Brassicaceae Family

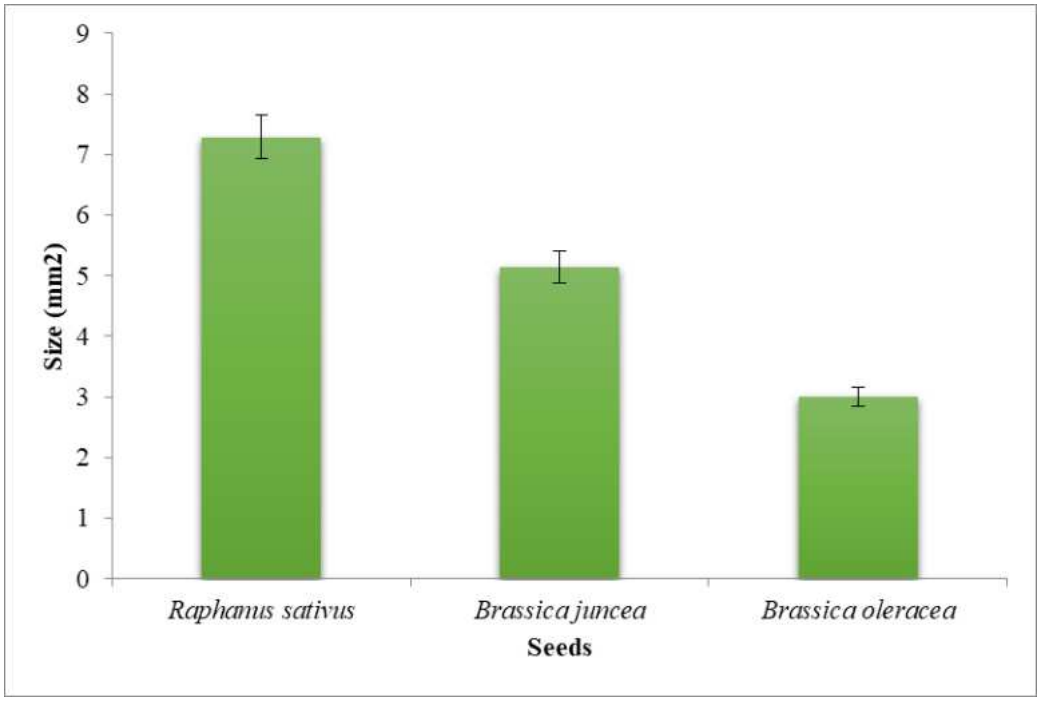


Table 3: Organoleptic characters of the Selected Seeds from Brassicaceae Family

S.No	Seed	Organoleptic character	Observed characters
1	*Raphanus sativus*	Shape	Round
		Colour	Reddish brown
		Odour	Pungent
		Taste	Bitter
		Texture	Soft and glittering
2	*Brassica juncea*	Shape	Round
		Colour	Black
		Odour	Pungent
		Taste	Oily
		Texture	Soft, smooth
3	*Brassica oleracea*	Shape	Round
		Colour	Brown
		Odour	Pungent
		Taste	Bitter
		Texture	Rough

rows of parenchymatous cells of hypodermis, which was more layered under the ridge region, embedded with crystalline masses of oil droplets , followed by a very wide zone of nonpitted sclereids of different sizes and shapes, and a layer of aleurone is also present.

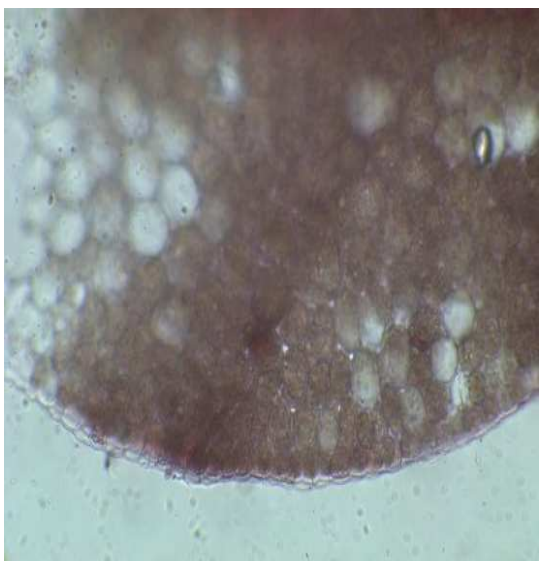
The T.S. of the *Brassica juncea* seeds are oval in outline, showed outer thin testa. encircled by endosperm. This is shown in Plate 3-B. Detailed T.S. shows an outer layer of epidermis, and 2-3 rows of palisade parenchymatous cells of hypodermis, which was more layered under the ridge region, which consists of oil droplets and storage materials. The endosperm is also seen clearly. Sclerides and tracheids are also observed.

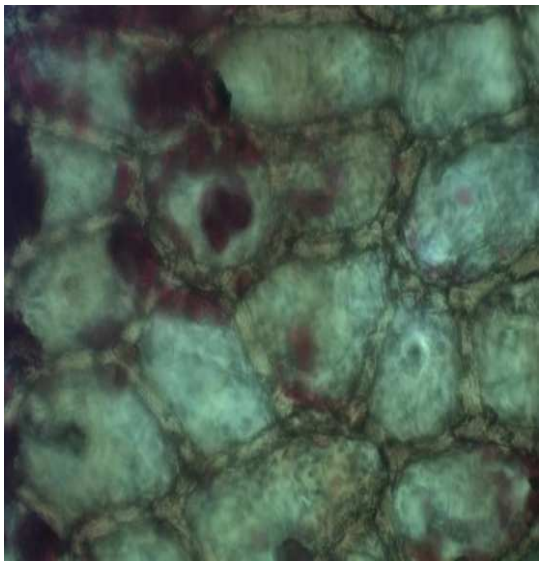
The T.S. of the *Brassica oleracea* seeds are oval in outline, showed outer thin testa. encircled by endosperm. This is shown in Plate 3-C. Detailed T.S. shows an outer layer of epidermis which is of barrel shaped cells, and 2-3 rows of simple palisade parenchymatous cells of hypodermis, which consists of oil droplets and storage materials. The endosperm is also seen clearly. Sclerides and tracheid are also observed.

The different seeds are also shown with different layers like the brassicaceae members. Every seeds will show the above characters since they store endosperm in the form of starch or oil droplets (Vijayan *et al*., 2007).

Plate 3-A

Transverse Section of *Raphanus sativus* Seed





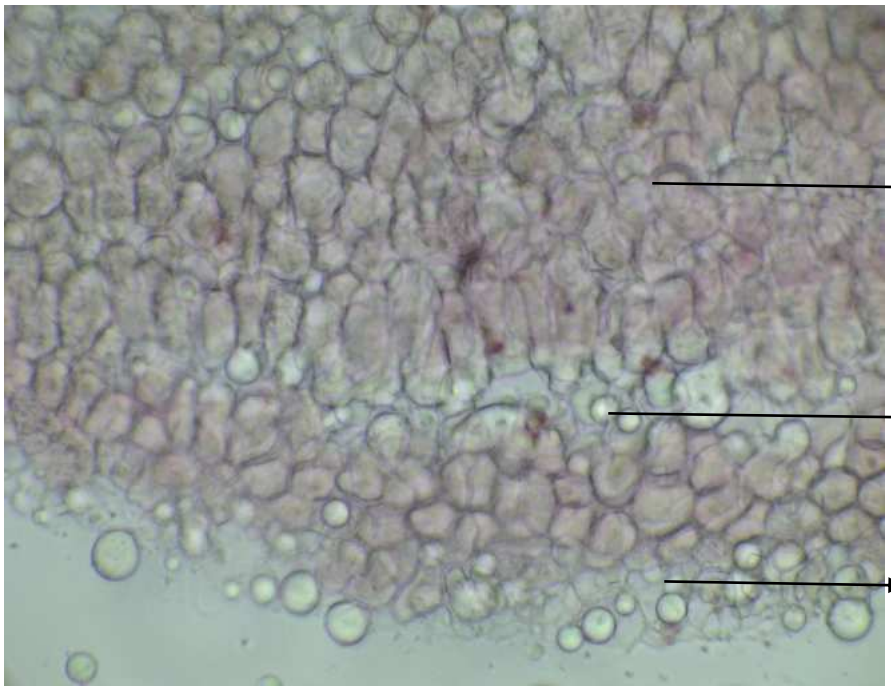
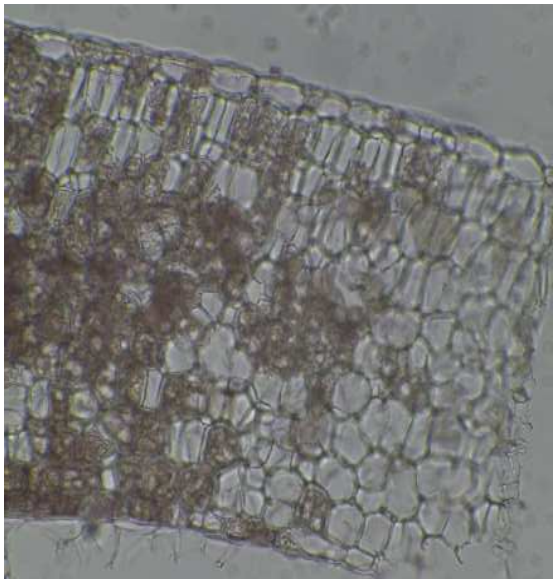
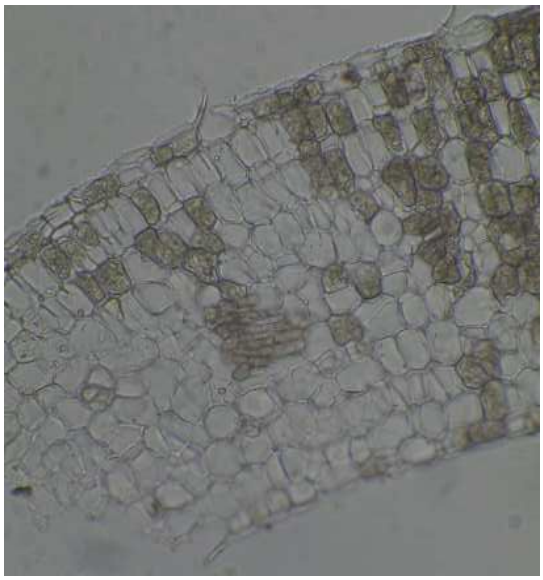


Plate 3-B

Transverse Section of *Brassica juncea* Seed





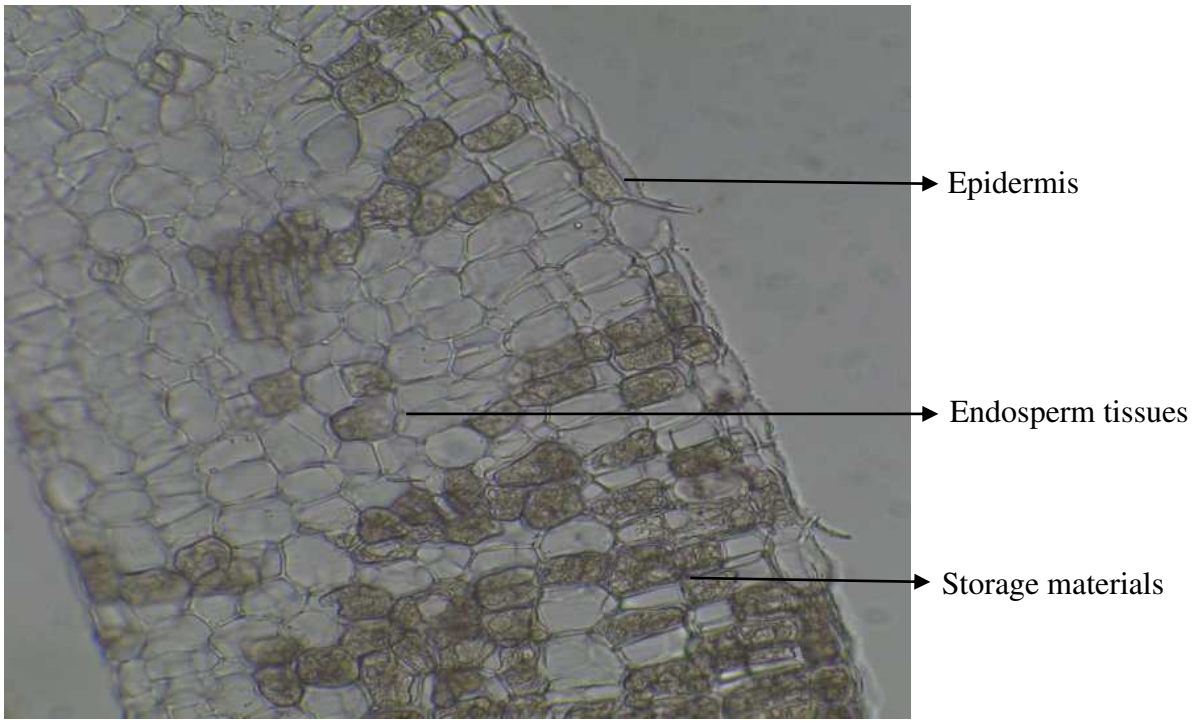
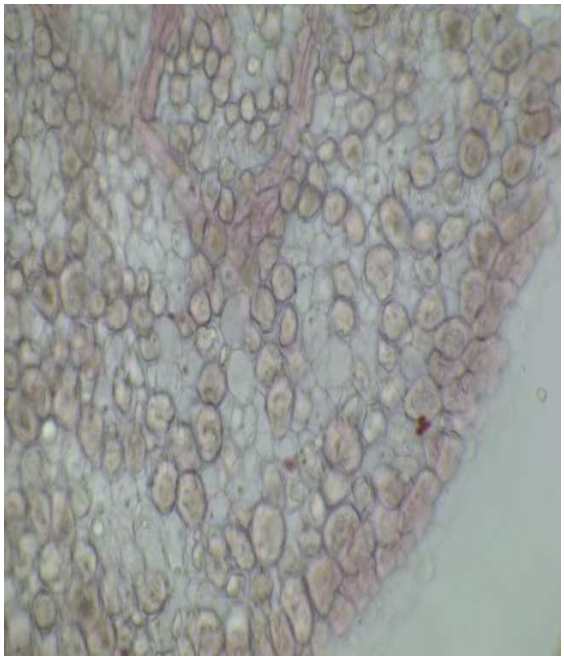
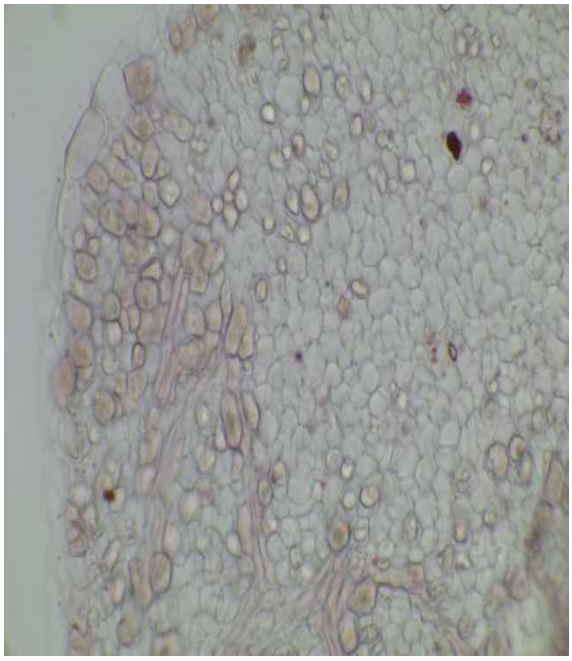
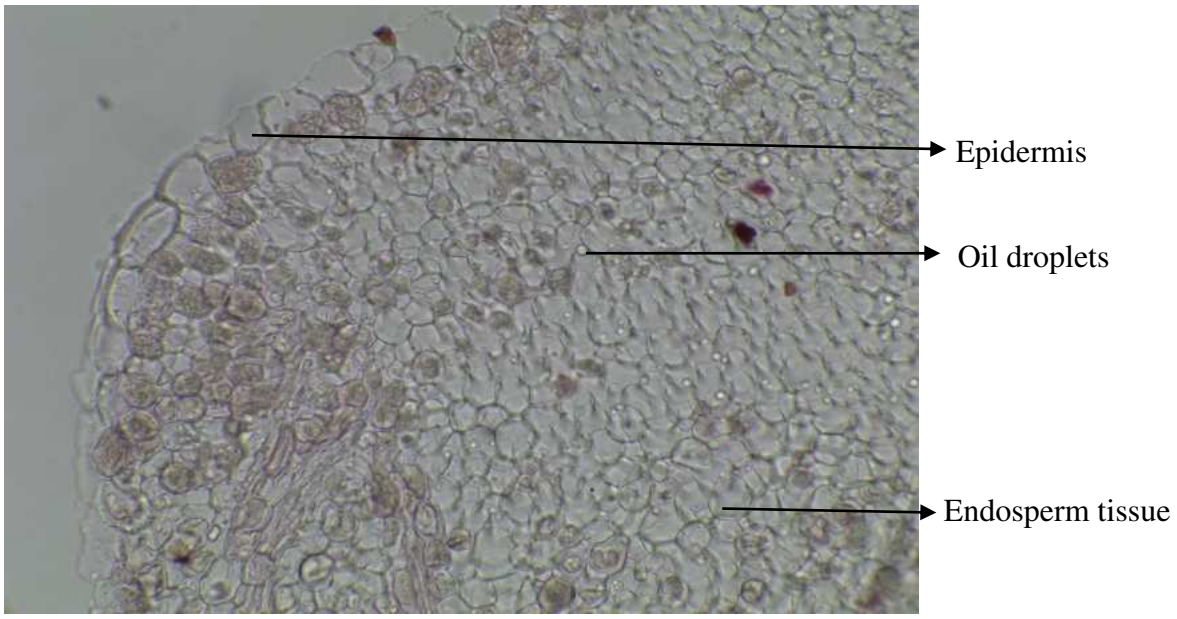


Plate 3-C

Transverse Section of *Brassica oleracea* Seed







Powder microscopy:

The Seed powders found to hold characteristic pungent odour and acrid taste. The samples are oily on touch. When studied under microscope, the diagnostic characters of seed powder such as transversely cut fragment of endosperm showing outer epidermis and underlined mesophyll cells with palisade parenchyma cells, tracheids and stone cells from innermost stony band, cells of hypodermis embedded with crystals, a parenchyma cells consisting of oil globules and aleurone grains are visible in all three seeds. This is shown in Plate 4-A, 4-B and 4-C.

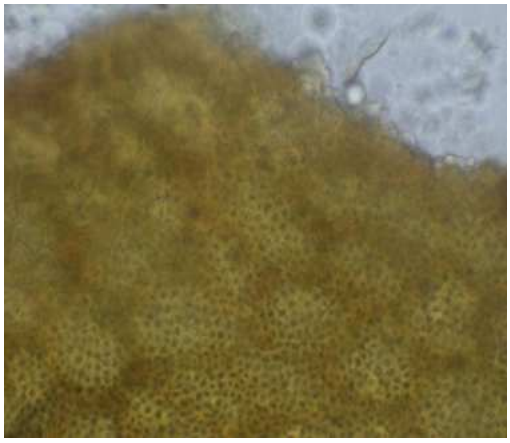
When the seeds of Abrus precatorius was obsersed for powder microscopy it also showed the significant characteristics for the presence of sclerids and stone cells thus making them an excellent medicine (Tabasum *et al*., 2017). **Histochemical evaluation:**

Thick sections of the three seeds, treated with phloroglucinol + concentrated HCL, observed red colour and dissolved indicating presence of lignified cells and calcium oxalate crystals. When it was treated with Iodine Sudan III, blue and red colour was observed indicating the presence of starch grains and oil globule. The sclerides were also observed. This is shown in Plate 5.

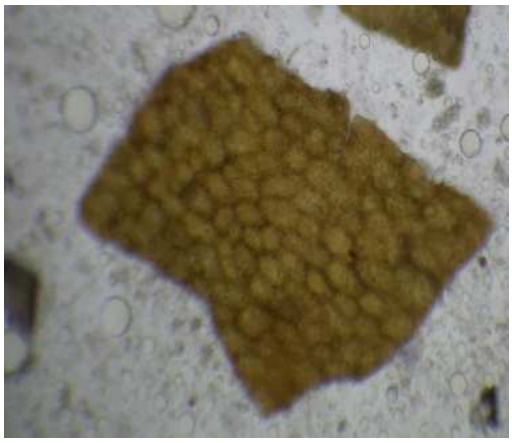
Tuvaraka is also dominated by the calcium oxalate crystals and it is rich in oil. Seed oil is used in Kustha (skin diseases) (Ambikadatta, 2010). Oil mainly consists of fixed oil containing glycerides of palmitic, hydnocarpic, and chaulmoorgic acids, these may able to help in curing the skin diseases (Mecalfe, 1950).

Plate 4-A

Powder Analysis of *Raphanus sativus* Seeds



Aleurone Layer



Palisade Layer

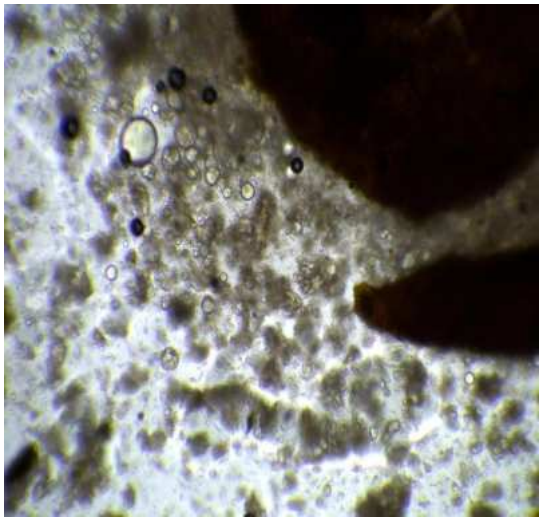
Tracheids

Plate 4-B

Powder Analysis of *Brassica juncea* Seeds

Aleurone Layer

Palisade Layer



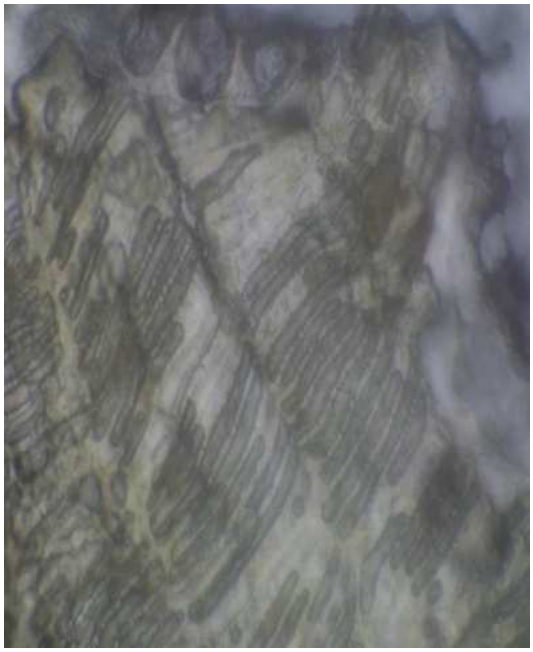
Oil droplets

Tracheids

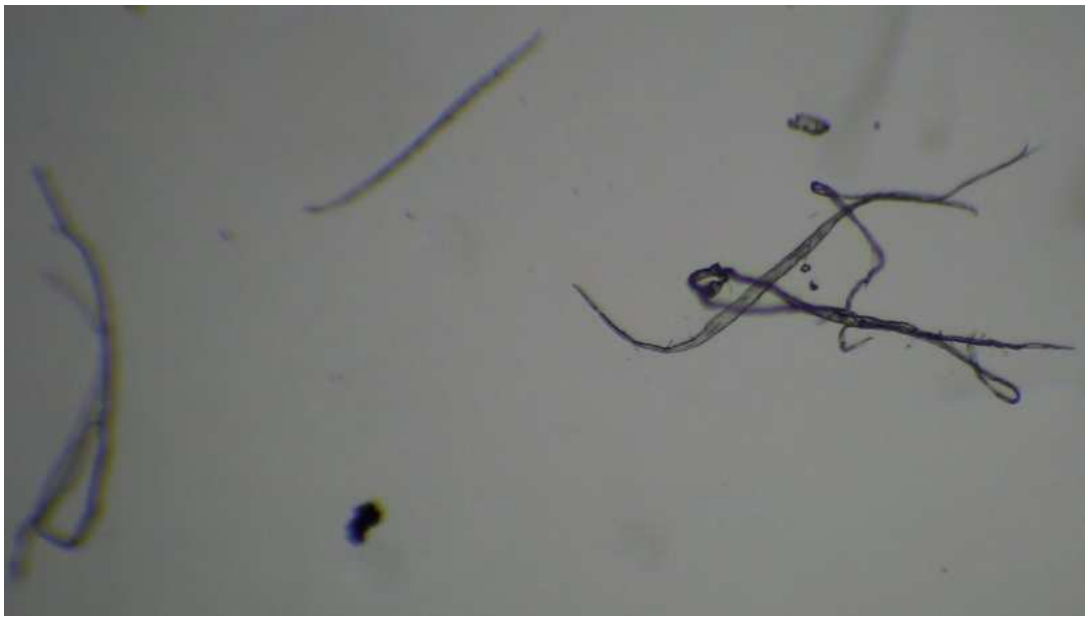
Plate 4-C

Powder Analysis of *Brassica oleracea* Seeds

Aleurone Layer



Palisade Layer



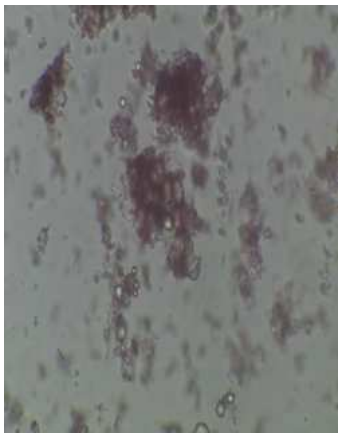
Tracheids

Plate 5

Histochemical Ananlysis of Selected Seeds from Brassicaceae Family

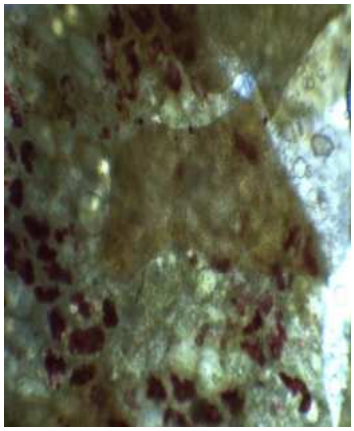
Raphanus sativus

Plalisade cells with starch



Raphanus sativus

Sclereid cells



Raphanus sativus

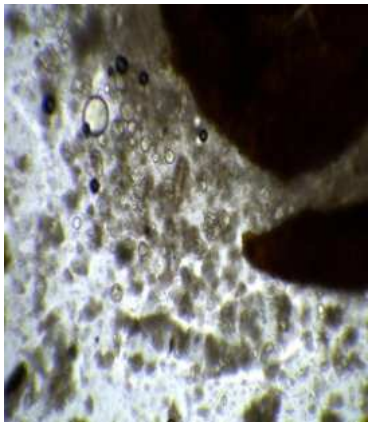
Oil droplets

Brassica juncea

Palisade cells with starch

Brassica juncea

Sclereid cells

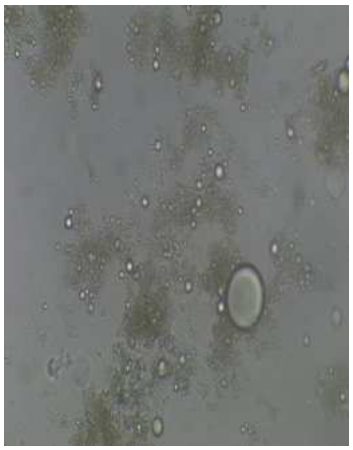


Brassica juncea

Oil droplets

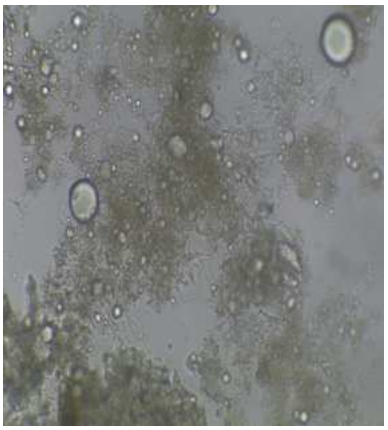
Brassica oleracea

Palisade cells with starch



Brassica oleracea

sclereids



Brassica oleracea

oil droplets

Imbibition percentage of seeds:

The imbibition percentage that is the water holding capacity of the three seeds are shown in Table 4 and Figure 3. Out of the three seeds, the *Brassica oleracea* seeds showed maximum imbibition capacity with 53% which is closely followed by *Brassica juncea* seeds with 50% and *Raphanus sativus* seeds with 47% This characteristic is attributed to the seed coat permeability and endosperm nature.

This is being supported by the work of (Swanson *et al*., 1984.) where they had done work in the legumes and their water holding capacity.

Table 4: Imbibition percentage of Selected Seeds from Brassicaceae Family

S. No	Seed	Initial Weight of the seed (gram)	Final weight of the seed (gram)	% of Imbibition
1.	*Raphanus sativus*	1	1.47 ± 0.18	47
2.	*Brassica juncea*		1.49 ± 0.36	50
3.	*Brassica oleracea*		1.53 ± 0.13	53

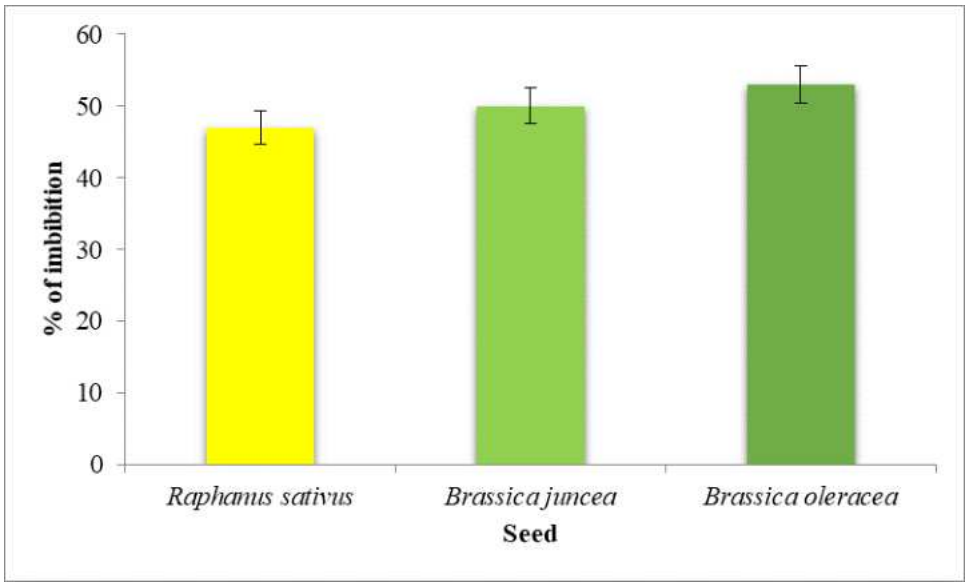


Figure 3: Imbibition capacity of Selected Seeds from Brassicaceae Family

SUMMARY AND CONCLUSION

Brassicaceae contain such important crop species which contribute to raise in the agriculture and its economy. Brassica plants are the oldest cultivated plants known to humans as the source of medicines and vegetables (Dhevi Vs et al., 2019). Keeping this mind, three economically important plant seeds such as *Raphanus sativus, Brassica juncea* and *Brassica oleracea* were collected for the present study.

The seed measurements showed greater difference in their size. Though all the three seeds looked small, the seeds of *Raphanus sativus* showed greater variation in their width, length and size, which was followed by *Brassica juncea* and *Brassica oleracea.*

The organoleptic characters of the seeds have certain similarities in their shape, taste and odour but they differ in their colour and texture. The microscopic evaluation portrayed the different cell structures such as a clear barrel shaped epidermis, mesophyll with palisade parenchyma cells, aleurone layer with endosperm and oil globules.

The powder microscopy indicated the presence of tracheids and sclerides in the three seeds. The histochemical analysis showed the presence of starch and oil globules in the seeds. The imbibition capacity of the seeds also differed from each other. The seeds of *Brassica olerace* showed maximum capacity when compared to the seeds of *Raphanus sativus* and *Brassica juncea.*

From the above study, it is concluded that all the three seeds consist of oil globules, starch granules and sclerides. This clearly attributes to their economic

importance since they are commonly used as food and in oil production. This showed only the preliminary efforts, hence a thorough study on the biochemistry of the seeds is recommended.

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STUDY ON ANTIOXIDANT POTENTIAL, FTIR, AND GC-MS ANALYSIS OF *JATROPHA INTEGERRIMA* Jacq.

A short term project work submitted to **St. Mary's College (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the Degree of Bachelor of Science in Botany

BY

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DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (Autonomous)

THOOTHUKUDI- 628001

2020-2021

CERTIFICATE

It is certified that this short term project work entitled "**STUDY ON ANTIOXIDANT POTENTIAL, FTIR, AND GC-MS ANALYSIS OF *JATROPHA INTEGERRIMA* Jacq.** submitted to **St. Mary's College (Autonomous)** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the degree of Bachelor 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 2020-2021 by the following students.

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INTRODUCTION

INTRODUCTION

The human civilization directly or indirectly depends upon plants for their very basic need of survival, food, fodder, fuel, fiber, fertilizer, timber, medicines and several raw materials. India possesses a variety of medicinal plants and it is one of the richest countries in the world in regard to genetic resources of medicinal plants. 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, antimutagenic, anti-carcinogenic, antibacterial, or antiviral activities. [Maridass and Britto, 2008].

Oxidation process occurs naturally in human body and defined as electron transfer from one to another. Since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP. But the problem may arise when electrons flow from oxidation process become unpaired and then subsequently generates free radicals, known as Reactive Oxygen Species (ROS), such as superoxide (O_2-), peroxyl (ROO-), alkoxyl (RO-), hydroxyl (HO) and nitric

oxide (NO-) [Pietta, 1999]. Free radicals are very reactive and rapidly attack molecules in nearby cells [Pietta, 2000].

Reactive oxygen species acts in damaging cell membranes, attacking proteins and DNA in tissues. Carcinogenesis may also be initiated through oxidatively induced DNA damage. Repeated damage caused by ROS throughout the span of human life increases with time, and is a major cause of age -related cancers and other oxidatively -induced diseases [Reynertson, 2007]. Antioxidants are substances those when present in foods or body at low concentrations compared with that of an oxidizable substrate significantly delay or prevent the oxidation of that substrate [Saha *et al.*,2004]. Antioxidants will help to minimize oxidative damage as the most important approaches to the primary prevention of age-related diseases, since antioxidants terminate direct ROS attacks and radical-mediated oxidative reactions [Tepe and Sokmen, 2007].

Therefore, dietary antioxidants are needed to protect the harmful action of ROS. Well established antioxidants derived from diet are vitamins A, C, E, polyphenols and carotenoids [Pietta, 1999 & 2000]. Current antioxidant research of free radicals also has confirmed that food with rich antioxidants play an essential role on the prevention of disease caused by oxidative stress. Therefore, plant derived antioxidants now receiving a special attention [Tepe *et al.*,2005].

Since 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 is desirable [Raja and Pugalendi, 2009].

The family Euphorbiaceae includes about 4000 species and 200 genera. The members of the family secrete an acrid juice of varying colours and density. Some species are slightly narcotic acrid and other aromatic. Most of the genera like *Phyllanthus, Jatropha, Acalypha, Euphorbia* are used in modern allelopathic and homeopathic systems of medicine. *Jatropha* is a diverse and widespread genus includes about 175 species (Airy Shaw, 1982). 12 species of Jatropha found to occur in India and 9 species in South India. *Jatropha* species are known for many biological activities such as anticancer, antitumor, antimicrobial, hepatoprotective and pesticidal. These plants are also used as antipyretic, diuretic, choleretic and purgative (Kaushik and Kumar, 2004; Panda *et al.*, 2009). The secondary metabolites present in the different plant parts of *Jatropha* species are used for maintaining health and for preventive diseases

Jatropha integerrima Jacq.is an erect ornamental evergreen shrub, native to West Indies and grows commonly in Southern parts of India (Krishan & Paramathma 2009). It is commonly known as Peregrina, Spicy *Jatropha, Chaya, Firecracker, Firecracker Jatropha,* Star of Bethlehem and belongs to the family Euphorbiaceae. It is an excellent ornamental perennial shrub or tree that blooms almost year round. It can be used as an accent or specimen or container plant in any garden. It serves as a lovely addition for the back of a sunny border. These bushes can be used to create an

informal hedge or screen for privacy and even suitable for landscaping along highways when planted in mass. It attracts butterflies and birds to the garden.

Various parts of *Jatropha integerrima* are traditionally used as purgative, styptic, emetic, in treatment of warts, tumours, rheumatism, herpes, pruritis, toothaches, scabies, eczema and ringworm (Kirtikar *et al.* 2002). The leaves and branches of the plant have been shown to hold cholinesterase activity while latex of the plant has demonstrated anti-cancer activity (Gupta and Gupta 1997, Wele *et al.* 2007, Sharma and Singh 2010). Antioxidant potential of *Jatropha integerrima* is not yet fully explored scientifically, so the present study was carried out with following objectives.

Scope and Objectives

- Collection of leaf and stem from *Jatropha integerrima* for extracts preparation..
- To quantitatively analyse and compare the total phenolics, flavonoids, vitamin C and vitamin E content of leaves and stem of *Jatropha integerrima* using spectrophotometric methods.
- To identify and compare the functional group of leaves and stem of Jatropha integerrima by Fourier transform infrared spectroscopy (FTIR) analysis.
- To assess the antioxidant potential of *Jatropha integerrima* using aqueous extract against DPPH radical scavenging activity.
- To identify the bioactive compounds of the methanol extract of leaf and stem of *Jatropha integerrima* using GC-MS analysis.

LITERATURE REVIEW

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 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-leukemia treatement. Ethanol extract of *Piper cubeba* showed high antioxidant activity.

Inbathamizh *et al.*, (2013) studied in vitro evaluation of antioxidant and anticancer potential of *Morinda pubescens* 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 *Saptum 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 citratus* 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).

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-dihydrobenzofuran and eugenol (Asmah *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, *Hyptis suaveolens* 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).

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 .Kareru *et 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 *Eclipta prostratea.* 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 *Aerva lanata* 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 *Ampelocissus lantifolia* 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 *et al.*, (2017).

antimicrobial, anticanserous and antitumerous activity (Alok prakash and Suneetha,2014).Tetrasilaxane identified in the ethanolic stem extracts of *Jatropha integerrima* is a main antimicrobial compound (Cai *et al*., 2018)

Cyclotrisiloxane and hexamethyl found in stem of *Jatropha integerrima* is a main antioxidant compounds that help remove harmful toxins and free radicals in the body (Anju Krishna*et al*., 2015)

ANTIOXIDANT ACTIVITY:

An antioxidant is a molecule capable of showing or preventing the oxidation 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 reactions by removing free radical intermediates, and inhabit other oxidation reactions by being oxidized themselves. They are believed to play a role in preventing the development of chronic disease like cancer, heart disease, stroke, AD, RA and cataracts (chakraborty et al.,2010).

Antioxidant chemicals found in nature inhibit or prevent 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 protect from oxidative stress related diseases, inflammatory diseases viz., arthritis, autoimmune disease, carcinogenesis, neurodegenerative diseases, inflammatory disease, cardiovascular disorders etc. Several food industries use butyated hydrouxyanol, butylated hydroxyl 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

MATERIALS AND METHODS

Plant material

Botanical name : *Jatropha integerrima* Jacq.

Classification :

Division	: Spermatophyta
Sub – Division	: Phanerogams
Class	: Dicotyledonae
Sub- Class	: monochlamydeae
Series	: Unisexuales
Order	: Euphorbiales
Family	: Euphorbiaceae

Distribution :

Jatropha integerrima Jacq. is a shrub to small tree native to cuba ,which has been introduced into tropical and subtropical areas worldwide as an ornamental speices. Although it has become naturalized in some areas, it is not listed as an invasive species.Most likely Mexico and cental America.

Description :

Jatropha integerrima Jacq. –An erect ornamental shrub, native to West Indies, grows up to 6 m tall, sparingly pubescent. Stipules filiform, petiole 1-3 cm long, glabrous, base attenuate, margin entire except usually subbasally glandular – denticulate, apex acuminate to cuspidate , tip acute ,venation basally 3-5 nerved, with up to 12 pairs nerves along the midrib. Inflorescence terminal , peduncle up to 10 cm long , bracts triangular, basally with glandular teeth .Scarlet or pinkish unisexual flower , monoecious. Stamens 10, the 5 outer filaments united for three quarters, the inner ones united for two-thirds of their length. Fruits trigonous, 1cm in diameter , dehiscive. Seeds are small , slender , ellipsoid to ovoid. Propagated by cuttings.

Materials And Methods

Collection and processing

The leaves and stem of *Jatropha integerrima* were collected from Thoothukudi, Tamil Nadu respectively. 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 the extraction of active constituents of the plant material.

Extraction (Maxon and Rooney,1972)

One gram of air- dried sample powder was taken in a 100ml flask, to which added 50ml of 1% (v/v) in methanol. The samples were shaken in a reciprocating shaker for 24h.at room temperature. The contents were centrifuged at 10,000 g for 5min.The supernatant was collected separately and used for further analysis.

Quantitative analysis of antioxidant

Total phenolic content:(Duan *et al.*,2006)

Reagents

- 50%Folin – ciocalteau reagent
- 20%sodium – carbonate
- Gallic acid – standard

cedure

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 $NaNO$, was added and again after 6min 0.3 ml of 10% $Alcl^3$ was added. After 5 minutes 1ml of 1M NaNH 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
- Indophenol 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 acid 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 KBrsalt, using amortar and pestle, and compressed into a thin pellet. Infra -red spectra were recorded as KBrpellets on a Thermo Scientific NicotiS5ID1 transmission, between 4000-400 cm^{-1} (Kareru et al., 2008).

· C-MS Analysis:

Extract Preparation

The 50g tuber powder of *Jatropha integerrima* 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 methanolic extracts was performed by using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia. and 0.25µm 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 programmed from 70 to 220°C at 10°C/min, held isothermal for 1 min and finally raised to 300°C at 10°C/min. 2 µl 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 WILEY8. 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 disttiled 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 was 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 vortexed 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.

RESULT AND DISCUSSION

Phytochemicals are natural bioactive compounds found in plants and their parts, such as vegetables, fruits, medicinal plants, aromatic plants, leaves, flowers and roots, which work with nutrients and fibres to act as a defense system against disease or, more accurately, to protect against disease. Plant derived natural products such as flavonoids, phenols, tannins, and ascorbic acids have diverse pharmacological properties including antioxidant activity.Various parts of *Jatropha integerrima* are traditionally used as purgative, styptic, emetic, in treatment of warts, tumours, rheumatism, herpes, pruritis, toothaches, scabies, eczema and ringworm (Kirtikar et al., 2002).These plants have some active principle which has this medicinal value

QUANTITATIVE ANALYSIS:

The total phenol; flavanoid, tannin, vitamin-C and vitamin- E were analysed in leaf and stem extract of *Jatropha integerrima* belonging to the family Euphorbiaceae.

TOTAL PHENOL:

Phenolics are the most wide spread secondary metabolites and are believed to be responsible for antioxidant activity. The total phenol contents of the leaf (6.8997 mg GAE/g) were higher than that stem (5.241 mg GAE/g) in *Jatropha integerrima* , phenolic compounds are as class of antioxidant agents act as free terminators .(Shahidi and Wanasundara, 1992). Phenolic compounds have a variety of beneficial activities. They have potential antioxidants and free radical scavenger (Meenakshi et al.,2012). The antimicrobials (most of the phenolics) may provide a microbe – free environment with in the body.

FIG:1 Total phenol content of *Jatropha integerrima*

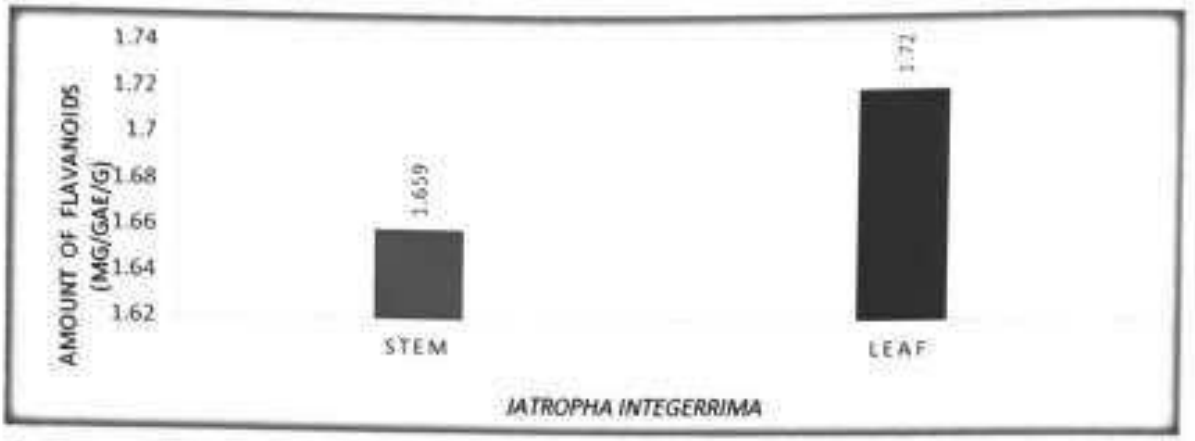


FIG:2 Total flavanoids content of *Jatropha integerrima*

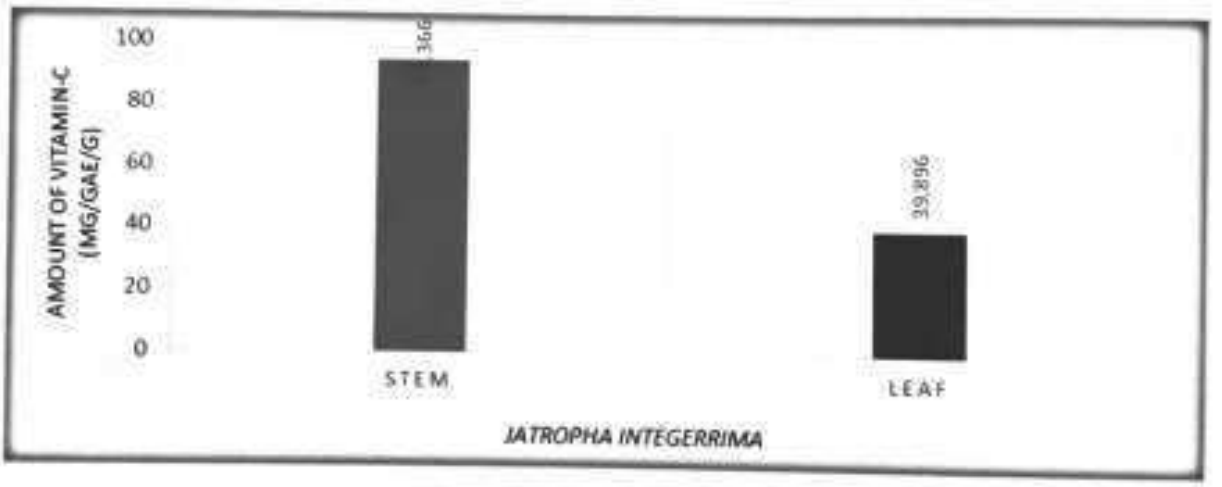


FIG:3 Total vitamin-C content of *Jatropha integerrima*

TABLE:1

TOTAL PHENOL CONTENT OF JATROPHA INTEGERRIMA		
Samples	Amount of phenol mg (GAE)/g	
	Leaf	Stem
Jatropha integerrima	6.957 ± 0.058	5.296 ± 0.049

Values are the mean of triplicates ± standard deviation. Dry samples were used for analysis.

Garlic acid equivalent (1mg/ml) was used as standard.

TABLE:2

TOTAL FLAVANOIDS CONTENT OF JATROPHA INTEGERRIMA		
Samples	Amount of flavonoids mg (GAE)/g	
	Leaf	stem
Jatropha integerrima	1.720 ± 0.018	1.659 ± 0.018

Values are the mean of triplicanes ± standard deviation. Dry samples were used for analysis.

Quercetin acid equivalent (1 mg/ml) was used as standard

TABLE:3

TOTAL VITAMIN-C CONTENT OF JATROPHA INTEGERRIMA		
Samples	Amount of Vitamin-C (mg/g)	
	Leaf	stem
Jatropha integerrima	39.896 ± 2.348	94.366 ± 4.736

Values are the mean of triplicates ± standard deviation. Dry samples were used for analysis. Vitamin-C Equivalent (1mg/ml) was used as standard.

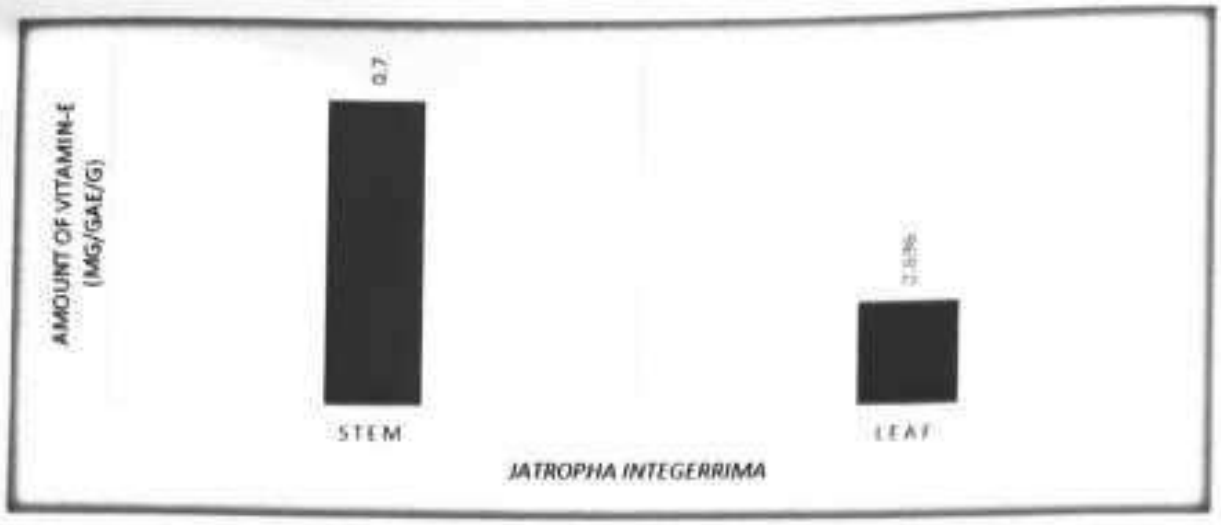


FIG :4 Total vitamin-E content of *Jatropha Integerrima*

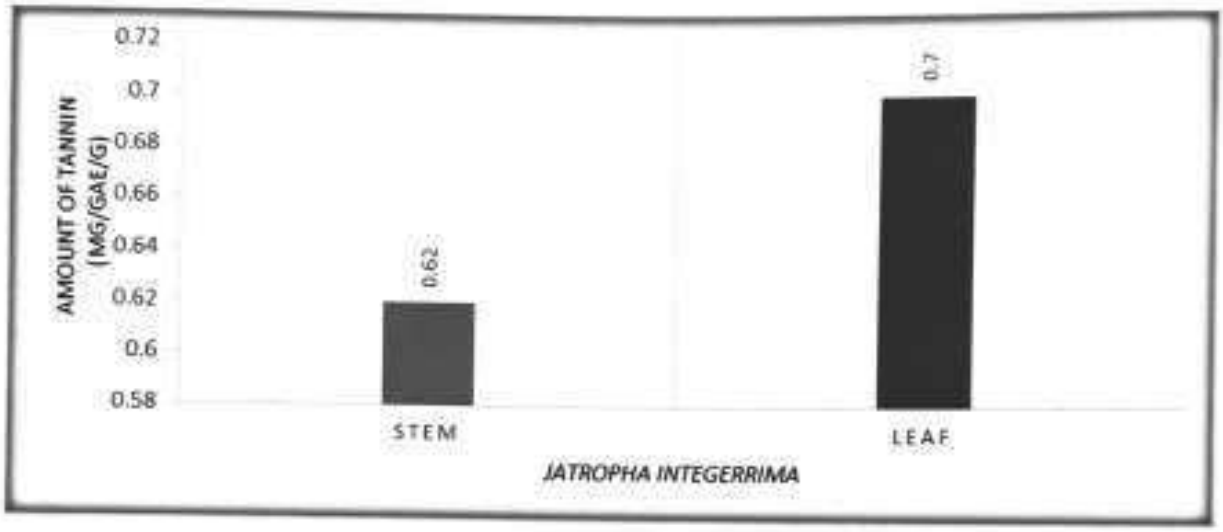


FIG:5 Total tannin content of *Jatropha integerrima*

TABLE:4

TOTAL TANNIN CONTENT OF JATROPHA INTEGERRIMA		
Sample	Amount of tannin mg (GAE)/g	
	Leaf	stem
Jatropha integerrima	0.7 ± 0.1	0.62 ± 0.01

Values are the mean of triplicates ± standard deviation. Dry samples were used for analysis.

Catechin equivalent (1mg/ml) was used as standard.

TABLE:5

TOTAL VITAMIN-E CONTENT OF JATROPHA INTEGERRIMA		
Samples	Amount of vitamin-E mg/g	
	Leaf	stem
Jatropha integerrima	0.696 ± 0.020	0.7 ± 0.02

Values are the mean of triplicates ± standard deviation. Dry samples were used for analysis.

Vitamin-E equivalent (1mg/ml) was used as standard.

TOTAL FLAVANOID:

Flavonoids are secondary metabolites and has responsible for antioxidant activity in medicinal field . The total flavanoids contents of leaf (1.701 mg QE/g) were higher than that stem (1.643 mg QE/G) in*Jatropha integerrima* . Flavanoids are potent antioxidants and epidemic studies indicate that high flavanoids in take is correlated with decreased risk of lifestyle disease like diabetes and cardiovascular disease (kaur et al., 2016). Flavanoids are potent water- soluble antioxidants and free radical which prevent oxidative cell damage and have strong anti-cancer activity(Havsteen, 2008).

TOTAL VITAMIN-C:

Jatropha integerrima leaf (37.208 mg/g) and stem (90.232 mg/g) contain significant amount of vitamin-C. Vitamin- C is a vital component in human diet with the highest concentrations in animal organs. Vitamin-C is a non- enzymatic, antioxidant water soluble antioxidant(Ueta et al., 2003). Vitamin –C functions in enzyme activation , oxidative strees reduction and immune function. It is protects against respiratory tract infection and reduces risk for cardiovascular disease and some cancer.

TOTAL TANNINS:

Jatropha integerrima leaf (0.69 mg CE/g) contain highest amount of tannin , and the stem of *Jatropha integerrima* contain the lowest amount of tannin (0.61 mg CE/g). Tannins are present primarily in the leaves of trees growing in stress conditions. They are accumulated in the vacuoles, especially these of the epidermal layer and the palised mesophyll. Tannins are useful in treating inflammation, ulcers and remarkable activity in cancer prevention and anticancer activities (Li et al.,2003,Akinpelu et al.,2009).

OTAL VITAMIN – E:

Total vitamin –E content in *Jatropha integerrima* leaf (0.68 mg /g) lowest and *Jatropha integerrima* stem (0.72 mg/g) highest (Table-5). Vitamin –E is a fat soluble nutrient found in many foods (Jacob,1995). In the body ,it acts as an antioxidants , helping to protect cells from the damages caused by free radicals. Free radicals are compounds formed when our bodies convert the food we eat into energy (Havsteen ,1983).

FTIR:

Fourier transform infrared spectroscopy was used to analyse the functional group present in the leaf and stem of the *Jatropha integerrima*.

The FTIR spectroscopy analysis of *Jatropha integerrima* leaf obtained peaks at 3424.38 cm^{-1} , 2920.99 cm^{-1} , 2849.63 cm^{-1} , 2360.71 cm^{-1} , 1745.46 cm^{-1} , 1628.77 cm^{-1} , 1450.37 cm^{-1} , 1318.25 cm^{-1} , 1245.93 cm^{-1} , 1163.00 cm^{-1} , 1114.78 cm^{-1} , 892.98 cm^{-1} , 780.15 cm^{-1} , 667.32 cm^{-1} , 515.92 cm^{-1}. These absorption peaks are known to be associated with the stretching vibration for N-H in Secondary amine , C-H in Ketones stretch , C-H in Carboxylic acids , N-H in Secondary amine , C=O in Dihaloketon , N-H in Primary amine , O-H in Symmetric , C-N in Aryl primary amine , N-H in Amino acids , C-N in Alkyl amine , C-F in Poly fluorinated compound , C-H in Benzene ring , C-Cl in Mono chlorinate Alicyclic equatorial , C-Br in Acyclic Axial groups , C-Br in Acyclic and Aromatic groups . Fig:6 , Table (6).

The FTIR spectroscopy analysis of *Jatropha integerrima* stem obtained peaks at 3442.70 cm^{-1} , 2919.06 cm^{-1} , 2849.63 cm^{-1} , 2360.71 cm^{-1} , 1611,41 cm^{-1} , 1442.66 cm^{-1} , 1383.83 cm^{-1} , 1319.22 cm^{-1} , 1113.81 cm^{-1} , 1028.95 cm^{-1} , 951.81 cm^{-1} , 661.54 cm^{-1} , 620.07 cm^{-1} , 516.89 cm^{-1} . These absorption peaks are known to be associated with the stretching vibration for N-H in Secondary amine , C-H in Ketmos

FIG: 6 FTIR spectroscopy analysis of *Jatropha integerrima* leaf

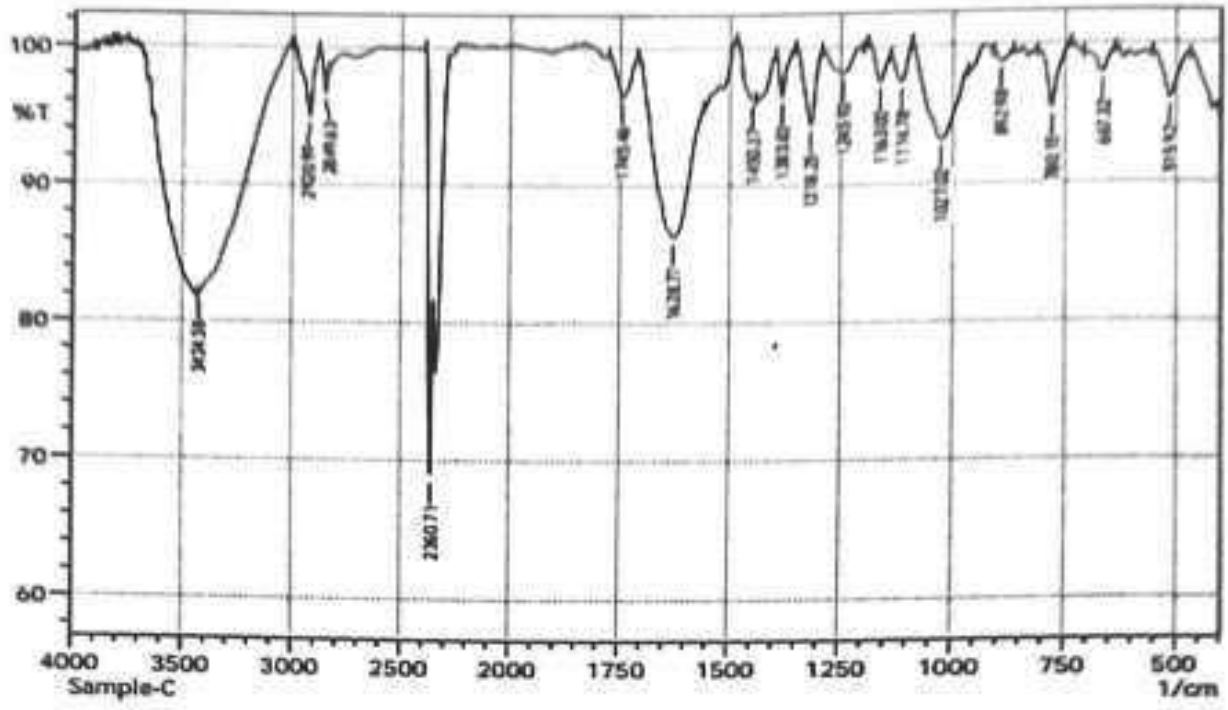


FIG:7 FTIR spectroscopy analysis of *Jatropha Integerrima* stem

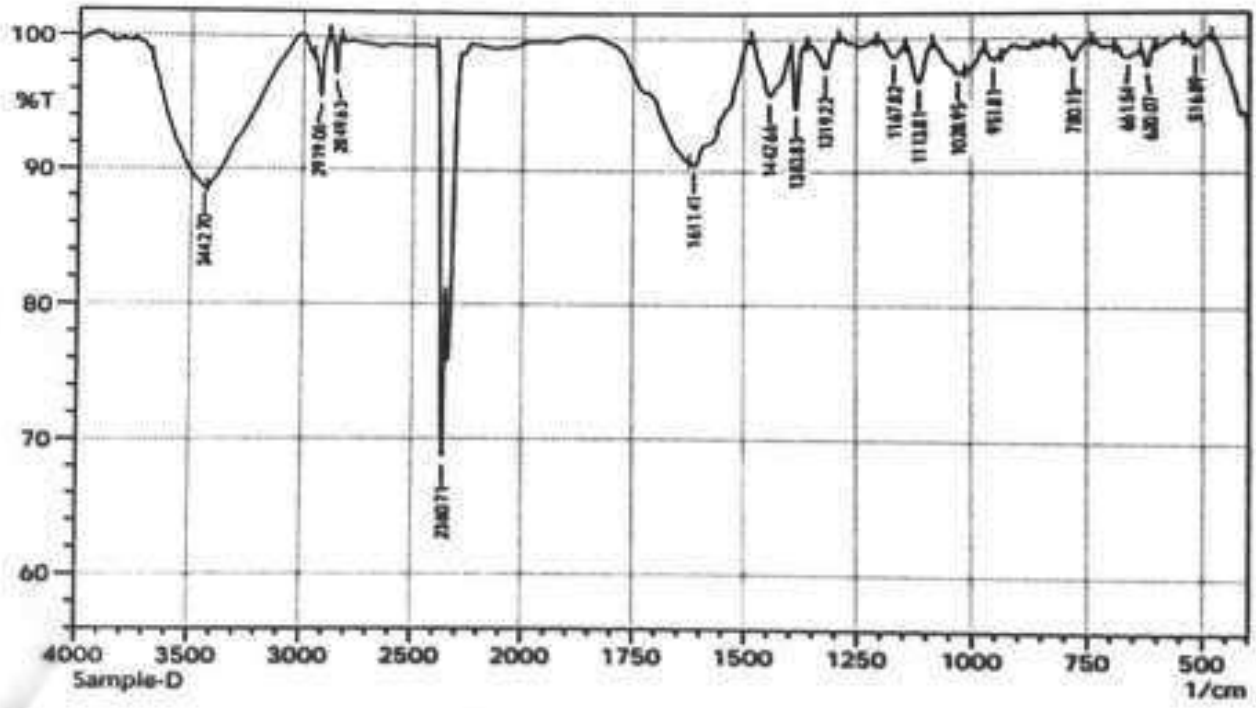


Table: 6 FTIR spectroscopy analysis of *Jatropha integerrima* leaf

PEAK VALUE	BOND	FUNCTIONAL GROUP
3424.38	MEDIUM ,SECONDARY AMINE	N-H
2920.99	WEAK,KETONES STRECH	C-H
2849.63	WEAK,CARBOXYLIC ACID	C-H
2360.71	STRONG,SECONDARY AMINE	N-H
1745.46	STRONG,DIHALOKETON	C=O
1628.77	STRONG,PRIMARY AMINE ,	N-H
1450.37	MEDIUM,SYMMETRIC	O-H
1383.83	MEDIUM,ASYMMETRIC	O-H
1318.25	STRONG,ARYL PRIMARY AMINE	C-N
1245.93	WEAK,AMINO ACIDS	N-H
1163.00	WEAK,ALKYL AMINE	C-N
1114.78	VERY STRONG , POLY FLUORINATED COMPOUND	C-F
1027.02	VERY STRONG,MONO FLUORINATED COMPOUND	C-F
892.98	MEDIUM, BENZENE RING	C-H
780.15	STRONG,MONO CHLORINATE ALICYCLIC EQUATORIAL	C-CI
667.32	STRONG,ACYCLIC AXIAL	C-Br
515.92	STRONG , ACYCLIC AND AROMATIC	C-Br

Table:7 FTIR spectroscopy analysis of *Jatropha integerrima* stem

PEAK VALUE	BOND	FUNCTIONAL GROUP
3442.70	MEDIUM, SECONDARY AMINE	N-H
2919.06	MEDIUM, KETMOS STRETCH	C-H
2849.63	WEAK ,BROAD CONJUGATE CHELATION	C=O
2360.71	STRONG ,SECONDARY AMINE	N-H
1611.41	STRONG , PRIMARY AMINE	N-H
1442.66	MEDIUM, CH2 BENDING	R-C
1383.83	MEDIUM, CH3 SYMMETRIC	R-C
1319.22	STRONG, SULPHONAMIDE	SO2
1167.82	STRONG, SULPHONAMIDE	SO2
1113.81	MEDIUM, CARBOXYLIC ACIDS	C-O
1028.95	STRONG,SULPHINIC ACIDS	S=O
951.81	WEAK,ALDEHYDE GROUP	C-H
780.15	WEAK, ALDEHYDE GROUP	C-H
661.54	MEDIUM,THIAZOLES	C-H
620.07	STRONG, TODO COMPOUND	C-I
516.89	WEAK, SULPHIDES	S-S

stretch , C=O in Broad conjugate chelation , N-H in Secondary amine , N-H in Primary amine , R-C in CH2 Bending , R-C in CH3 Symmetric , SO2 in Sulphonamide , C-O in Carboxylic acids , S=O in Sulphonic acids , C-H in Aldehyde group , C-H in Thiazoles , C-I in Todo compound , S-S in Sulphides group. Fig :7 ,Table (7).

From the spectral data presence of N-H , C-H, C=O, O-H, C-N, C-F , C-Cl , C-Br, C-O, S=O, C-I, S-S were identified. These bending are responsible for the presence of amine group, Carboxylic acids group ,Dihaloketon group, Asymmetric group, Alkyl amine group , Poly fluorinated group , Mono chlorinate Alicyclic group , Acyclic and Aromatic group , Carboxylic acid group, Sulphinic acid group , sulphides group, carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer, jaundice, head ache, stomatitis ,hemicranias, fever, pain in lever ,treatment of edema and rheumatic joint pain .Amides, amine are the main groups which are involved in protein synthesis. The study revealed that the whole plant of *Jatropha integerrima* contain a considerable amount of secondary metabolites and it may considered in future to be used human disease management.

GC-MS Analysis:

The GC-MS analysis of ethanolic leaf extract of *Jatropha integerrima* was confirmed the presence of 5 compounds with retention time. Interpretation of mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of this 5 compounds 3 compounds are majority present in the leaf extract of *Jatropha integerrima* respectively 2-Ethylacridine(52.89%), Benzo(h)quinoline,2,4-dimethyl(52.89%) and 5-methyl 2- phenylindolizine (35.86 %).

FIG:8 GC-MS chromatogram of leaf extract (ethanol)
Jatropha integerrima

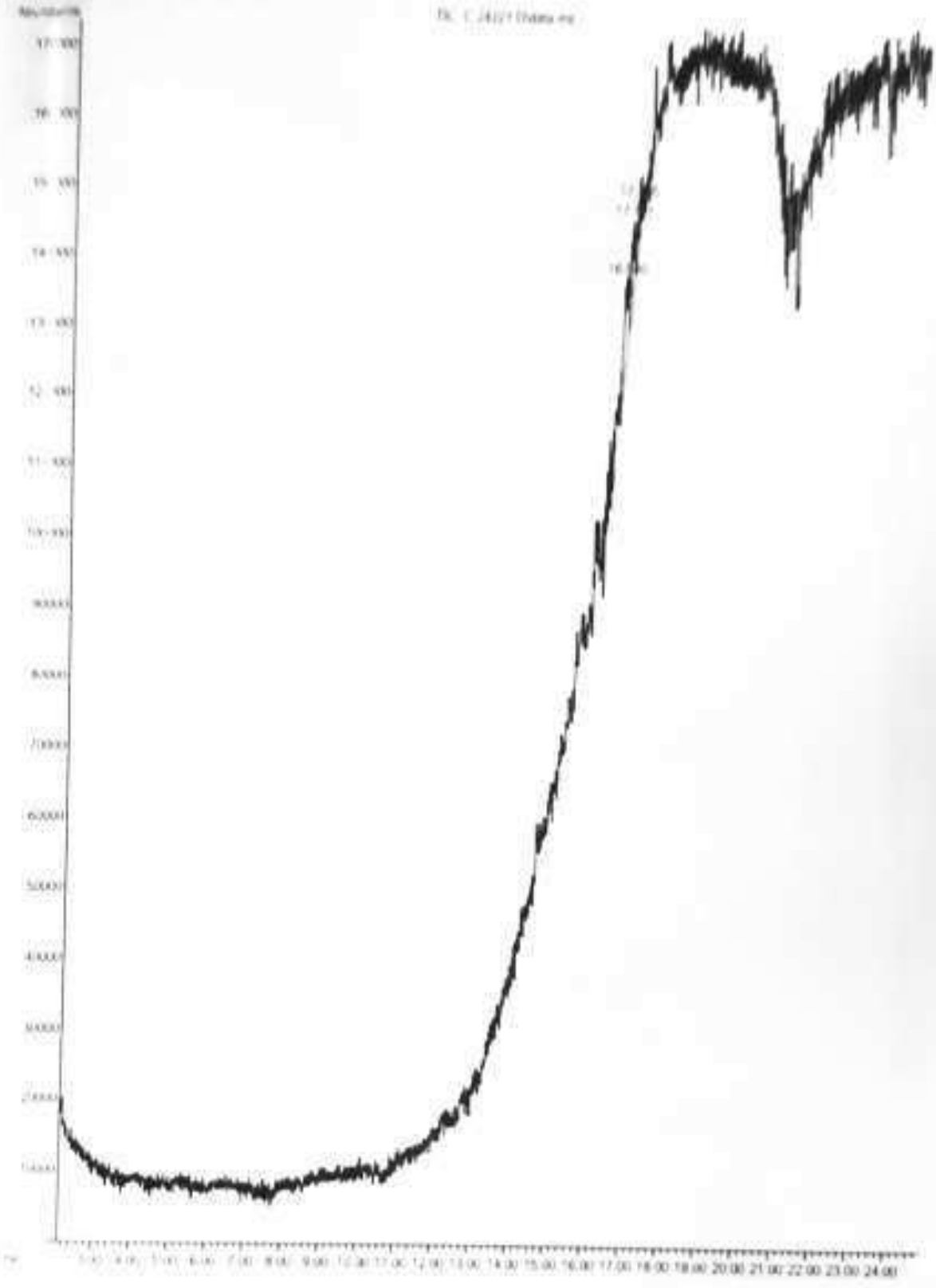


TABLE:8 *Jatropha integerrima* leaf mass spectrum

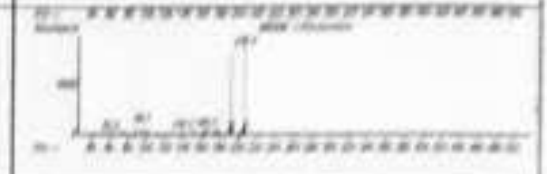
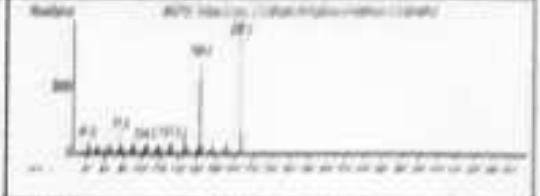
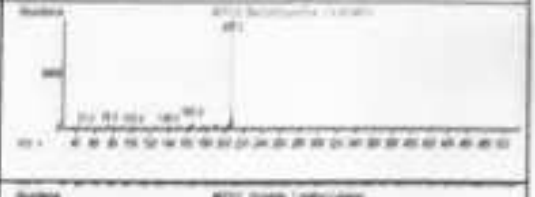
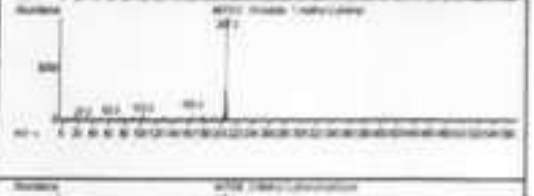
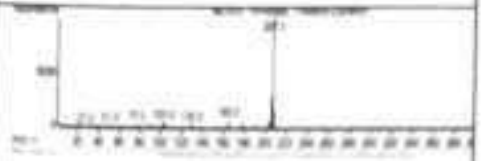
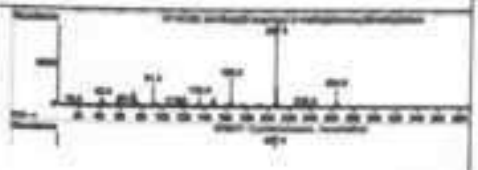
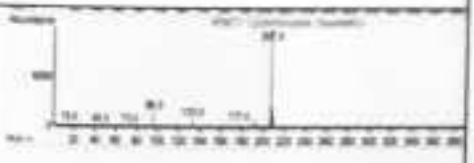
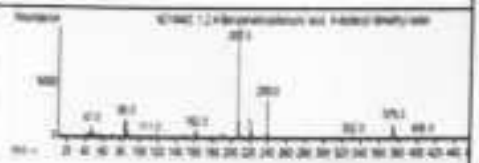
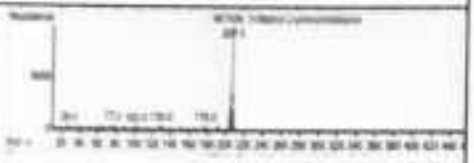
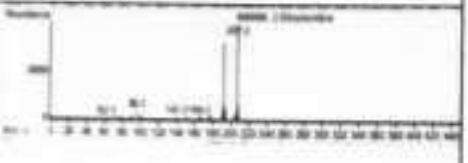
S.No	RT	Name of the compounds	Area%	Mass spectrum
1.	16.941	2-Ethylacridine	52.89	
2.	16.941	Indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	52.89	
3.	16.941	Benzo[h]quinoline,2,4-Dimethyl-	52.89	
4.	17.111	1H-Indole,1-methyl-2-Phenyl-	35.86	
5.	17.111	5-Methyl-2-Pheylindolizine	35.86	

Table: 9 List of chemical compounds identified from ethonal leaf extract of *Jatropha integerrima* through GC-MS analysis

S.NO	RT	Name of the compound	Area%	Biological Activity
1.	16.941	2-Ethylacridine	52.89	Antiulcerative, Antiviral, Muscular dystrophy treatment,kidney function stimulant
2.	16.941	Indole-2-one,2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	52.89	Antidiabetic, Antiviral, Antiseptic, Antibacterial, Antidiarrheal, Antiinflammatory and kidney function stimulant
3.	16.941	Benzo(h)quinoline2,4-Dimethyl-	52.89	Antiviral. Antiprotozal, Antimutagenic, kidney function stimulant, Diabetic neuropathy treatment, Renal disease treatment
4.	17.111	1H-Indole,1- methyl-2-phenyl	35.86	Antiviral, Antialcoholic and Antihelmintic
5.	17.111	5-Methyl-2-phynylindolizine	35.86	Antiviral, Antineurotic, Antiinfective, Antiuremic and Antihypoxic

FIG. 9 GC-MS chromatogram of stem extract (ethanol) *Jatropha integerrima*

TABLE:10 *Jatropha integerrima* stem 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	

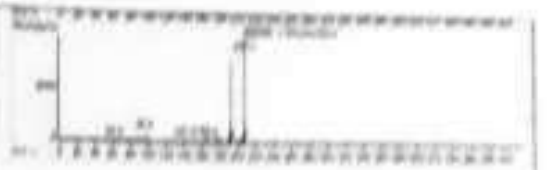
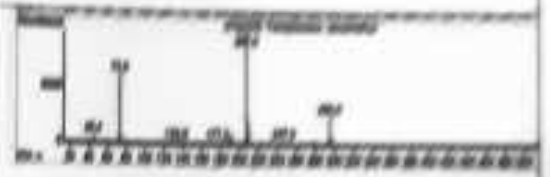
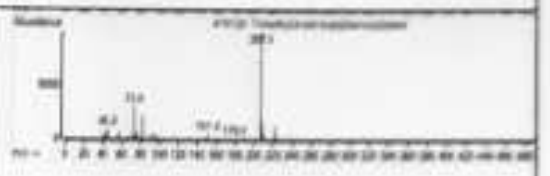
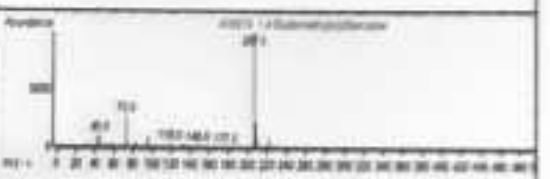
7.	17.197	2-Ethylacridine	28.85	
8.	17.509	Methyltris(trimethylsiloxy) Silane	38.37	
9.	17.509	Tetrasiloxane,decamethyl-	38.37	
10.	17.509	Trimethyl[4-(1,1,3,3,-tetramethyl butyl) silane	38.37	
11.	17.669	1,2-Bis (trimethyl silyl) benzene	15.01	
12.	17.669	Trimethyl (4-tert-butyl phenoxy) silane	15.01	
13.	17.669	1,4-Bis (trimethyl silyl) benzene	15.01	

Table:11 List of chemical compounds identified from ethanol stem extract of *Jatropha integerrima* through GC-MS analysis

S.NO	RT	Name of the Compound	Area%	Biological activity
1.	16.951	1H-Indole,1-methyl-2-phenyl	8.63	Antiviral, Antihelimintic, Antiprotozol and Antihypoxic
2.	16.951	tert-Butyl(5-isopropyl-2-methyl phenoxy)dimethyl silane	8.63	Antiviral, Antiparasitic, Antiparasitic and Insecticide
3.	16.951	Cyclotrisiloxane, hexamethyl-	8.63	Antibacterical activity and Antioxidant
4.	17.074	1,2,4-Benzenetricarboxylic acid,4-dodecyl dimethyl ester	9.13	Antiviral, Antiseptic, Antiinflammatory and Antiprotozoal
5.	17.074	5-Methyl-2-phenylindolizine	9.13	Antiviral, Antipyretic, Antimycobacterial and Antiinfective.
6.	17.074	Benzo(h)quinoline, 2,4-dimethyl-	9.13	Antiviral, Antialcoholic, Antimutagenic and Antineurotic.

7.	17.197	2-Ethylacridine	28.85	Antiviral, Antihelmintic, Antiinfective and Antineoplastic
8.	17.509	Methyltris(trimethylsiloxy) silane	38.37	Antiviral, Antiprotozol, Antimyopathies and Antidyskinetic
9.	17.509	Tetrasiloxane, decamethyl-	38.37	Phobicdisorders treatment, Antiviral, Antiinfective
10.	17.509	Trimethyl[4-(1,1,3,3,-tetramethyl butyl)phenoxy]silane	38.37	Antiviral, Antiprotozol, kidney function stimulant and Antineoplastic
11.	17.669	1,2-Bis(trimethysilyl)benzene	15.01	Antiviral, Antifungal, Antineoplastic, Antihelmintic And Prostate disorder treatment
12.	17.669	Trimethyl(4-tert-butyl phenoxy)silane	15.01	Antiviral, Antioxidant, antineoplastic, Antifungal and vascular dementia treatment
13.	17.669	1,4-Bis(trimethylsilyl)benzene	15.01	Antiviral,Antiinflammatory, Antianginal and kidney function stimulant

The two minor compounds such 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 ethanolic leaf extract of *Jatropha integerrima*. The chemical constituent's analysis result of *Jatropha integerrima* were 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-hydroxyl-4-methoxy-3,3-dimethyl, Benzo(h) quinoline, 2-4-dimethyl whereas 1H-Indole,1-methyl-2-phenyl and 5-Methyl-2-phenylindolizinewas 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 ethanolic stem extract of *Jatropha integerrima* was confirmed the presence of 13 compounds with retention time. Interpretation of mass spectrum of GC-MS was conducted using the database of NIST and WILEY libraries. Out of this 13 compounds 2 compounds were majority present in the stem extract of *Jatropha integerrima* 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 [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 (trimethylsilyl)1 Benzene (15.01%)were also reported from the ethanolic leaf extract of *Jatropha integerrima* . The chemical constituents's analysis result of *Jatropha integerrima* stem 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-isoprophyl-2- methyl phenoxy) dimethyl silane, cyclotrisiloxane, hexamethyl- whereas Methyltris (trimethyl siloxy) silane, tetrasiloxane, decamethyl, trimethyl [4-(1,1,3,3-tetra methyl butyl)phenoxy] silane was the last compound which took longest retention time (17.509 min) to identify. At (17.508 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-Buthyl (5-isopropyl-2-methyl phenoxy) dimethylsilane, cyclotrisil oxane, hexamethyl, The above mentioned isolated compounds from the ethanolicleaf and stem extract of *Jatropha integerrima* have a medicinal important.

Benzo (h) quinoline ,2,4-dimethyl in the leaf ethanolic extact of *Jatropha integerrima* 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 stem ethanolic extract of *Jatropha integerrima* is a main antimicrobial compound.Phytocompound 1,2-Bis (trimethylsilyl) benzene at retention time15.651 and 16.055 min has antioxidant,

Table: 12

Antioxidant activity in aqueous extract of stem and leaf of *Jatropha integerrima*

S.NO	AQUEOUS EXTRACT	DPPH free radical assay (%)
		Jatropha integerrima
1	Stem	99.46
2	Leaf	99.16

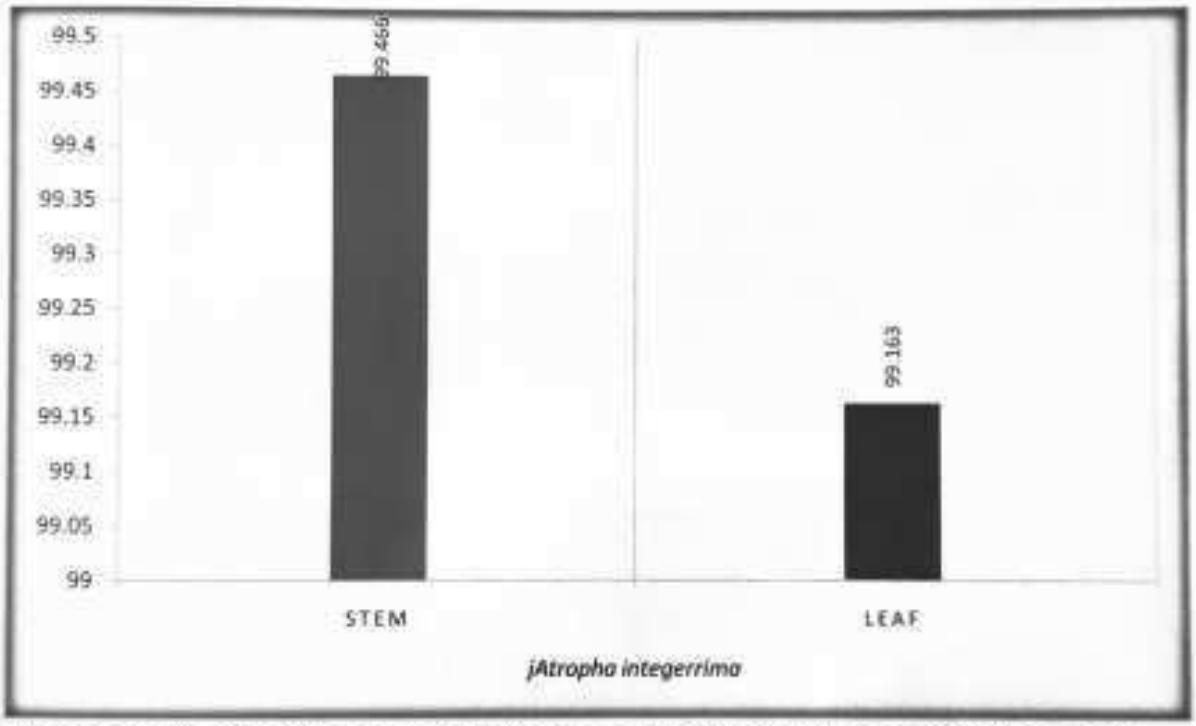


FIG: 10 Antioxidant activity in aqueous extract of stem and leaf of *Jatropha integerrima*

wang 2004). Natural antioxidants comprised non-detrimental chemical combinations are considered to be rather safer for use in food products. Further, uncared wastes are if exploited as resource of antioxidants, will be more beneficial to human kind and protecting the environment. Flavanoids are water soluble polyphenolic molecules with antioxidant activity which has many beneficial effects on the cardiovascular system (Evans,1989). Vitamin C acts as ROS scavenger, thus potentially protecting cells from harmful oxidative products (Fossati et al.,). Vitamin E supplement elevates the activities of antioxidant enzymes (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 plant extracts (Wei et al.,2012). DPPH solution shows a strong absorption bandat 517 nm appearing as a deep violet colour. The absorption vanishes and the resulting decolourization is stoichiometric with respect to degree of reduction. The leaf and stem extract of *Jatropha integerrima* was able to reduce stable DPPH radical to yellow colourdiphenyl picrylhydrazine. The degree of reduction in absorption is the reflection of radical scavenging power of the compound.

The antioxidant activity of aqueous extract using leaf and stem of *Jatropha integerrima* plants was evaluated by using DPPH scavenging essay Fig (10).Aqueous extract using *Jatropha integerrima* leaf has higher scavenging activity (99.16%) followed by stem (99.46) as shown Fig (10) and Table (12).

This result indicated aqueous extract using leaf and stem of plants shows higher scavenging activities. It has been reported that the antioxidant activity of aqueous extract using leaf and stem of *Jatropha integerrima* was due to presence of phenolics and it is responsible for redox properties, which allow them to act reducing agent, hydron donors and singlet oxygen quenchers. (Arasali and Kadimi 2009).

SUMMARY AND CONCLUSION

Jatropha integerrima well known plant of family Euphorbiaceae is used as a therapeutic agent. Various parts of *Jatropha integerrima* are traditionally used as purgative, styptic, emetic, in treatment of warts, tumours, rheumatism, herpes, pruritis, toothaches, scabies, eczema and ringworm (Kirtikar et al., 2002). The medicinal effects of plants are considered to be due to metabolites, especially secondary compounds, produced by plant. In this study, we were determined flavonoid, tannin and phenol,vitamin E and vitamin C content of leaf and stem of *Jatropha integerrima* using spectrophotometric methods. The result of this study showed that the leaf of *Jatropha integerrima* have signifigant amount of phenol, flavonoid and tannin compared to stem. The stem of *Jatropha integerrima* have signifigant amount of vitamin E and vitamin C compared to leaf.

The FTIR spectrum of *Jatropha integerrima* showed strong IR bands characteristics of Amine (3424.38 cm^{-1}) , Carboxylic acids (2849.63 cm^{-1}) , Dihaloketon (1745.46 cm^{-1}) , Amino acids (1245.93 cm^{-1}) , Sulphonamide (1319.22 cm^{-1}) , Sulphinic acid (1028.95 cm^{-1}) , Aldehyde (951.81 cm^{-1}) , Thiazoles (661.54 cm^{-1}) , Sulphides (516.89 cm^{-1}) , functional group. From the spectral data, presence of N-H , C-H , C=O , O-H , C-N , C-F , C-Cl , C-Br , C-O , S=O , C-I , S-S were identified. These bonding are responsible for the presence of Amine group , Carboxylic acid group , dihaloketon group , Asymmetric group , Alkyl amine group , Poly fluorinated group , Mono chlorinate Alicyclic group , Acyclic and Aromatic group , Carboxylic acid group , Sulphinic acid

group , Sulphides group. Carboxylic acid present in the medicinal plant serves as main pharmaceutical product in curing ulcer, jaundice, head ache, stomatitis, hemicranias, fever, pain in lever, treatment of edema and rheumatic joint pain. Amides, amine and amino acid are the main groups which are involved in protein synthesis.

The GC-MS analysis of ethanolic leaf extract of *Jatropha integerrima* was confirmed the presence of 5 compounds with retention time. Out of this 5 compounds 3 compounds were majority and two minor compound present in the leaf extract of Jatropha integerrima.. The GC-MS analysis of ethanolic stem extract of Jatropha integerrima was confirmed the presence of 13 compounds with retention time. The above mentioned isolated compound from the ethanolic extract of Jatropha integerrima. leaf and stem have a medicinal important. Benzo (h) quinoline ,2,4-dimethyl in the leaf methanolic extact of Jatropha integerrima 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 stem methanolic extract of Jatropha integerrima is a main antimicrobial compound.Phytocompound 1,2-Bis (trimethylsilyl) benzene at retention time15.651 and' 16.055 min has antioxidant, antimicrobial, anticanserous and antitumerous activity (Alok prakash and Suneetha,2014).Tetrasilaxane identified in the methanolic stem extracts of *Jatropha integerrima* is a main antimicrobial

compound.(Cai et al., 2018). Cyclotrisiloxane and hexamethyl found in stem of Jatropha integerrima is a main antioxidant compounds that help remove harmful toxins and free radicals in the body.(Anju Krishnaet al., 2015)

The antioxidant or free radical scavenging activity of leaf and stem extracts of this selected medicinal plant is investigated by using methods like DPPH scavenging activity. The leaf and stem extracts of *Jatropha integerrima* show maximum antioxidant activity. The findings of the present study suggest that *J. integerrima* could be a potential source of natural antioxidant that could have great importance as therapeutic agent in preventing or slowing the oxidative stress related degenerative diseases.

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ROLE OF *COLOCASIA ESCULENTA* (L.) Schott IN PHYTOREMEDIATION OF DETERGENTS UNDER LABORATORY CONDITIONS

A short term project work submitted to

ST.MARY`S COLLEGE (Autonomous), THOOTHUKUDI

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfilment of the requirement for the degree of

BACHELOR OF SCIENCE IN BOTANY

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CERTIFICATE

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INTRODUCTION

Water is the mother liquid of all forms of life. The essentiality of water for living systems is quite evident as without water, there is no life. No other substance on earth is abundant as water. All aspects of cell structure and functions are adapted to the physical and chemical properties of water. Water is a universal solvent. Water can dissolve most of the biologically important molecules. It is the solvent of life. The life originated in water and adapted to survive only in the presence of water. About 70 to 90% of a cell occupies water. Water acts as a medium for the diffusion of molecules in the cell. Osmotic concentration of cell is maintained by water and dissolved solutes. The turgidity of the cell is maintained by the water, translocation of inorganic and organic compounds in the living system takes place through the water. Carbohydrates the product of photosynthetic, in plants are transported through the water. Water is the source of H+ ions for photosynthesis.

Oxygen is released by the hydrolysis of water during photosynthesis. Water acts as a reactant in the hydrolysis of water reaction. Water supports aquatic plants and animals. Flagellated and ciliated organisms can swim in the water. Organisms with flagellated gametes require water for their fertilization. Lower plants such as algae, fungi, bryophytes and pteridophytes require the presence of water to complete their fertilization. Pollination and dispersal of seed in plants can be done through water bodies. Transpiration in plants is due to the presence of water. Transpiration ensures water uptake and transport of minerals in plants. Transpiration also cools the leaves and makes them stay in the open sunlight. Seed germination requires water (Aksungur and Firidin, 2008).

Water can form buffers with acids and bases. Condensation reaction, a common type of reaction in the cells, results in the release of water molecule. Water protects the cells from temperature fluctuations. Water is an essential a biological component of the ecosystem (Hansen and Brask, 2020).

Wastewater contains suspended organic matter and dissolved substances such as cohydrate, fat and protein. 80% of the causes of freshwater pollution are liquid waste (Hermawati, 2005) and 20% of the total wastewater is detergent (Nair and K. Swarnalatha, 2015). Detergents that contain surfactants such as Sodium Dodecyl Benzen Sulfonate (NaDBS) and sodium Tripolyphosphate (STPP) are found in laundry soap, bath soap, shampoo, toothpaste, and others. Detergents are chemical compounds made from synthetic materials so that detergents have a negative effect on humans and the environment. Surfactant in detergents is a material that is very difficult to degrade naturally so that it can cause pollution. Surfactants can cause skin irritations such as itchy and blistered skin. In the chlorination of PDAM drinking water treatment. Surfactants can form chlorobenzane which is toxic and harmful to health. Detergents also contain phosphate (builders) which can reduce water hardness by binding to calcium and magnesium ions even though Phosphate itself is non-toxic and even becomes an important nutrient for living things. In large quantities, phosphate causes eutrophication of waters and causes a reduction in the amount of aquatic oxygen which can endanger the aquatic ecosystem (Pattusamy *et al.,* 2013).

The quality of water has been growing concerns amongst people as many rivers facing high level water contamination problems with the rapid urbanization and industrialization. Water pollutants consists of the harmful compound founded in water or river significantly give an adverse effects to humans, wildlife and ecosystems. Either in nature or synthetics, these pollutants are highly toxic, resist to degrade, semi-volatile, bio-accumulative, and mobile over considerable distances (Mohammad and Sapawe, 2021).

Among the different contaminants, detergent as an important pollutant has serious risks to natural ecosystems. Furthermore, detergents can pass into the wastewater treatment plants and have bad effect on their performance. They are part of human life and consumed for different aims especially hygienic purposes. Therefore, detergent components can enter to soil and water bodies from different sources. Detergents affect fauna and flora, and they have direct and indirect effects on ecosystems. Eutrophication, foaming, and altering parameters such as temperature, salinity, turbidity, and pH are more important, and their effects need to be managed and controlled. Researchers confirmed that aerobic processes are able to degrade the most of detergents but anaerobic degradation is not possible because of restricted metabolic pathways and toxicity of them. Therefore, production of environment-friendly detergent is an important issue around the world (Mousavi and Khodadoost, 2019).

Surfactant (XP-100) biodegradability with yeast extract was studied in the presence of the pollution active hydrocarbons naphthalene and hexadecane. Surfactant biodegradation was faster with yeast extract than without. Increased surfactant concentration did not inhibit its biodegradation over the period studied. The addition of

organic contaminants, on the other hand, enhanced surfactant biodegradation due to their synergistic effect. Naphthalene degraded more than hexadecane. The results outlined contribute to a better understanding of the bioremediation mechanism and the fate of the compounds studied in the aquatic environment (Abd-Allah and Srorr, 1998).

Lecithin, cephalin and sphingomyelin prevent the inhibition of bacterial metabolism which is caused by synthetic anionic and cationic detergents. The phospholipids must be added either before or simultaneously with the detergent. Addition after the detergent is without effect. Bacteria still exhibit this phenomenon after they have been exposed to the phospholipid and thoroughly washed. SDS is one of the most widely used anionic detergent in households and in industry. After use it is discharged in large amounts. In water systems like ponds and rivers etc. It is now well established that SDS is toxic to the health and survival of aquatic organisms (Baker *et al.,* 1941).

Commercial household detergents are diverse group of chemical that is best known for their wide used in laundry industries and household cleaning product. After use, residuals (surfactant) detergents are discharge into sewage system directly or indirectly into the surface water and most of them end up dispersed into the different environment compartment of soil and water. Water is facing lots of problems due to domestic waste. These toxic effects of surfactant damaging biodiversity of aquatic environment (Chaturvedi and Tiwari, 2013).

If detergent was added to a plant, it would block the transportation of water to the plant because the detergent will reduce the surface tension of the water which will force it to break the hydrogen bonds between them, depriving the plant of its necessary

nutrients. Detergents containing harmful ingredients cause damage to the soil structure by raising the alkalinity of soil. Consequently the damaged soil deteriorates healthy plants. Some bleaching detergents kill the good bacteria in the soil (Rouse *et al.,* 1994).

The detergents also brought about increase in electrical conductivity, PH and salinity of the soil which adversely affected plant growth. The present study has shown that high detergent concentration is unhealthy for plant growth and brings about unfavorable changes in soil physiochemistry. In addition to nitrogen and phosphate, detergents also contain sodium salts. These salts can build up overtime and become toxic to plants, especially poisoning the soil. To avoid this avoid using products that contain softening agents which are generally high levels of salts. Detergent will affect plant growth because detergent has chemicals that will damage the plant roots, making the plant die (Anbia and Maghsoodlu, 2020).

A number of synthetic detergents and soaps contain volatile chemicals and substances that can have a negative effect on the normal growth of plants. According to some scientific researchers, low concentration of some detergents may be beneficial for plant growth. It is best, however not to risk the health of the plant. Detergents containing sodium, chlorine bleach and boron may have negative effects while potassium, ammonia and phosphate show good effects on plant growth, according to harvestingrainwater.com (Ferrer *et al*., 1996).

Phytoremediation is considered an effective, aesthetically pleasing, cost effective and environmental friendly technology for the remediation of potentially toxic metals from the environment. Plants in phytoremediation accumulate contaminants through their roots and then translocate these contaminant in the aboveground part of

their body (Ashraf *et al.,* 2018; Sharma *et al.,* 2015). The notion of using metal accumulator plants for the removal of heavy metals and several other contaminants in phytoremediation was first introduced in 1983, but this idea has already been implanted for the last 300 years (Blaylock, 2008). Phytoremediation is known by different names such as agro-remediation, green remediation, vegetative remediation, green technology and botano remediation (Sarwar *et al.,* 2017; Kushwaha *et al.,* 2018). Use of vegetation, soil and micro biota along with other agrochemical practices makes the vegetative remediation an appealing green technology for the accumulation of different heavy metals (Helmisaari *et al.,* 2007; Mahar *et al.,* 2017). The application of in situ and ex-situ remediation is applicable in a phytoremediation process. In situ application is used more commonly because it reduces the multiplication of contaminant in water and airborne waste, which ultimately minimize the risk to the adjacent environment (Ensley, 2000). More than one type of pollutant can be treated on site by the phytoremediation without the need for a disposal site. It also reduces the spread of contamination by preventing soil erosion and leaching (Sová *et al.,* 2009). The clean up cost of phytoremediation is far less than other conventional techniques of remediation, which is the utmost advantage of this technique (Gerhardt *et al.,* 2017).

Plants should have the following characteristics in order to make the phytoremediation an eco-sustainable technology: native and quick growth rate, high biomass yield, the uptake of a large amount of heavy metals, the ability to transport metals in aboveground parts of plant, and a mechanism to tolerate metal toxicity (Ali *et al.,* 2013; Arslan *et al.,* 2017; Burges *et al.,* 2018; Cunningham and Ow, 1996). Other factors like pH, solar radiation, nutrient availability and salinity greatly influence the

phytoremediation potential and growth of the plant (Reeves *et al.,* 2018; Tewes *et al.,* 2018).

Interaction of plants with low concentration of a detergent, show signs of better growth and development. In some situations, such as in the case of a drought people use recycled laundry water for irrigation. Therefore, detergent plant interaction becomes inevitable avoid using detergent water for watering plants as the toxic chemicals can cause the plants to die.

Water plants are commonly used to control water pollution are *Pistia stratoites* L, *Eichornia* crassipes and *Ipomoea aquatica*. Plants that can be used to purify water usually have the ability to reduce the content of organic and inorganic substances in waters so that these types of plants can be used for water remediation. The process of water remediation using aquatic plants is called phytoremidiation. Phytoremediation aims to extract, reduce or clean pollutants in soil and surface water (Drake *et al.,* 2020). Phytoremediation technology is relevant to be applied in developing countries because it is effective, economical and sustainable (Ghosh and Singh, 2005; Ahalya and Ramacandra, 2006; Laghlimi *et al.,* 2015) and environmentally friendly (Emmanuel *et al.,* 2014).

The objective of the current study is to reduce the toxic effect of detergent using plants

REVIEW OF LITERATURE

Feltrium *et al.* (2021) reported that the plants can actively reduce the hydraulic resistance in the xylem by moving water from the neighbourhood living cell via aquaporins. They can also produce substances known as surfactants which stabilize nanobubbles avoiding embolism. Transcriptomic and proteomic data were used to test the presence of these two mechanisms in stems of *Eucalyptus gradis* and *Eucalyptus globulus* grown at two temperature treatment. The result suggest that aquaporins and surfactants can be involved in the reduction of embolism in *Eucalyptus* under high xylem tension, by allowing radial transport of water in the stem and stabilising nanobubbles,respectively.

Wisetkomolmat *et al.* (2021) analyzed the existing awareness of nearly forgotten Thai detergent plants by the use of chemometrics tool. A Northern Thai forest dependent community was chosen as it played vital role on knowledge to retaining of plant utilization. For phytochemical analysis plant extracts showed positive variable of bioactive ingredients and the main compounds in the extracts was sapanins. These findings confirmed that the knowledge of indigenous plant utilization was reserved by the forest dependent community and the information is beneficial toward local plant conservation method.

Appah *et al.* (2020) analyzed the molecular constituents of surfactants into cationic, anionic, non-ionic and amphoteric groups. The extent of surfactant emulsification is determined by its hydrophilic-lipophilic balance (HLB) value. In plant

cells, Surfactant reacts with lipoproteins of the cuticular layer to attenuate cell membrane

Zhiheng *et al.* (2020) studied the application way to inhibit the migration of organic contaminants from soil to plants. The result showed that the decrease in bioavailable OCs in soil and the increase in sorption of OCs on roots should be taken into consideration when predicting the concentration of OCs in plants in the presence of surfactants.

Aleksandar *et al.* (2020) investigated the contamination of surfactants in the natural environment and represent potential threat to terrestrial higher plants. The study aimed to prove how changes in lipophilicity of surfactant and their various structural modification influence toxicity towards investigated plants. The investigation was finally concluded that cation ability to mimic the structure of bilayer have less harmful effect on plant development.

Nowrouzi *et al.* (2020) reported that non-ionic surfactant extracted from soapwort plant under ultrasonic extraction and saponin purification processes. The focus of this study was on the use of the plant surfactant in EOR by ASP injection process. However, other applications of this surfactant for injection in various scenarios based on chemical water in EOR process can be developed.

Penfold and Thomas. (2019) investigated the biosustainable surfactants and surface active protein for a range of applications. This review focuses on two plant derived biosurfactants, the surface active glycoside, saponin and the surface active

globular protein, hydrophobin. A particular emphasis in the review is on the role of neutron reflectivity in probing the adsorption, structure of the adsorbed layer and their mixing at the interface with a range of more conventional surfactant and protein.

Freeling *et al.* (2019) investigated the seven day composite effluent samples from a german monitoring campaign including 33 conventional wastewater treatment plants were analysed for linear alkylbenzene sulfonates and alkyl ethoxysulfates were screened by wide scope suspect screening for 1564 surfactants and their transformation product by UHPLC-ESI-QTOT-MS.The study reveals the risks for all analyzed surfactants were below the commonly accepted PEC/PNEC ratio of 1 for single compound.

Kumari *et al.* (2019) investigated the effects of three surfactants (CTAB,TX-100 and SDS) against AgNP induced plant toxicity was studied in *Fagopyrum esculentum* L. importantly ,use of surfactants significantly decreased the intercellular AgNP content in plant perhaps due to increase in the particle size of AgNP. Overall findings clearly demonstrated the AgNP induced toxicity in *Fagopyrum esculentum* was substantially alleviated by the surfactants, which not only improve the plant tolerance against AgNP, but also reduced the excess Ag accumulation by plants.

Zhiheng *et al.* (2019) studied a series of mixed surfactants were utilized to reduce the uptake of PAHs by crops in farming period and enhance the plant microbe associated biodegradable in fallow period. The mixture of SDBS and Tween 80 increased the water soluble fraction of PAHs in soil, modified bacterial community structure and enriched the functional genes involved cell motility and signal

transduction. This systematic technology provided an effective solution to remediate and plant on PAH-contaminated farmlands.

Fagerstrom *et al.* (2013) investigated the mechanism of molecular transport across the cuticle of Clivia leaves. The results show that addition of surfactants allows for higher concentrations of tebuconazole available for penetration. In leaves, surfactants induced the same quantitative increase in both flux and diffusion coefficient of solute in the cuticle, while the cuticle water partition coefficient was unaffected.

Mohammad and Moheman (2012) studied the effects of anionic and nonanionic surfactants on dry biomass and nutrient uptake in tissues of wheat under pot. The result showed that nutrient concentrations in shoots were less affected with increase in concentration of Surfactant in soil as compared to roots, Shoot dry biomass seemed less affected than root dry biomass while anionic surfactant was found to be more toxic to plant growth.

Malekian *et al. (*2011) reported the feasibility of using surfactant modified zeolite in comparison with Zeolite clinoptilolite application to reduce nitrate leaching and enhance crop growth.The effects of size and application rate if Cp and SMZ on nitrate leaching and crop response were also evaluated.Using soil lysimeters,it was determined that the maximum and mean nitrate concentration in the leachate of SMZ-amended soil were significantly lower than those of Cp amended soils.The result implicity suggest that plants may have a better response if Cp is used as a fertilizer carrier rather than SMZ when applied at a rate of 60 g Kg -1.

Lu and Zhu (2009) studied the transfer of contaminants from soils to plants is a promising approach to produce safe agricultural products grown on contaminated soils. Concentrations of phenanthrene and pyrene in vegetables grown in contaminated soils treated with the cationic surfactants were lower than those grown in the surfactant- free control. Considering the impacts of cationic surfactants on plant growth and soil microbial activity CTMAB was more appropriate to employ and the cost effective dose was 100-200 mg/kg.

Almeida *et al.* (2009) analyzed the possible effect of surfactants commonly found in the aquatic environment on the remediation potential of the salt marsh plant. Experiments were carried out in the laboratory, either in hydroponic or in sediment soaked in elutriate, using sediment and water from an estuarine salt marsh, Cu was determined in solutions, sediments and in different plant tissues before and after experiment. The non-ionic surfactant Triton X-100 and to a lesser extent,the anionic surfactant SDS too, favored Cu accumulation in the plant roots but not Cu translocation, indicating that surfactants may favor Cu adsorption to the roots.

Yang and Xiaomei. (2008) recorded the effect of a low concentration of Surfactant Tween 20 on plant growth and physiology of two nursery crops. In laboratory soil columns,the initial wetting of a commercial substrate fafard 3B was accelerated 40.5% compared to that of water treatment at initial wetting.

Lunney and Irene. (2007) reported that the plants grown in weathered DDT contaminated soil collected from a former LORAN station in northwestern Canada in a series of greenhouse studies.Howden pumpkin is used. The study reveals that DDT concentration in shoots of surfactant treated sedge were greater than the control.

Amounts of DDT in roots were significantly lower in soils with high organic matter content but DDT uptake in shoots was not significantly different between all treatment group.

Garland *et al.* (2004) anionic, amphoteric and non-ionic Surfactant were added to separate nutrient film technique hydroponic systems containing dwarf wheat in a series of 21 day trials. Microbial communities associated with both the plant roots and wetted hardware surfaces actively degraded the surfactants. The studied indicate that relatively small area of hydroponic plant systems can process per capital production of mixed surfactants with minimal effects on plant growth.

Bruschi *et al.* (1998) studied the action of several concentrations of an anionic synthetic surfactant alkyl benzene sulphonate and a nonionic surfactant arylalkylpolyglycol ether were studied using a yes different in vitro culture systems. Three Parameters were analysed i) callus or total biomass increase ii) shoot regeneration iii) cell damage. A clear callus growth stimulation was found in *Pittosporum tobira* cultured in the presence of ABS at lower doses. Surfactants at higher concentrations caused strong damage at cytological level. The effect of these surfactants on cytoplasm and nuclei were determined.

Spurrier and Jackobs. (1955) reported that UPFACE active agents or surfactants are being used in the manufacturer and processing of commercial fertilizer to improve the blending of materials and to speed up the manufacturing process. The surfactants in fertilizer has stimulated on interest in the effects of the additives on seed and growing plant. The study reveals the effect of surfactant, in various concentrations,

upon seed germination and plant growth. In addition, the effect of surfactant upon water movement in soil was observed.

MATERIALS AND METHODS

Collection of soil and analysis

Soil sample was collected from Keelamangalam. The soil sample was characterized by both physical and chemical properties namely, pH, EC, OC. pH of the soil was analysed by digital pH Meter. Soil nutrient contents like N, P and K were also analysed.

Selection of plant:

The plant used for the study is *Colocasia esculenta* (L.) Schott. Healthy-looking young plants were collected from Kanniyakumari District. The selected plant species have not yet been tested extensively for wastewater treatment. Some studies have been reported that these species showed good performance in a growth studies and Hg (II) and nutrient removal from wastewater (Skinner *et al.,* 2007; Bindu *et al.,* 2008). *Colocasia esculenta* species are readily found in the native flora of Kanniyakumari District.

Systematic position

Class: Moncotyledons

Order: Alismatales

Family: Araceae

Genus: *Colocasia*

Species: *esculenta*

Botanical description:

Perennial herb up to 1.5 m, with thick shoots from a large corm; slender stolon's are often produced, along with offshoot corms.

Treatment:

The surfactant used to treat *Colocasia esculenta* is zoom. The plants were treated for 20 days with surfactant in different concentrations (2%, 4%, 6%, 8% and 10%). Blank (without plant) and control (plant without treatment) were maintained separately. After treating the plants for 10 days the difference, in their petiole, tuber and root were observed under Trinocular microscope.

RESULTS AND DISCUSSION

Phytoremediation of heavy metals with aquatic plants has gained significant consideration due to its elegance and cost-effectiveness (Sharma *et al.,* 2015. Plants remove heavy metals via absorption or through surface adsorption and integrate them into their system, and then accumulation them in certain bounded forms (Rai *et al.,* 1995). Effluents from wastewater mitigated through the plants, thus causing less harm to the surrounding environment.

The physicochemical properties of soil sample of the current study is depicted in Table 1. In the present study, different soil parameters like, Electrical Conductivity, Organic carbon, macronutrients and micronutrients. The pH, bulk density, porosity, moisture content, organic matter content and texture were recorded as 9.8, 1.12g/cm3, 50%, 5.387% and 11.58%. In blank, the chemical Parameters like EC, OC, macronutrients (N, P, K) and micronutrients (S, Zn, B, Fe, Mn, Cu) were analysed. The results obtained for EC and OC are 1.26 ds/m and 0.47%. The macronutrient content in the soil were recorded as N-185.4 Kcal/g, P-131.3 Kcal/g, K-419.3Kcal/g. The micronutrient content in the soil for S, Zn, B, M is zero. While, for Fe and Cu is 7.39 ppm and 4.30 ppm.

In control the pH, EC, OC, N, P, K value are recorded as 8.74, 0.493 ds/m, 0.35%, 169.5Kcal/g,139.4Kcal/g,401.2Kcal/g. After treatment, In T1 the values are recorded as 9.32, 4.20 ds/m, 0.64%, 210.8 kcal /g, 148.7Kcal/g, 421.7Kcal/g. Likewise for T2 is 10.03, 7.76 ds/m, 0.67%, 214.0Kcal/g, 149.2Kcal/g, 391.4Kcal/g . For T3 is 10.51, 11.68 ds/m, 0.69%, 217.2Kcal/g, 159.9Kcal/g, 332.9Kcal/g. For T4 is 10.63, 13

.49 ds/ m, 0.71%, 220.3Kcal/g, 168.9Kcal/g, 339.9Kcal/g. For T5 is 10.75, 19.65 ds/m, 0.73 %, 223.5Kcal/g, 169.8Kcal/g, 280Kcal/g.

Table 1: Physicochemical properties of soil sample

Sl.No	Soil Samples	PH	EC	OC	N	P	K
1.	Blank	9.8	1.26ds/m	0.47%	185.4Kcal/g	131.3Kcal/g	419.3Kcal/g
2.	Control	8.74	0.493ds/m	0.35%	169.5Kcal/g	139.4Kcal/g	401.2Kcal/g
3.	T1	9.32	4.20ds/m	0.64%	210.8Kcal/g	148.7Kcal/g	421.7Kcal/g
4.	T2	10.03	7.76ds/m	0.67%	214.0Kcal/g	149.2Kcal/g	391.4Kcal/g
5.	T3	10.51	11.68ds/m	0.69%	217.2Kcal/g	159.9Kcal/g	332.9Kcal/g
6.	T4	10.63	13.49ds/m	0.71%	220.3Kcal/g	168.9Kcal/g	339.9Kcal/g
7.	T5	10.75	19.65ds/m	0.73%	223.5Kcal/g	169.8Kcal/g	280.0Kcal/g

The increase in exposure time and decrease in initial concentration of the detergent resulted in an increased removal of detergent. Furthermore, increasing the time beyond 10 days did not significantly affect the percentage of detergent removal. Treatment with a longer exposure time and lower concentration resulted in a higher percentage removal of detergent. The findings showed that the response and percentage removal of detergent were highly influenced by the exposure time and initial concentration of detergent. This finding concurred with a study by Du *et.al.* (2020),

whereby heavy metal accumulation had a limit beyond which water hyacinth uptake could no longer increase with the contamination level.

The decline in percentage removal at high detergent concentration was probably due to the higher concentrations of detergent, while the active sites for the media and plant surface adsorption remained constant. Yadav *et al.* (2010) stated that adequate adsorption sites were available for metal ion adsorption at lower initial ion concentrations. Therefore, the increase of percentage removal relies on the lower initial metal concentration, and decreases with an increased initial metal concentration. Mwanyika *et al.* (2016) have reported that sedimentation is one of the processes involved in reducing heavy metal.

The plant underwent treatment, as days passed by plants growing in high concentration developed yellow leaves. Symptoms of toxicity have been observed in some plant species as a result of metal uptake and accumulation at high concentration, causing structural and ultrastructural changes that alter their development and physiology.

GENERAL ANATOMY OF COLOCASIA ESCULENTA PETIOLE:

The anatomy of *Colocasia esculenta* petiole is described based on the transverse sections observed under the light microscope, the outline of petiole is circular, which showed the following types of cells: epidermis, cortex which includes collenchyma and parenchyma and vascular bundles. Air cavities are present.

Epidermis:

Epidermis forms peripheral layer which is composed of barrel shaped thin walled parenchyma cells. It is a outermost layer. They are compactly arranged without intercellular spaces. They form the protective covering of the inner lying tissues

Cortex:

Cortex is distinguished into outer cortex and inner cortex. It is otherwise know as ring and core region. Rind region it contains ring formed vascular bundles, scattered form of vascular bundles are presented in core region. Thick walled collenchymas cells are present only in rind region of vascular bundles, collenchymas cells is absent in core region of vascular bundles, collenchyma cap are presented in below the epidermis some collenchymas cells are directly connected to epidermal cells. They provide more mechanical strength and flexibility to the petiole. Inner cortex consists of thin walled parenchyma cells with air cavity. More number of air cavities is present which are formed by separation and differential cell expansion of parenchyma cells. Aerenchyma cells are meant for gaseous exchange.

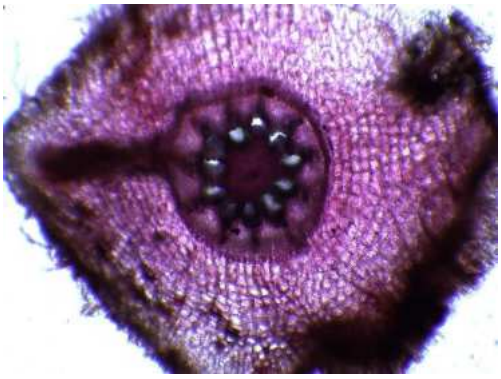
Vascular bundles:

Ring formed vascular bundles are presented in rind region, scattered formed vascular bundles in core region of cortex. Vascular bundles are closed and their arranged collaterally. There are numerous simple collateral vascular bundles and they consist of xylem and phloem, Number of vascular bundles depends on the number of air cavities present. Rind region vascular bundles contain xylem, phloem with collenchymas cells, but core vascular bundles it contain only xylem and phloem. The vascular bundles present in the periphery are smaller than those present in the centre. Poorly developed collateral vascular bundles are also present in periphery region. Vascular bundles contain metaxylem and protoxylem Xylem consists of tracheids.

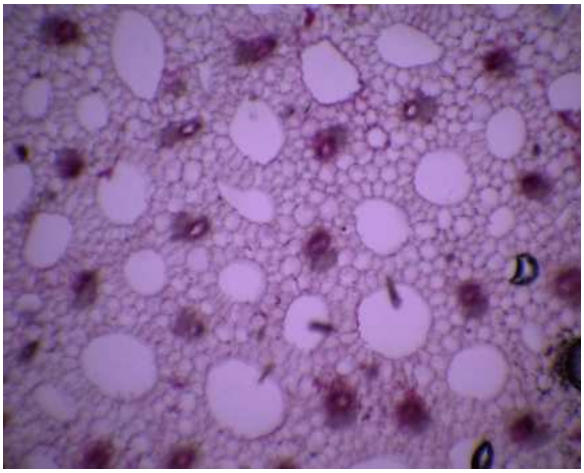
Morphologically the plant showed variations. The morphological change is likely due to the oxidation process in the plant. At higher concentration levels, Ni can cause several toxicities in plant tissue, where it affects the physiological modification in the plant species. Ni, which is a transition metal, can cause oxidative stress in plant tissue, and as a non-redox active metal, possesses the ability to indirectly inflict oxidative stress via multiple mechanisms (Emamverdian, 2015). Exposure to heavy metals triggers a wide range of physiological and biochemical alterations, and plants must develop or adopt a

Plate 1: Trinocular micrograph of cross section of control and treated plants

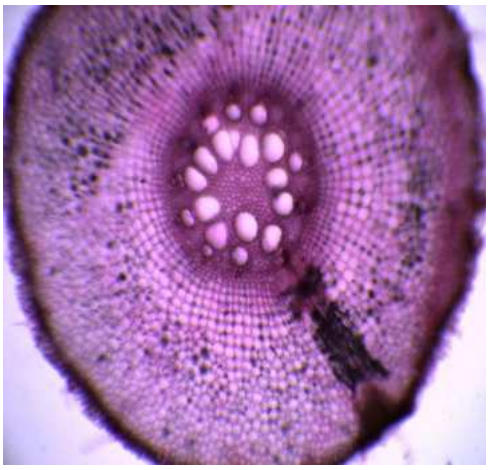
Control petiole



Control root

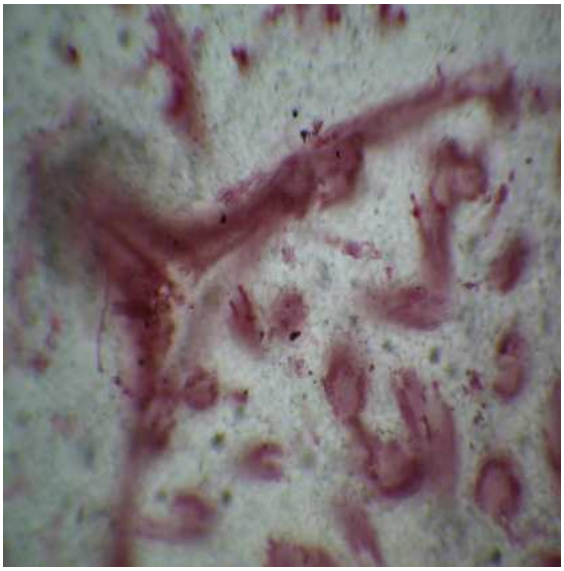


T2 petiole

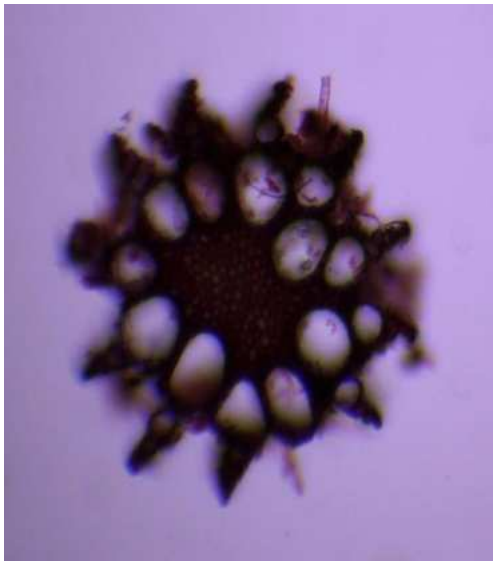


T2 root

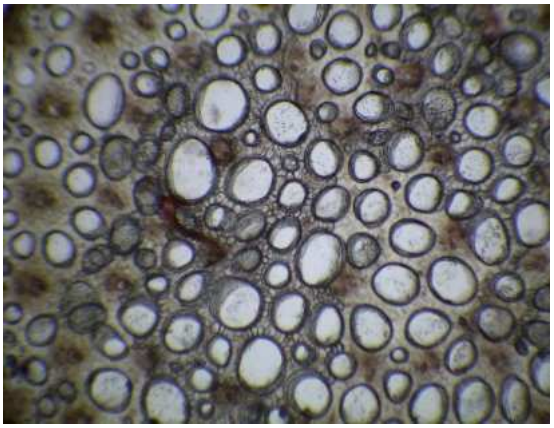
Plate 2: Trinocular micrograph of cross section of control and treated plants



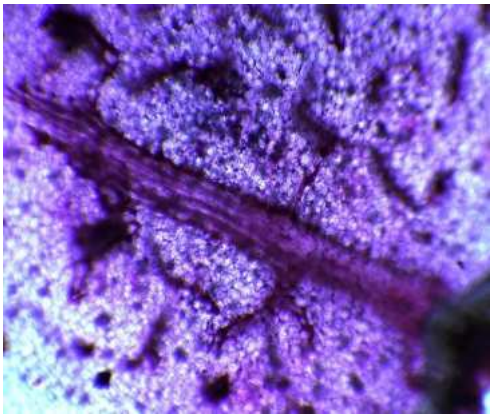
T4 tuber



T4 root



T5 petiole



T5 tuber

series of strategies that allow them to cope with the negative consequences of heavy metal toxicity (Singh *et al.,* 2016)

The plant underwent T3, T4 and T5 the cross sections shows loss of root hairs, epidermis and hypodermis. The reduction in number and length of root hairs in the treated samples suggests that the plant restricted contaminant uptake by adopting the root hair-based strategy. Indeed, Balasubramaniyam and Harvey (2014) stated that this move was practical, because it prevents the absorption of contaminants from the root hair into the main root. Therefore, the increase in root hair death is beneficial for the plant.

Previous studies have reported the presence of electron-dense granules in the cytoplasm, which appear to be a common occurrence in metal-stressed plants (Jiang *et al.,* 2009). According to Einicker-Lamas *et al.* (2002), the formation of these granules in the cells exposed to Ni might be a purification pathway to avoid cell damage. Hall (2002) and Sinha *et al.* (2007) agreed that intracellular changes are inevitable when there is a metal tolerance in plants. These changes include alterations in organelles, such as the cell wall, cytoplasm, and mitochondria. Absorption, cellular localization, and the transport of metals like Ni determine the toxicity level in plants (Singh *et al.,* 1997).

In the roots with detergent exposure, the uptake of toxic contaminants into the inner core, which contains conducting and storage cells, was blocked by the thickening of the plant cell walls. Toxic chemicals enter the roots by extracellular (apoplastic) or intracellular (symplastic) pathways. The apoplastic network is the continuous network of cell walls and extracellular spaces in plants where substances can pass through without having to go into the cell itself (Salt *et al.,* 1995). Rogers and Campbell (2004) explained that the thickening process would alter the cell wall in a way that will make

the cell wall an apoplastic apoplastic network, impermeable to water; this reduces transfer, thus restricting cell expansion.

Piechalak *et al.* (2002) reported that heavy metals would first attach to roots, since they provide a principal pathway for the penetration of metal ions. Growth is the most recognizable morphological parameter of plants undergoing metal stress, where the root growth is commonly the most affected. Furthermore, slow growth will result in low crop production if it occurs in cultivated plants (Hamim *et al.,* 2018).

CONCLUSION

Detergent toxicity in our environment as a persistent pollutant needs absolute elimination for a completely remedial objective. Utilization of phytoremediation seems to be a less disruptive, economical and environmentally sound clean-up technology. Choice of appropriate plant is the most significant feature in phytoremediation.

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A COMPARATIVE STUDY OF PHYTOCHEMICAL ATTRIBUTES, ANTIBACTERIAL AND ANTHELMINTIC ACTIVITY OF *SOLANUM XANTHOCARPUM* SCHARD AND WENDL AND *DATURA METEL* L. LEAVES

A Short term Project Work Submitted to St. Mary's College (Autonomous) Affiliated to Manonmaniam Sundaranar University in Partial Fulfillment for the Degree of

BACHELOR OF SCIENCE IN BOTANY

By

X. IRUTHAYA SHARAN	**-**	**18AUBO16**
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DEPARTMENT OF BOTANY

ST. MARY' S COLLEGE (AUTONOMOUS)

THOOTHUKUDI - 628001

2020 -2021

CERTIFICATE

It is certified that this short term project work entitled "**A COMPARATIVE STUDY OF PHYTOCHEMICAL ATTRIBUTES, ANTIBACTERIAL AND ANTHELMINTIC ACTIVITIES OF *SOLANUM XANTHOCARPUM*** SCHRAD AND WENDL **AND *DATURA METEL* L. LEAVES"** submitted to St. Mary's College (Autonomous) affiliated to **MONONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment of the requirements for the degree of Bachelor 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 2020 -2021 by the following students.

By

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It is our humble attempt to present this project **"A COMPARATIVESTUDY OF PHYTOCHEMICAL ATTRIBUTES, ANTIBACTERIAL AND ANTHELMINTIC ACTIVITY OF *SOLANUM XANTHOCARPUM* S C H R A D AND WENDL AND *DATURA METEL L. LEAVES"*** First and foremost our sincere gratitude belongs to **Dr. Sr. A.S.J. LUCIA ROSE., M.Phil., PGDCA., Ph. D.,** principal, St.Mary's College (Autonomous) for providing us an opportunity to do this project.

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Above all we humbly bow in gratitude to the **GOD LORD** for showering abundant graces on us and for helping us to yield fruitful results.

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CHAPTER - I

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 . Medicinal plants are 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 molecules, making them a rich source of different types of medicine. Higher plants medicinal compound source have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural origin. Natural products have an important role to play in drug development programme in the pharmaceutical industry (Baker *et al., 1995).* In comparison of synthetic drugs medicinal plant contain numerous phytochemical 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. Nowadays,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 flavouring 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.

Solanum xanthocarpum Schrad and Wendl.,is commonly known as 'Yellow berried night shade' (English) and kantakari (Sanskrit). It's a spiny perennial herb with prominent nodes and inter nodes that's herbaceous and spiny.The roots are almost cylindrical and taper.Flowers are purple in colour, with a few flower axillary cymes with glabrous globular berries that are green when young and yellow when mature. The seeds are smooth,compact and have a reniform bitter flavour. It's found throughout India,mostly in dry places as a weed along road sides and waste lands.

Kantakari is very effective in respiratory disorders such as asthma and cough. This herb is useful in treating urinary and stomach disorders.It is helpful for gum problems and sore throat.It is also beneficial in muscular pain fever,gonorrhea,cardiac disease and liver disorders.Kandakari also treats acidity and constipation. The whole plant is useful in vitiated conditions of vata and kapha. Panchang (whole herb including roots) and berries, have anthelmintic property. The decoction of the plant are used in gonorrhea. The leaves are applied for piles. The fruits are laxative. The plant is bitter, acrid, hot, anthelmintic, anti-inflammatory, anodyne, digestive, carminative,appetizer, stomachic, depurative, sudorific, febrifuge, expectorant, laxative, stimulant, diuretic, rejuvenating, emmenagogue and aphrodisiac.

Datura gets its name from the Sanskrit word Dultura. It also known as thorn apple fruit. A perennial herbaceous plant in the *Solanaceae* family that can grows up to 3ft high. The leaves are about 10-20 cm long and 5-18 cm broad and covered with soft and greenish hair. Flowers are big, solitary and trumpet shaped with a sweet fragrance enjoyed in the morning and evening and come in variety of colour ranging from white to dark purple, insects pollinate the flowers, which are hermaphrodite. The fruit is capsule with shard spines on it. Datura can grow in average soil but it prefers rich moist soil or even very alkaline soil, and it doesn't do well in the shade. It's inflammatory properties it is one of the most widely used medicinal herb in the world (Harbone,1999). The entire plant especially the leaves and seeds it used to treat aesthetic, antispasmodic, anodyne, hallucinogenic, hypnotic and mydriatic complaints (Satyavati *et al., 1997;* Duke and Ayensu, 1984). The powdered leaves or seeds sometimes combined with cannabis and smoked in Indochina and Africa to relieve asthma, rheumatism (Richard ,1992;Satyavatiet *et al.,1997).* It is well known and commonly used in India for the treatment of epilepsy, hysteria, heart disease, insanity, fever with catarrh, cough, convulsion, diarrhoea skin disease etc (Chopra *et al.,*1986;Ngyyen and Doan,1989). Crusted *Datura* leaves have been used recently to relieve pain (Chopra *et al.,1986;* Ngyyen and Doan 1989)

With the advent in science and technology many natural and synthetic drugs have been discovered resulting in remarkable success in the field of medicine. Antibiotic are undoubtedly one of the most significant medicinal advances of the twentieth century with potent antibacterial properties. Antibiotic microorganisms have become immune as a result of continued use. In addition to this

problem antibiotic have been linked to host side effects such as hypersensitivity, immunosuppression and allergic reaction. This has resulted in a slew of clinical issue with infectious disease treatment. Screening local medicinal plants for antimicrobial properties is one process. Plant materials are also an effective tool in the fight against serious disease around the world. Plant have been observed developing a number of compounds to protect themselves from pathogens. Plant extract with target sides other than those used by antibiotics are expected to be effective against drug resistant pathogens for thousand of years and in many part of the world. Medicinal plant have been used as traditional medicines for a variety of human disease. As a result , it is critical to expand medicinal plant research in order to access their future applications. Therefore the current study aimed to validate the phytochemical screening, antibacterial and anthelmintic activity of two medicinal plants from the *Solanaceae family* (*Solanum xanthocarpum* and *Datura metel*) .

CHAPTER - II

SCOPE AND OBJECTIVES

Medicinal plants have started to consider an essential source in preventing a various kind of disease . Each plant consists of several important ingredients that can be used in medical field, and can be involved in the development of different kind of drugs. A lot of under developed countries or even developed countries are using herbal medicine in maintain human well being, personal health condition, and treading certain type of disease such as cough . Some plants species are considered as a weed, but they are also medicinally important too. In current era, the trend of using herbal medicine is in scope. New drugs are discovered by the invention of modern biotechnological and bio informatics techniques. In current era, herbal medicine are used more often as human believe in natural therapies is increase day by day. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of disease. Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agent. However it is essential to work on locally available resource to bring out their pharmaceutical values and antimicrobial in medicine. Hence the present investigation was undertaken with the following objectives.

- To identify the phytochemicals present in the leaf of *Solanum xanthocarpum* and *Datura metel.*
- Elucidating the effectiveness of medicinal plants (*Solanum xanthocarpum* and *Datura metel)*in controlling human pathogenic bacteria such as *Escherichia coli, Bacillus cereus, Vibrio cholerae* and *Staphylo cocus aureus*
- To evaluate the anthelmintic activity in different leaf extracts (benzene, chloroform, aqueous extract, ethanol, acetone) of *solanum xanthocarpum* and D*atura metel*
- To identify the functional groups present in benzene leaf extract of *Solanum xanthocapum* and *Datura metel* by Fourier Transform Infra-red Spectroscopy.

The results of the study also underline the cost effective, bio friendly resources which would be tapped for development of effective drug in futur

CHAPTER –III

LITERATURE REVIEW

Plants are a source of many potent and strong drugs that are used medicinally in various countries (Srivastva *et al*., 2008).In various traditional literature, a variety of herbs with significant antimicrobial activity have been published (Jones *et al*., 1996; Sathish *et al*., 1999).Ayurvedic traditions use a variety of medicinal plants on a regular basis. More than 7000 medicinal plants have been identified in medicine meets the primary healthcare needs of more than 80% of the world's population (Umamaheswari *et al*., 2008) Because of the lack and high cost of new generation antibiotics, researchers are turning to alternative medicines for anti micribial action (Poovendran *et al*.,2011).

DISTRIBUTION AND MEDICINAL VALUES OF PLANTS

Solanum surattense is a highly potent Solanaceae plant that is widely used as a medicine in Ayurveda. Its various plant parts (stem, leaves, root, and fruit) have remarkable antibacterial and antifungal properties against Gram positive and Gram-negative bacteria, as well as fungal pathogenic microbes. Also, its plant parts have, anthelmintic, anti-convulsant, antihyperlipidemic, anti-malarial, anti-urolithiasis, natriuretic, anti-ulcer, wound healing, anti-asthmatic, hypoglycemic, anti-oxidant, hepatoprotective activity and cytotoxicity. Chemical constituents such as alkaloids, saponins, steroids, tannins, flavonoids, glycosides, oleanolic acid, proteins, phenolic compounds, and many other amino acids have been extracted and reported using enriched proof. (Sahar, 2018)

Solanum L. of the Solanaceae family is a wide and diverse cosmopolitan genus of over 1500 species, making it suitable for wired taxonomy in perplexing tropical plant groups (Knapp *et al*., 2004). There are 90 genera in the Solanaceae family, with approximately 3000 species. (Knapp and Vorontsova, 2012) This cosmopolitan family is widely distributed between tropical and temperate regions, with Latin America and Australia serving as major dispersal centers (Barroso *et al.,* 1991) and Central and South America serving as diversity hotspots. The giant genus was described by (Frodin 2004). *Solanum* is one of the flowering plant genera with a large number of species.

The genus *Solanum* is divided into 13 major clades, with the spiny Solanums being the most common (the Leptostemonum clade) reflect the largest, with approximately 450 species spread across the globe (Knapp *et al.,* 2013). *Solanum* species can be found on both temperate and tropical continents and display a wide range of characteristics.

Solanum is an economically significant genus that includes well-known crop species such as *Solanum lycopersicum, Solanum tuberosum*, and *Solanum melongena*, as well as a variety of minor food crops. Secondary compounds in plants and animals that are poisonous or medicinally useful (Weese and Bohs 2007). The genus *Solanum* is well-known for its traditional and modern uses all over the world.

In Tripura, a total of 15 Solanum species have been described. The inhabitants make use of the majority of the species in some way. Among these, S. viarum has gotten a lot of attention. The world's attention was drawn to it because of its solasodine material, an alkaloidal steroid sapogenin (Chandra, 2008)

Datura stramonium L., a Solanaceae family wild-growing herb, is widely distributed and easily available. It contains a variety of toxic tropane alkaloids such as atropine, hyoscamine, and scopolamine. In Eastern medicine, especially in Ayurvedic medicine, *D. stramonium* has been used for curing various human ailments, including ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruises and swellings, fever, asthma and bronchitis, and toothache. *Datura stramonium* has long been recognised for its euphoric and hallucinogenic properties. For hallucinations and complete relaxation, the herb was dried and smoked. Also, treat to asthma, stomach issues, aches, abscesses, arthritis and used another ailment, boils, headaches, hemorroids, rattlesnake bites, sprains, swelling and tumours are some of the most common ailments. It was used as an ointment to alleviate rheumatism and sciatica symptoms, as well as to ease Parkinson's disease spasms. The leaves' juice in warm milk was used to remove intestinal worms, including cestodes, and seeds with palm oils were used to expel cestodes. In Nigeria, it is used externally to treat insect bites (Al-snafi 2017)

The insecticidal, herbicidal, antifungal, antibacterial, anti-cancer, anti-inflammatory, and anti-rheumatoid properties of *Datura metel* are well established. Alkaloidal compounds abound in Datura. The current paper summarises phytochemistry, common applications, and current research. *Datura metel* has pharmacological properties.

Datura metel L., also known as "Dhutura" in Bengali, is an erect shrub with spreading branches. It A perennial herbaceous plant, belonging to the Solanaceae family can reach a height of 1.5m. Leaves are simple, alternate, dark green, broadly ovate, shallowly lobed and glabrous. Datura can tolerate average soil but prefers soil which is rich and moist or even very alkaline soil but hardly survives under shade It prefers a warm temperature and is distributed in warmer regions of the world (Drake *et al.,* 1996).

Datura is most likely an American plant that is widely grown in both tropical and subtropical regions for its lovely flowers. (Glotter *et al.*,1973). *D. metel* can also be found in East Asia or India, and is used in traditional Bangladeshi herbal medicine. In Traditional Chinese Medicine, the flowers of *D. metel* are known as baimantuoluo and used for skin inflammation and Psoriasis (Wang *et al.,* 2008).

Datura metel (Solanaceae), also known as thorn's apple, Indian apple, or devil's trumpet, is a temperate zone annual herb that is found all over the world. The existence of massive chemical compounds such as flavonoids, tropane alkaloids, tannins, saponins, and withanolides was confirmed by *D. metel. Datura metel* has been found to be pharmacologically important species because of its different pharmacological and traditional uses, such as hepatoprotective, antiviraleffect, antibacterial effect, anti-asthmatic, analgesic, antipyretic and nephroprotective effect, anticancer and antifungal effect .

ANTIBACTERIAL ACTIVITY

Hossain *et al.,* 2014 studied the different crude extracts from the fruits of *Datura metal* were subjected to determination of total phenolics, flavonoids, antioxidant and antimicrobial activities by established method, ethyl acetate, extract was the most efficient (60.26%) compared to hexane, chloroform, butanol and methanol extracts which had phenolic content of 50.08, 35.50, 52.54 and 26.49% respectively. All extracts displayed moderate antibacterial potential against the tested bacteria.

Musfara Anjum and Hussain 2015 studied the leaves, stem, flowers and seeds of *Datura alba* Nees were extracted successively with various organic solvents. The ethanol, methanol, chloroform and acetone crude extracts of selected plant parts had significant antibacterial activities on both gram positive and gram-negative bacteria. Extracted methanolic and ethanolic extracts of leaves and flower of *D. alba* exhibited prominent activities against tested bacteria. The crude extracts of the selected plant parts were more active against gram positive bacteria than gram negative bacteria.

Sheeba 2010 studied the Ethanol extracts of *Solanum surattense* used in traditional medicine for treatment of various infections found to have antibacterial activity. The highest antibacterial activity was observed in 500µg concentration of leaf extracts of all bacteria screened except Shigella dysenteriae.

Monira *et al.,* 2012 investigated the crude aqueous and ethanol extracts of *D. metel* leaf, stem bark, and roots were tested against eight clinical bacterial strains. isolates (*Staphylococcus aureus, E. coli, Streptococcus betahemolyticus , Bacillus cereus, klebsiella)* The bark of the leaf and stem extracts were found to be antagonistic to the test Bacteria with inhibitory regions.

Udhaya kumar*et al.,* 2003 investigated the antibacterial activity of various parts of *Solanum xanthocarpum*(leaf , stem and fruit) using different solvents (petroleum ether, alcohol and acetone) against *E. coli, Salmonella typhi* and *Bacillus cereus*. *Solanum xanthocarpum* extracts found high sensitivity to *Klebsiella pneumoniae* and *Salmonella typhi,* moderate sensitivity to *Escherichiacoli* and less sensitivity and resistant to *Bacillus cereus*.

Nithya *et al.,* 2018 studied the methanolic extract of plant had a maximum quantity of phenol and flavonoids than others. The methanolic extract showed excellent antibacterial activity and exhibited the highest inhibitory effect against *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Escherichia coli* is compared to average minimum inhibitory concentration and minimum bactericidal concentration.

Gavimath *et al.,* 2012 studies the three plants belonging to family Solanaceae were evaluated for antimicrobial potentials against some selected pathogenic microorganisms. Five different solvents were used for extraction of different bioactive constituents from fresh leaves. The highest antibacterial activity was shown by methanolic and ethanolic extracts.

Kodura *et al.,* 2006 investigated the fruit and leaf extracts of *Solanum aculeastrum* for *in vitro* antimicrobial activity against 10 selected bacterial and 5 fungal strains. The methanolic extracts of both the fruits and the leaves showed appreciable activity against Gram-positive and Gram-negative bacteria. The water extracts showed the least activity against the bacteria. The methanolic extracts of both the fruits and the leaves showed appreciable activity against Gram-positive and Gram-negative bacteria. The methanol extracts were particularly inhibitory to the growth of the fungi.

Eftekhar *et al.,* 2005 investigated the antibacterial function of methanol extracts of *Datura innoxia* and *Datura stramonium* aerial sections. In a dose-dependent manner, the extracts showed activity against Gram (+) bacteria. Antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* was found to be limited.

Alabri *et al.,* 2014 investigated the antibacterial activity of different crude extracts from dry and fresh leaves of *Datura metel* determined by agar diffusion method. All organic crude extracts could be used as potential sources of new antioxidant and antibacterial agents. The methanol crude extract showed small and moderate antibacterial potential with one gram positive (*Staphylococcus aureus)* and three gram negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa)*

Gachande and khillare 2013 tested the various parts of four *Datura* plant species were examined for their potential antibacterial activity. In aqueous and ethanolic extracts against pathogenic bacteria such as *Bacillus subtilis, Escherichia coli Staphylococcus aureus and Proteus vulgaris*. The pattern of inhibition depends largely upon plant part used and organism tested.

Gomathi *et al.,* 2017 studied the Silver nanoparticles of 15–20 nm size with spherical shape were synthesized from green synthesis method using *Datura stramonium* leaf extract. Synthesized Ag NPs showed antibacterial activity against *E. coli* and *S. aureus* bacteria. FTIR analysis exhibits the possible reducing bio-molecules within the leaf extract.

Rao *et al.,* 2016 studied Silver nanoparticles are the metal of choice for anti-microbial agents. Phytochemicals extracted from roots of *datura metel* using n-hexane as solvent by soxhlet extract technique. Nanoparticles have potential antibacterial activity against *Pseudomonas aeruginosa andE. coli*.

Abbas Khizar *et al*., 2014 investigated antimicrobial activity of fruit extracts of two *Solanaceous* plants found in Pakistan. The highest percentages of polar components of both the species were extracted by water. Antimicrobial activities were estimated by measuring zones of inhibition through hole-plate diffusion method. Doses of 5, 10 and 15 mg/mL prepared through methanolic extracts of each plant displayed significant inhibition against all three Gram positive bacteria.

Nithya *et al.,* 2018 studied the methanolic extract of *Solanum xanthocarpum*had a maximum quantity of phenol and flavonoids. The methanolic extract showed excellent antibacterial activity and exhibited the highest inhibitory effect against *Pseudomonas aeruginosa*. GC-MS analysis revealed the presence of six major bioactive compounds. This study suggests *S. xanthoarpum* as a potential candidature for having better antibacterial and antioxidant property.

Parmar *et al., 2010* reported the antibacterial activity to be potentially toxic, glycoalkaloids and hydrolysis products without the carbohydrate side chain also have beneficial effects. These include lowering of cholesterol, protection against infection by *Salmonella* and against cancer.

Devi *et al*., 2015 tested the antibacterial activity of *Solanumvirginianum*. It was found that the aqueous extract of *S. virginianum* inhibited the growth of bacterial pathogens. The most susceptible Gram-negative bacterial pathogens were *Salmonella typhi* leaf (2.5 cm), stem, root, and fruit (1.4 cm), and *Escherichia coli.*

PHYTOCHEMICAL ACTIVITY

Tekuri*et al.,* 2019 reported that the medicinal plants are unique in having the ability to produce diverse chemical compounds. *Solanum surattense* is

widely used in the traditional medicine Phytochemical compounds from different plant parts like roots, stem, leaves, fruits, and seeds reported to possess a wide range of pharmacological activities. Intensive investigation on phytochemical constituents resulted in isolation of alkaloid and steroidal compounds solasonoine, solamargine, campesterol, and diosgenin.

Monira *et al.,* 2012 studied the phytochemical action of many different Alkaloids are found in the whole plant of *Datura*, which increased gradually with increase in age of the plant. Main constituents are a huge number of tropane alkaloids and various trigloyl esters of tropine and pseudotropine. The root contains higher amount of atropine compared to the other parts.

Kalbar *et al.,* 2019 studied the Sequential phytochemical extraction of the shade dried fruits was carried out using four different solvent (petroleum ether, chloroform, water and methanol). The qualitative phytochemical analysis of methanol and water extracts showed the presence of alkaloids, flavonoids, terpenoids, steroids tannins, saponins and glycosides. This study indicates that *Solanum macranthu*m fruit possesses various bioactive phytoconstituents and low ash value. The ash values obtained signified low amount of inorganic constituents.

Kalita, Lawrance *et al.,* 2017 studied the species of *Solanum torvum* (family-*Solanaceae*) was found to possess promising antimicrobial activity when compared with the standards. All the extracts of the plant showed. the highest presence of phytochemicals. It can be concluded that the extract of whole aerial parts of the *Solanum torvum*contain the high presence of. phytochemistry.

Singh *et al.,* 2010 studied the phytochemical screening of *Solanumxanthocarpum*contains alkaloids, phenolics, flavanoids, sterol, saponins and their glycosides, and has a wide range of medicinal values. This review presents chemical constituents of *S. xanthocarpum*and its biological activities.

Kumar *et al.,* 2012 investigated the free radicals have been implicated in many diseases. They attack biological macromolecules in healthy human cells and cause protein and DNA damage. *Solanum xanthocarpum*root extracts

exhibited significant free radical scavenging activity. Antioxidant activity of the extracts was compared with standard antioxidants and could be attributed to the presence of other phytochemicals like tannins and terpenoids.

Gogoi and Islam, 2012 studied the scientifically validates the use of both species in traditional medicine. Protein is more abundant on *Solanum nigrum* L of shady areas than *S. myriacanthus* of dry areas. Other phytochemical constituents such as alkaloids, saponins, tannins, flavonoides are more or less presence in both species.

Al-snafi and Ali Esmail2017 investigated the methanolic and hydroalcoholic extract of *Datura fastuosa (*syn*: Datura metel*) revealed the presence of alkaloids, tannins, cardiac glycosides, flavonoids, carbohydrates, amino acids and phenolic compounds, while, phytochemical analysis of *Datura stramonium* showed that it contained alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides. The current review highlights the chemical constituents and pharmacological effects of *Datura fastuosa* and *Datura stramonium*.

Dhawan and Jeena Gupta, 2017 studied the bioactive components present in the *Datura metel* plant are known to be responsible for its medicinal properties, to cure many diseases like asthma and bronchitis. The extraction method for these bioactive components is not yet standardized. This study compared the effect of using different extraction solvents to extract the active components like alkaloids, flavinoids, saponins, steroids and tannins from the dried leaves. The results show that using methanol as an extraction solvent works best.

Khan and Nasreen 2010 studied the phytochemicals and quantification of alkaloids, phenolic compounds and flavonoids and to evaluate the nematicidal activity of ethanolic leaf extracts of *Datura*. Extracts of *Daturta metel ,Datura innoxia* and *B. suaveolens* were studied against the root knot nematode M. incognita. Leaf extracts of *D. metel*, *D. innoxia* and *B. suaveolens*were found to contain phytochemicals. Plants exhibited nematicidal activity against root knot nematode *M. incognita*. Study may be useful for the identification and isolation of novel active compounds from the leaf.

Nanda kumar *et al.,* 2017 studied the phytochemicals analysis of alkaloids, phenolic compounds and flavonoids. The nematicidal activity of extracts of *Datura. metel, D. innoxia* and *B. suaveolens* were studied against the root knot nematode *M. incognita.* The present study may be useful for the identification and isolation of novel active compounds from the leaf.

Devi *et al.,* 2015 studied the phytochemical analysis of *Solanum virginianum* belongs to the family *Solanaceae.* The objective of the present study was to scientifically evaluate typhoid potential of *Solanum virginianum*. Phytochemicals present in leaves, stem, roots and fruit of *Solanum virginianum* was studied in the current study by biochemical tests. It was found that various phytochemical was present in high proportion alkaloids, terpenoids, glycosides, flavonoids, saponins, coumarins, tannin, proteins and amino acids.

ANTHELMINTIC ACTIVITY

Gunaselvi *et al.,* 2010 studied the various extracts from the fruits of *Solanum xanthocarpum* are prepared and out of it methanolic and aqueous extracts of the fruits shown the amazing anthelmintic activity of parasite.

Nayak *et al.,* 2009 studied the species of *Solanum surattense* Linn is found in the tribal area of Koraput district and extensively used traditionally by the tribal people. The various doses of aqueous and ethanolic extracts were evaluated for their anthelmintic activities on adult Indian earthworms, *Pheretima postuma*. All extracts of both the solvent were able to show anthelmintic activity at 10 mg/ml concentration.

Zebsaddiqe and sabakhalid, 2013 studied the crude methanol extract and subsequent solvent fractions of *Solanum nigrum L. (Solanaceae*) were evaluated for anthelmintic activity against sheep intestinal worms *Haemonchu scontortus*. Extracts were also evaluated for total phenolic and total flavonoid contents using colorimetric methods.

Yadav *et al.,* 2012 studied the species of *Solanum myriacanthum* is a perennial shrub used in the folk medicine of Tangkhul Naga tribe of India for treating intestinal worms. This study evaluates the anthelmintic activity of its ripe fruit extract. A single oral dose of 800 mg/kg of extract showed 60.49% reduction in the EPG counts and 56.60% reduction of worm counts in the extract-treated group. The effects of the extract were more apparent on the adult stages than larval or immature stages.

Gupta *et al.,* 2013 studied the *Solanum xanthocarpum* (*Solanaceae)* is distributed all over India and has been used by triable as well a local people widely. Alcoholic and aqueous extracts of *Solanum xanthocarpum* were evaluated for its anthelmintic activity against adult earthworms, *(Pheretima postuma).* The activities of the extracts were compared with standard Albendazole. The alcoholic and aqueous extract showed significant anthelmintic activity and it was found that the aqueous extract activity is higher than alcoholic extract.

CHAPTER- IV

MATERIALS AND METHODS

Collection and processing of the plant materials

The leaves of *Solanum xanthocarpum* and *Datura metal* were collected from in and around Thoothukudi district in TamilNadu, during December 2020 to January 2021.The leaves were collected, then washed carefully with water to remove dust and foreign materials. The leaves were dried under shade and coarsely powdered.

Preparation of extracts:

10 grams powdered sample was sequentially extracted with 200 ml of benzene , acetone, ethanol, chloroform and aqueous solution using in soxhlet apparatus. The prepared extracts were tested for phytochemical screening, antibacterial, FTIR and antihelmintic activities.

Plate 1: ***Solanum xanthocarpum***

Class	:	Dicotyledons
Sub class	:	Gamopetalae
Series	:	Bicarpellatae
Order	:	Polemoniales
Family	:	Solanaceae

Description:

The plant body stout herb or shrubs, sometimes subscandent , rarely small trees, unarmed or prickly leaves are alternate or sub opposite ,entire lobed or pinnatified. Flowers in dichotomous or racemose,lateral or terminal cymes. Calyx-lobes not abruptly acute; Flowers in few flowered cymes,blue –purple ; Leaves ovate or elliptic, acute,pinnatifid half –way down, Sometimes only sinute ,upto 4 in long 2 in broad ,sparsely stellate –pubescent to nearly glabrous.Prickles very numerous ,yellow,straight,often 5 in long ,on stems,leaves and calyx ;berry globose, yellow or white with green blotches.5-75 in diameter;Seeds are small smooth . Fruiting sepals are prickly sparsely pubescent berry pale yellow 1.3-2.2 cm in diameter. Flowering wormly appears around November and May.

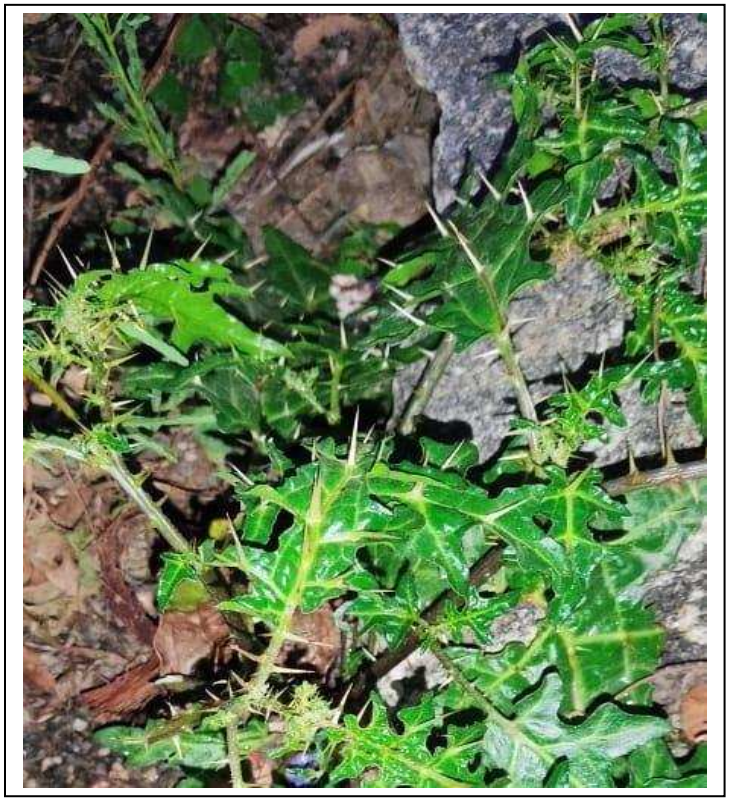


Plate 2 : *Datura metel*

Description:

The plant body stout herb or short lived shrub, usually circa 1m in height,occasionally as much as 2m. Roots are branched tap root system. Stem hellow,green or purple – black,some woody ,with a strong odour. Leaves are large , purple or white ,solitary,eract or pendulous . Calyx long tubular, herbaceous,5- lobed , in fruit the upper part deciduous leaving the circumsciss base . Corolla long tubular,funnel – shaped,the mouth wide; limb platted,entirely or shortly 5 or 10 lobed.Stamens 5 attached near the base of the tube;filaments filliform;anthers included, linear,longitudinally dehiscing ovary 2 or spuriously 4 celled; style filliform;stigma 2-lobed fruit an ellipsoid spinescent 4 celled capsule ,4 volved or irregularly bursting near the apex seeds very many, compressed, rugose, embryo peripheric .Capsule covered with long slender spines;Flowers are white tinged with green ,the fruiting calyx very large , leaves ovate –lanceolate,acute base very unequal;upto about 4 in .long or more,minutely grey –tomentose.

Phytochemical qualitative analysis:

The phytochemical tests were done for analysising different chemical groups present in the extracts.These were done to find out the presence of bioactive chemical constituents such as alkaloid, flavonoids, tannis, phenol, terpenoids, glycosides, cardiac glycosides, anthroquinone, steriods 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:-

Mayer's 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,1 ml of 10% 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:-

$Fecl_3$ Test:-

About 2 ml of plant extracts was taken and warmed at 45-50^{0}c.Then 2ml 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 H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Glycosides:-

2ml of extract was dissolved in chloroform and 2 ml of acetic acid was added to the mixture. The 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:-

Keller-killiani Test:-

1ml of extract was dissolved in 5 ml of water . 2ml of glacial acetic acid containing one drop of ferric chloride solution was added.This 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 forAnthraquinone:-

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.The chloroform layer was pipetted into another test tube followed by addition of 1 ml of dilute ammonia.The resulting solution was observed for colour changes to violet indicates presence of anthroquinone.

Test for Steroids;-

Sal kowski 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.

ANTIBACTERIAL ACTIVITY

Bacterial strains used

The test organisms were obtained from the Department of Microbiology, St. Mary's 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 cholerae* were used in the present study. Bacillus subtilisis responsible for causing food borne gastroententis. E.coli, S*taphylococcus 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 was evaluated by agar disc diffusion method. Test solutions were preparedwith 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 ul of these extracts and allowed to dry 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 37^0 c incubation, the diameter of the inhibition zone was measured. For positive control, amphicillin disc (100ug/ml) was used, whereas for negative control, respective solvents were loaded on the sterile disc.

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

(Vijayabaskar and Shiyamala, 2012)

Ten milligram of *S. xanthoocarpum* and *D. metel* leaf powder was mixed with 100 mg of dry potassium bromide (FT-IR grade) and then compressed into a pellet using hydraulic press (5000 -10000 psi). The pellet was immediately put into the sample holder and FT-IR (Systronics 166) spectra were recorded in the range of 400 to 4000 cm^{-1}.

ANTHELMINTIC ACTIVITY:

This antibacterial activity assay was carried as per the method followed by Islam *et al.,* 2015 with minior modification.

Preparation of extracts:

Dried powder of (*Solanum xanthocarpum* and *Datura metel*) 10 (Grams) was extracted with 200 ml of various type of solvent extracts (acetone,ethanol,chloroform,benzene, and aqueous extract).The filtrate was collected and concentrated till a syrupy mass was obtained and dried at room temperature.The dried extracts were dissolved in normal saline and used for anthelmintic activity.

Experimental animal:

Anthelmintic activity was performed on adult earth worm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round warm parasite of human beings(Chatterjee 1967). The Indian adult earthworms were collected from moist soil of the field and washed with normal water and saline solution to remove soil and faecal matter.Earthworms were identified from the Department of Zoology, St. Mary's College (Autonomous), Thoothukudi.The earthworms of 10-15cm in length and 0.22-0.3cm in width were used for all experimental parameters.

Experimental design:

In the present investigation the earthworms were divided into the following 13 groups. Each group consists of 6 earthworms.

Group I : Earthworms were placed in normal saline and served as control.

Group II : Earthworms were placed in standard drug albendazole at the dose of 25mg/ml served as standard.

Group III : Earthworms were placed in standard drug albendazole at the dose of 50mg/ml served as standard.

Group IV : Earthworms were placed in ethanol extract of *Solanumxanthocarpum* and *Datura metel* at the dose of 25mg/ml.

Group V : Earthworms were placed in ethanol extracts of *Solanum xanthocarpum* and *Datura metel* at the dose of 50mg/ml.

Group VI : Earthworms were placed in benzene extracts of *Solanum xanthocarpum* and*Datura metel* at the dose of 25mg/ml.

Group VII: Earthworms were placed in benzene extracts of *Solanum xanthocarpum* and *Datura metel* at the dose of 50mg/ml.

Group VIII : Earthworms were placed in chloroform extracts of *Solanum xanthocarpum* and *Daturametel* at the dose of 25mg/ml.

Group XI : Earthworms were placed in chloroform extracts of

Solanum xanthocarpum and *Datura metel* at the dose of 50mg/ml.

Group X : Earthworms were placed in acetone extracts of *Solanum xanthocarpum* and *Datura metel* at the dose of 25mg/ml.

Group XI : Earthworms were placed in acetone extracts of *Solanum xanthocarpum* and *Datura metel* at the dose of 50mg/ml.

Group XII : Earthworms were placed in aqueous extracts of *Solanum xanthocarpum*and *Datura metel* at the dose of 25mg/ml.

Group XIII : Earthworms were placed in aqueous extracts of *Solanum xanthocarpum* and *Datura metel* at the dose of 50mg/ml.

CHAPTER -V

RESULT AND DISCUSSION

Phytochemical:

Phytochemical constituents in plant samples are considered to be biologically active compounds with a variety of functions including antioxidant, antimicrobial, antifungal, hypoglycaemic, anti-diabetic, anti inflammatory, anticarcinogenic, antimalarial, anticholinergic properties. (Hossain and Nagooru, 2011, Suresh and Nagarajan, 2009). The phyto chemical analysis of different leaf extracts (ethanol, acetone, chloroform, benzene and aqueous extract) of *Solanum xanthocarpum* and *Datura metel* were found to contain alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and anhraquinone (Table1&2). Alkaloids were found in various alcoholic and aqueous leaf extract of *D. metel* but were absent in ethanolic and aqueous extract of *S. xanthocarpum*. 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. (Kam, 2002). However, phenols were detected in ethanolic, chloroform and benzene extracts of both plants and the cardiac glycosides and glycosides were found in leaf extracts of the solvents chloroform, acetone, ethanol and benzene. Flavonoids are powerful antioxidants and free radical scavengers that protect cells from oxidative damage (Salah *et al*., 1995). Ethanol and benzene leaf extracts of both plants showed the presence of flavonoids. All extracts of both plants contained terpenoids and tannins. Tannins react with proteins to produce the tanning effect, which is beneficial in the treatment of inflamed or ulcerated tissues. (Parekh and Chanda, 2009). Herbs are those that contain tannins as one of their main components and are used to treat intestinal problems like diarrhoea and dysentery.(Dharmananda and Gallnuts, 2003). Leaf extracts of *D. metel* revealed the presence of anthraquinone and steroid. In *S. xanthocarpum* saponin content was absent in ethanol, chloroform and aqueous water whereas, saponin wanti-inflammatory, anti-hepatotonic, wound healing, veinotonic, expectorant, spasmolytic, hypoglycemic, antimicrobial, and antiviral properties (Rahaman Onike, 2010). Benzene leaf extracts had a higher number of secondary metabolites with a higher degree of precipitation than all other solvent extracts. Aqueous leaf extracts of both plants showed the less variety of these secondary metabolites. Alkaloids, glycosides, tannin, phenols, flavonoids, steroid and saponin are these secondary metabolites found in both plants. They have a high therapeutic value and are commonly used in the pharmacy and drug industrie

Table 1: Preliminary phytochemical analysis of *Solanum xanthocarpum* leaf (+ : present; - : absent)

Phytochemical analysis of *Solanum xanthocarpum* extracts						
S.NO	Phytochemical	Extracts				
		Ethanol	Acetone	Chloroform	Benzene	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 *Datura metel* leaf (+ :present ; - : absent)

Phytochemical analysis of *Datura metel* extracts						
S.NO	Phytochemical	Extracts				
		Ethanol	Acetone	Chloroform	Benzene	Aqueous
1.	Alkaloids	+	+	+	+	+
2.	Flavanoids	+	+	_	+	+
3.	Tannins	+	+	+	+	+
4.	Phenols	+	_	+	+	+
5.	Terpenoids	+	+	_	+	+
6.	Glycosides	+	+	+	+	_
7.	Cardic glycosides	_	+	_	+	+
8.	Anthroquinone	+	+	_	+	+
9.	Steriods	+	+	+	+	+
10.	Saponins	_	+	+	+	_

Antibacterial activity of *Solanum xanthocarpum* and *Datura metel:*

In the present study, antibacterial activity of two medicinal plant leaf extracts (*Solanum xanthocarpum* and *Datura metel*) of five different solvents (ethanol, acetone, benzene, chloroform and aqueous) were tested against four human pathogenic bacteria (*Escherchia coli, Bacillus cereus, Staphylo coccus, Vibrio chlorea)* and were presented in table (3 to 4). The diameter of the inhibition zones against these species ranged from 7 to 30 mm. The study revealed that all extracts inhibited the growth of all the pathogens tested. As shown in Table 4, Benzene and acetone extracts of *Datura metel* exhibited maximum activity against different bacterial species, *E. coli* and *Vibrio chlorea* (30 mm).Similarly ethanol, acetone and benzene extract of *Datura metel* inhibited the growth of *E. coli, Bacillus cereus* and *Vibrio chlorea* by showing 27 mm of inhibition zone. The moderate sensitivity was noted in ethanol, chloroform and acetone extract in all pathogens tested and the inhibition zone is ranged between 20 and 25 mm. The acetone extract of *Datura metel* showed less sensitivity and resistant to *staphylo coccus* (8 mm). Sundaram Ravikumar *et al*., (2010) investigated the antibacterial and antifungal efficacy of chloroform extracts of seventeen different coastal medicinal plants against gram positive and gram negative bacteria, as well as fungal ornamental fish pathogens *in vitro*. *Datura metel*, one of the plants chosen, had a wide range of antimicrobial activity against a variety of fish pathogens. He came to the conclusion that *Datura metel* has been used as a potential antimicrobial drug in aquaculture. *E. coli, Bacillus cereus* and *V. chlorea* was seemed to be more sensitive to ethanol, aqueous and chloroform extract of *S. xanthocarpum* in comparison with *S. aureus* and the inhibition zone is 25 mm. According to Prasanna and Raghunathan (2014), in most cases the ethanol extract exhibited higher antibacterial effects than the corresponding extracts. However, acetone extract of *S. xanthocarpum* exhibited less inhibitory activity against S*taphylo coccus* and the zone of inhibition is 7 mm (Table 3). The high antibacterial activity in the leaf extract of both plants may be due to the presence of tannins, flavonoids and terpenoids. Antimicrobial 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 (Mamtha, 2004). Saponins have the potential to induce protein leakage (Zablotowicz *et al.,*1996). Terpenoids cause the breakdown of a microorganism cell wall by compromising the membranous tissue (Hemandez *et al.,* 2000). 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 (Marjorie, 1999). Steroids are also known for their antibacterial activity, which is related to membrane lipids and induces liposome leakage (Epand *et al*., 2007). It is observed that the leaf content has more phytochemicals as compared to other plant parts (Jamdhade *et al.*, 2010). Therefore it is concluded that leaf extracts of *D.metel* and *S. xanthocarpum* used in the present study could be effectively processed to be utilized as a source for antibacterial therapeutic drug preparations.

Table 3: Antibacterial activity of different solvents extracts of *Solanum xanthocapum*

Concentration of *S.xanthocarpum* extracts (5 mg/ml) Diameter zone of inhibition(mm)						
Bacterial cultures	streptomycin	Chloroform	Benzene	Ethanol	Acetone	Aqueous extract
Eschercia coli	15mm	23mm	20mm	25mm	9mm	21mm
Vibrio chlorae	25mm	25mm	15mm	24mm	23mm	15mm
Bacillus ceresus	30mm	20mm	20mm	16mm	10mm	25mm
Staphylo cocus aureus	20mm	13mm	8mm	9mm	7mm	21mm

Control - Streptomycin (5 mg/ ml)

Leaf extract - (5 mg/ml)

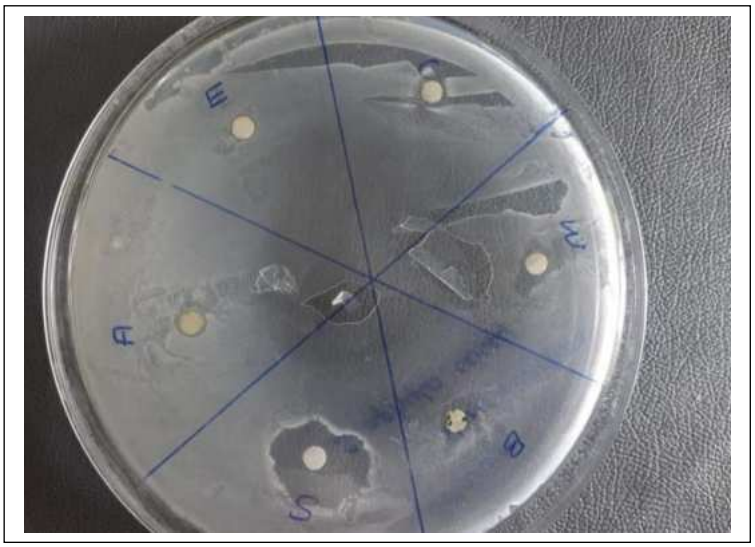
Table 4: Antibacterial activity of different solvents extracts of *Datura metal*

Concentration of *D.metel* extracts(5mg/ml) Diameter zone of inhibition(mm)						
Bacterial cultures	Streptomycin	Chloroform	Benzene	Ethanol	Acetone	Aqueous extract
Eschercia coli	22mm	20mm	30mm	27mm	25mm	25mm
Vibrio chlorae	25mm	20mm	27mm	20mm	30mm	25mm
Bacillus ceresus	17mm	25mm	15mm	25mm	20mm	15mm
Staphylo cocus aureus	20mm	20mm	8mm	25mm	27mm	10mm

Control - Streptomycin (5 mg/ml)

Leaf extract - (5 mg/ml)

Plate 3 : In vitro antibacterial activity of *Solanum xanthocarpum* leaf against human pathogens



Solanum xanthocarpum leaf extract against *Staphylococus*

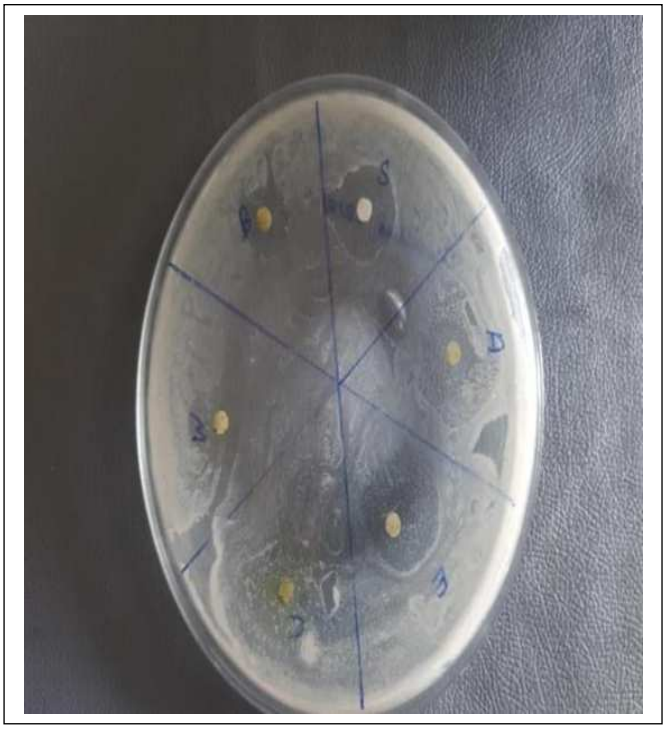
Solanum xanthocarpum leaf extract against *E.coli*

Solanum xanthocarpum leaf extract Against *Vibrio chlorae*

Solanum xanthocarpum leaf Extract against *Bacillus cereus*

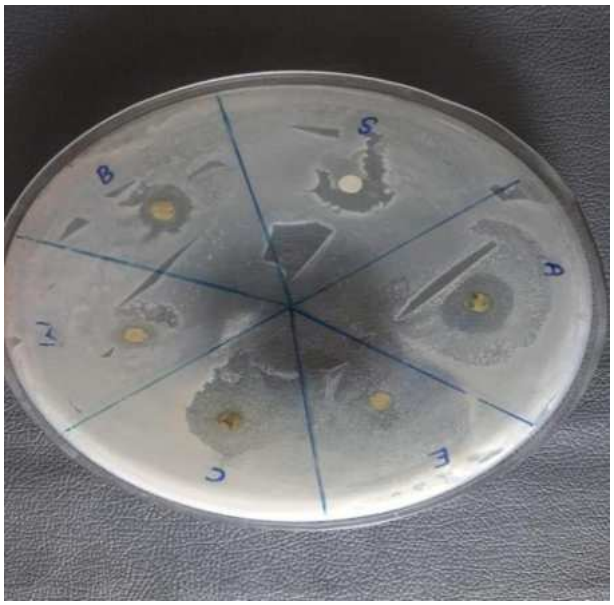
Antibacterial activity is revealed as clear zone around the disc and is represented as zone of E- Ethanol, B- Benzene, C- Chloroform, A-Acetone , A- Aqueous extract

Plate 4 : *In vitro* antibacterial activiy of *Datura metel* leaf against human pathogens



Datura metel leaf extract against *Staohylo coccus*

Datura metel leaf extract against *E. coli*



Datura metel leaf extract against *Bacillus cereus*

Datura metel leaf extract against Vibro chlorae

Antibacterial activity is revealed as clear zone around the disc and is represented zone of inhibition .E-Ethanol,B-Benzene,A-Acteone,C-Chloroform,A-Aqeuous extract of plants (2.5mg/ml). Sterptomycin(100µg/ml)-positive control.

Fourier Transform Infra - red Spectroscopy analysis:

Fourier Transform Infra - red Spectroscopy measurement spectrum were carried out to identify the possible biomolecules responsible for the antimicrobial properties. The FTIR spectra to medicinal plant were depicted in fic 1 &2. The representative spectrum of *Solanum xanthocarpum* shoewed absorption peaks located at 3640. 62, 3034. 12, 2975. 39, 2850. 55, 2548. 70, 1803. 83, 1690. 34, 1415. 62, 1252. 51, 1169. 13, 889. 19 and 749. 64 corresponds to biomolecules such as Primary alcohol (variable), Aromatic Methane (medium), Vinyl terminal (medium), Methoxy (medium), Acids (medium), Acid Peroxides (very strong), Aromatic acid (very strong), Vinyl terminal (medium), Tertiary butyl (strong), Isopropyl (strong), Cycloalkanes (medium), Alkanes (strong). FTIR spectrum of *Datura metel* showe peaks at 3620. 14, 3451. 28, 2981. 51, 2521. 21, 1815.73, 1584. 39, 1120. 07, 735.11 and 650.34 cm-1. Showing the presence of C-O Tertiary alcohol (variable), N-H Primary amines (weak to medium), C-C Cycloalkanes (medium), Acids (medium), O-O Acid peroxide (very strong), N-H Secondary amines (weak), N-H Secodary amines (weak to medium), H-N-H Ortho substituted (very strong) and Aromatic methane (strong). Amines, amides and amino acids are the main groups of protein synthesis and herbs serve as herbal oil and hair tonic. The result of the present study lead to conclude that compound such as amines, aromatic acid, methane, cycloalkanes etc., present in both pants were responsible for the antimicrobial proberties exhibited by respective plants

Figure : 1 FTIR spectrum of *Solanum Xanthocarpum*

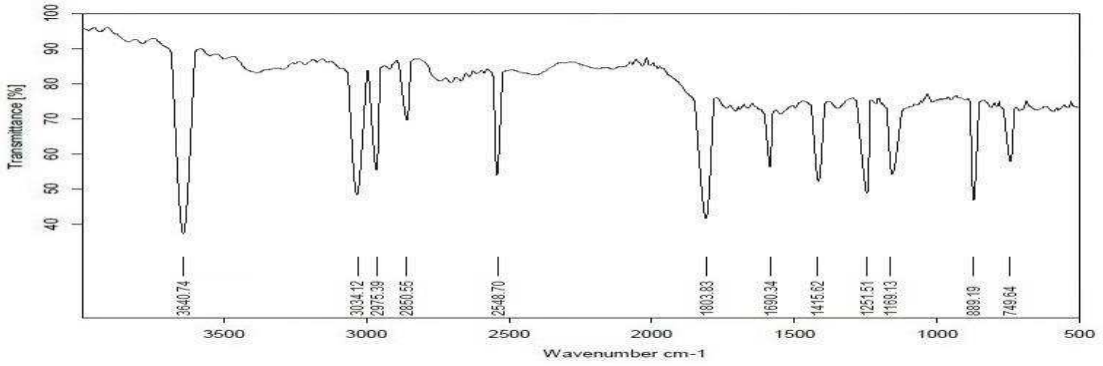


Table 5: Interpretation

FTIR spectral qualities interpretation of the comparative shift in functional peaks of critical value (S*olanum xanthocarpum*)

S.No	Absorption frequency (cm^{-1})	Intensity
1	3640.74	Primary Alcohol (variable)
2	3034.12	Aromatic Methane (Medium)
3	2975.39	Vinyl terminal (Medium)
4	2850.55	Methoxy (Medium)
5	2548.70	Acids (Medium)
6	1803.83	Acid Peroxides (Very strong)
7	1690.34	Aromatic acids (very strong)
8	1415.62	Vinyl terminal (Medium)
9	1252.51	Tertiary butyl (Strong)
10	1169.13	Isopropyl (strong)
11	889.19	Cycloalkanes (Medium)
12	749.64	Alkanes (Strong)

Figure : 2 FTIR spectrum of *Datura metel*

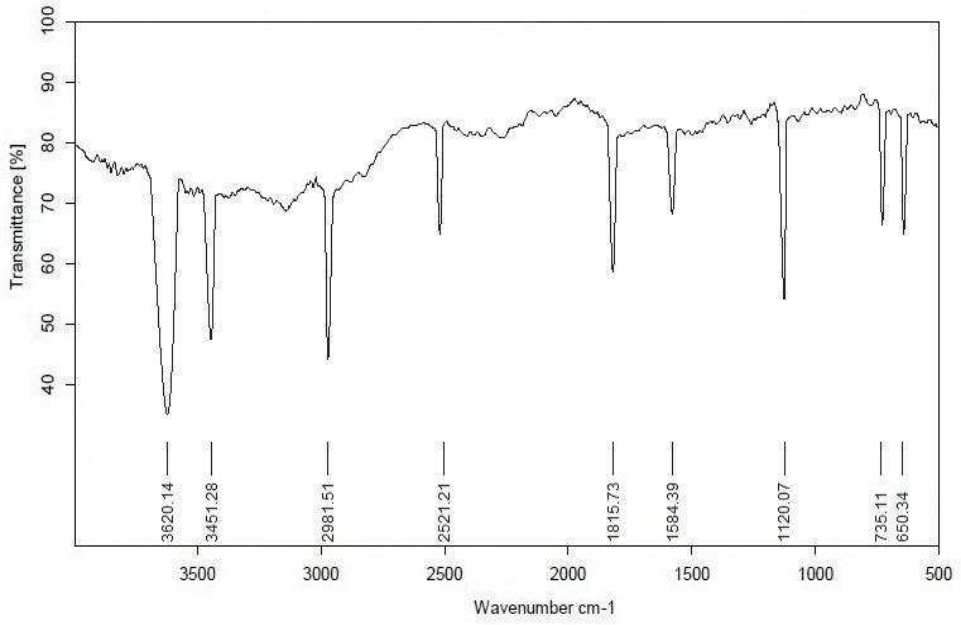


Table 6: Interpretation

FTIR spectral qualities interpretation of the coomparitive shift in functional peaks of critical value (*Datura metel)*

S.No	Absorption frequency (cm^{-1})	Intensity
1	3620.14	Tertiary alcohol (variable)
2	3451.28	Primary amines (weak to medium)
3	2981.51	Cycloalkanes (Medium)
4	2521.21	Acids (Medium)
5	1815.73	Acid Peroxides (Very strong)
6	1584.39	Secondary amines (weak)
7	1120.07	Secondary amines (weak to medium)
8	735.11	Ortho- substituted (Very strong)
9	650.34	Aromatic Methane (Strong)

Anthelmintic activity

Anthelmintic drugs are known to work by paralyzing worms or destroying their cuticle, causing partial digestion or injection through the immune system. It also disrupts worm metabolism, as the metabolic needs of these parasites differ greatly from one species to the next (Aisawanya *et al*., 2010). Albendazole has been shown to have an effect on worms by destroying the worm's cytoskeletal system, resulting in paralysis (Nikesh *et al*., 2011). Ethanol, acetone, benzene, chloroform and aqueous extract of *S. xanthocarpum* and *D. metel* leaves were used for anthelmintic activity. *Pheretima posthuma* worms can be successfully used for anthelmintic activity research because they are simple, visible, adaptable to laboratory conditions, and reproducible in all aspects, such as worm age, size, and weight (Murugamani *et al*., 2012). In the present anthelmintic activity study, when the time of paralysis and time of death of earthworms were compared between plants extract and standard, the results showed that the time taken for paralysis and death is more closely to standard. Five extracts were tested at 25 mg/ml and 50 mg/ml concentrations and showed significant results that were similar to standard and some of the extracts take less time to paralysis and death. Among two plant extracts *D. metel* (25 mg/ml and 50 mg/ml) was showed higher significant performance (Table 8) compared to *S. xanthocarpum* (25 mg/ml and 50 mg/ml) (Table 7). Acetone, chloroform and ethanol leaf extract of *D. metel* (50 mg /ml) was taken 5 minutes to bring paralysis and 10 minutes to bring death of worms. Acetone and chloroform leaf extracts of *S. xanthocarpum* is showing paralysis at 5 minutes and death of worms at 17 minutes while death is comparable with that of albendazole as death of worms was observed at 17 minutes. The secondary metabolites identified in *S. xanthocarum* and *D.metel* during the qualitative phytochemical screening (**Table 1and 2**) may be responsible for screened anthelmintic activity (**Table 7 and 8**) and could be promising alternative approach to control helminth infections (Bauri *et al*., 2015; Sutthaya Poolperm and Wannee Jiraungkoorskul, 2017). Alkaloids have been confirmed to have neurotoxic effects, causing worm paralysis by affecting acetylcholine-stimulated body wall muscle contraction and acting as an acetylcholinesterase inhibitor. Because glycosides have an antiparasitic effect due to their neurotoxic potential, low concentrations of glycosides in plant materials, when ingested by humans, can contribute to the killing of gastrointestinal worms through its toxic effects (Jain *et al*., 2013; Velebny, 2013). Phenol and tannins (phytoconstituents) are the active ingredients in anthelmintics. Tannins can bind to free proteins in the host

animal's gastrointestinal tract or glycoprotein on the parasite's cuticle (earthworms) and cause death (Kane, 2009; Gnaneswari *et al*., 2013). Flavonoids found in this study can inhibit larval growth and arachidonic acid metabolism, which can lead to neurodegeneration in the worm's body and death. (Sutthaya Poolperm and Wannee Jiraungkoorskul, 2017). Saponin works as an anthelmintic by inhibiting the enzyme acetyl cholinesterase, which causes worms to become paralyzed and die. They have an effect on the permeability of worm cell membranes and can irritate the gastrointestinal mucous membrane channel of worms, interfering with food absorption. (Melzig *et al*., 2001). The wormicidal activity is due to the presence of alkaloids, glycosides, flavonoids, saponin, phenol and tannin content in both plants. Further, in future it is necessary to identify and isolate the possible active phytoconstitutents responsible for the anthelmintic activity.

Table 7: Anthelimintic activity of various extracts of S*olanum xanthocarpum*

Treatment	Group	Concentration Mg/ml	Time taken Paralysis(min)	Time taken For death(min)
Control (saline)	I	-	-	-
Standard (albentazole)	I	50mg/ml	15	17
	II	25mg/ml	20	25
Ethanol	I	50mg/ml	18	23
	II	25mg/ml	25	25
Benzene	I	50mg/ml	10	24
	II	25mg/ml	15	35
Chloroform	I	50mg/ml	5	17
	II	25mg/ml	15	20
Acetone	I	50mg/ml	8	25
	II	25mg/ml	15	30
Aqueous extract	I	50mg/ml	15	29
	II	25mg/ml	30	45

Table 8:Anthelimintic activity of various extract of *Datura metal*

Treatment	Group	Concentration Mg/ml	Time taken Paralysis	Time taken for Death
Control (saline)	I	-	-	-
Standard (Albentazole)	I	50mg/ml	10	15
	II	25mg/ml	15	25
Ethanol	I	50mg/ml	5	10
	II	25mg/ml	12	19
Benzene	I	50mg/ml	10	15
	II	25mg/ml	19	25
Chloroform	I	50mg/ml	5	10
	II	25mg/ml	8	15
Acetone	I	50mg/ml	5	10
	II	25mg/ml	12	20
Aqueous extract	I	50mg/ml	15	20
	II	25mg/ml	25	45

Plate 5: Invitro* antihelminthic activity of *Solanum xanthocarpum

Control - normal saline

Ethanol extract of *Solanum xanthocarpum* leaf *(50*mg/ml)

Aqueous extract of *Solanum xanthocarpum* Leaf (50 mg/ml)

Standartd drug Albendozole (50 mg/ ml)

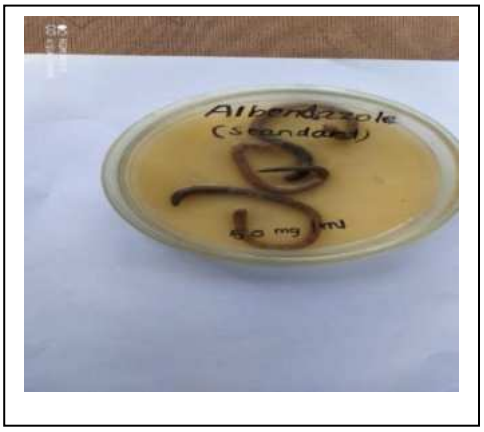
Benzene extract of *Solanum xanthocarpum* Leaf (50mg/ml)

Acetone extract of *Solanum xanthocarpum* leaf (50mg/ml)

Control - Albendazole (50 mg/ml

Plate 6: *Invitro* anthelminthic activity of *Datura metel* leaf extract

Control - saline



Standard drug
Albentozole(50mg/ml)

Chloroform extract of *Daura metel* leaf (50mg/ml)

Aqueous extract of *Datura metel* leaf (50mg/ml)

Benzene extract of *Datura metel* leaf (50 mg/ml)

Ethanol extract of Datura metel leaf (50mg/ml)

Control - Albendazol

Solanum xanthocarpum leaf extract (50mg/ml)

CHAPTER-VI

SUMMARY AND CONCLUSION

India is richly endowed with a wide variety of plants 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. Phytochemicals with biological activity have a lot of applications in terms of pharmaceuticals and pharmacological effects. The preliminary phytochemical studies revealed the presence of phytoconstituents such as terpenoids, flavonoids, alkaloids, phenols, steroids, saponins, anthroquinones, cardic glycosides, glyconides and tannins. In this study the leaf of *Solanum xanthocarpum* and *Datura metel*have a various chemical group in their chemical composition. It revealed some differences in the constituent of the one part of the plant tested. Alkaloids, Steroids and Tannins were found in all five extracts of *Datura metel.* Tannins, Terpenoids and Cardic glycosides were found in all five extracts of *Solanum xathocarpum.* Rests of the phytochemicals was moderate / trace amounts. Compared to *Datura metel,Solanumxanthocarpum*showed the presence of more phytochemicals. Hence this plant should be evaluated further to assess its phytotherapeutic properties.

Medicinal plants have antimicrobial properties. The extracts of *Datura metel*and *Solanum xanthocarpum*have the antibacterial activity. As shown in Table 4, ethanolic extracts of *Datura metel*leaf exhibited maximum activity against *E. coli* (27mm). In case of leaf extract of *Solanum xanthocarpum,* the highest activity (inhibition zone of 25mm). The acetone extract of *Solanum xanthocarpum* leaf showed lowest activity of against *Staphylococcus* (7mm). In case of leaf extract of *Datura metel*, the highest activity (zone of inhibition of 30mm) was demonstrated against *staphylo coccus* while the lowest activity (inhibition zone of 8mm) was demonstrated against *E. coli.* The chloroform, benzene, ethanol and acetone extracts of *Solanum xanthocarpum* leaf shown the most effective and highest activity against *E. coli, Vibrio chlorae*and *Bacillus ceresus*(above15mm). It is concluded that, in the present study, both the plants contain potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments. Although the extract active plant principles such as flavonoids, alkaloids, phenol and tannins were observed in these extracts. The study has therefore justified the use of the plant is ethnomedicine. Further studies are needed with this plant to identify the unknown functional groups, isolate, characterized and elucidate the structure of the bioactive compounds which are responsible for the antimicrobial activity and other medicinal values.

Benzene extract of *Solanum xanthocarpum* and *Datura metel* consist of compounds such as amines, aromatic acid, methane, cycloalkanes responsible for antimicrobial activity.

Helminthiasis is a worldwide and one of the common diseases of all ages. The natural sources play a key role in the treatment of the anthelmintic. Medicinal plants have anthelmintic properties. The extracts *Solanum xathocarpum*and *Datura metel*have the anthelmintic activity. As shown in Table the crude extracts of *Solanum xanthocarpum*not only demonstrated paralysis but also caused death of worms especially at higher concentration of 250 mg / ml in nearly same time as compared to reference drug albendazole. The extracts potency was found to be inversely

proportional to the time it took for the animal to be paralysed or die. It’s a worm the behavoiur was compared to that of ethanolic and aqueous solution.

CHAPTER - VII

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ANALYSIS OF SOIL QUALITY USING PHYSICO-CHEMICAL PARAMETERS FROM SELECTED SITES OF THOOTHUKUDI

A Short – term project submitted to
ST.MARY'S COLLEGE (AUTONOMOUS)

Affiliated to
MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfilment of the requirements for the degree of
BACHELOR OF SCIENCE IN BOTANY

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APRIL 2020-21

CERTIFICATE

It is certified that this short-term project work entitled "**Analysis of Soil Quality using Physico-Chemical Parameters from Selected Sites of Thoothukudi**" submitted to **St. Mary's College (Autonomous)**affiliated to **ManonmaniamSundaranarUniversity** in partial fulfilment of the requirements for the degree of Bachelor 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 2020-2021by the following students.

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INTRODUCTION

Soil is derived from the Latin word “Solum” which means earthly material in which plant growth takes place. Soil is a natural body consisting of layers (soil horizons) of mineral constituents of variable thicknesses, which differ from the parent materials in their morphological, physical, chemical, and mineralogical characteristics. It is composed of particles of broken rock that have been altered by chemical and environmental processes that include weathering and erosion. Soil is essential for survival of the living world, especially for human population. Soil is a dynamic medium made up of minerals, organic matter, water, air and living creatures including bacteria and earthworms. It was formed and is forever changing due to physical factors, the parent material, time, the climate, the organisms present. (Anu *et al.,* 2010)

Soil, the biologically active, porous medium that has developed in the uppermost layer of Earth’s crust. Soil is one of the principal substrata of life on Earth, serving as a reservoir of water and nutrients, as a medium for the filtration and breakdown of injurious wastes, and as a participant in the cycling of carbon and other elements through the global ecosystem. It has evolved through weathering processes driven by biological, climatic, geologic, and topographic influences. The soil forms the intermediate zone between the atmosphere and the rock cover of the earth, the lithosphere. It also forms the interface between water bodies (hydrosphere) and the lithosphere and thus forming a part of biosphere. The soil may be defined as the uppermost weathered layer of the earth’s crust in which are mixed organisms and products of their death and decay. It may also be

defined as the part of the earth's crust in which plants are anchored. Soil sampling is perhaps the most vital step for any soil analysis. As a very small fraction of the huge soil mass is used for analysis, it becomes extremely important to get a truly representative soil sample of the field. Soil test based nutrient management has emerged as a key issue in efforts to increase agricultural productivity and production since optimal use of nutrients, based on soil analysis can improve crop productivity and minimize wastage of these nutrients (Soni, 2016)

Soils differ widely in their properties because of geologic and climatic variation over distance and time. Even a simple property, such as the soil thickness, can range from a few centimetres to many metres, depending on the intensity and duration of weathering, episodes of soil deposition and erosion, and the patterns of landscape evolution. Yet, despite this variability, soils have a unique structural characteristic that distinguishes them from mere earth materials and serves as a basis for their classification: a vertical sequence of layers produced by the combined actions of percolating waters and living organisms.

The nature of soil primarily depends upon its continued change under the effect of physical factors like the parent material, time, the climate, the organic activity in it etc. (Solanki and Chavda, 2012). Since soil is made up of such diverse materials like weathered rock particles and organic material (humus), it can be classified into various types based on the size of the particles (Tan, 1996; Ganguly, 2007). The modern concept of soil quality is the ability to sustain plant and animal productivity, to increase water and air quality and to contribute plant and animal health (Doran and Zeiss, 2000; Emnova, 2004). Although all physico-

chemical properties are involved in soil functioning, bio chemical properties tend to react most rapidly to get change in the external environment (Nannipieri *et al,* 1990; Trasar-Cepeda *et al*, 2008).

Human activities such as industrial production, mining, agriculture and transportation, release high amounts of heavy metals into surface and ground water, soils and ultimately to the biosphere. Accumulation of heavy metals in crop plants is of great concern due to the probability of food contamination through the soil root interface. Though the heavy metal like, Cd, Pb and Ni are not essential for plant growth, they are readily taken up and accumulated by plants in toxic forms. Heavy metal concentration in the soil solution plays an important role in controlling metal bioavailability to plants. These heavy metals may adversely affect soil ecology, agricultural production or product quality, and ground water quality, and will ultimately harm to health of living organism by food chain. These effects are closely related to the biological availability of heavy metals, which in turn are controlled by the metal ion speciation in the soil. Pollution of heavy metals in aquatic environment is a growing problem worldwide and currently it has reached an alarming rate. There are various sources of heavy metals; some originates from anthropogenic activities like draining of sewerage, dumping of hospital wastes and recreational activities (Nazir *et al.,* 2015).

In this study, the focus is confined to the Thoothukudi city region. Because Thoothukudi city and its environmental components such as soil and lake and marine sediments are under increasing stress owing to urbanisation and industrial activities. Recently, the city has garnered attention with people

Protesting the industries there. Therefore, it is important to understand the chemical and physical characteristics of soil periodically to assess their environmental condition and to understand the changes that happen over time. Hence, the current study was carried out with the following objectives

- Analysis of physical parameters like soil pH, electrical conductivity (EC), moisture content, specific gravity (Gs), bulk density and porosity of the nine representative soil samples of Thoothukudi.
- Examination the selected soil samples for its macronutrient level like Nitrogen (N), Phosphorus (P), Potassium (K) and Sulphur (S)
- Observation of micro nutrients namely Iron (Fe) and Copper (Cu) in selected soil samples

STUDY AREA

Thoothukudi District is located in extreme southern parts of Tamil Nadu. The district is located lies between 0.8 and 45 of northern latitude and 78 and 11 of the eastern longitudes. The district is roughly triangular in shape and is bounded by Virudhunagar and Ramanathapuram districts in the north, Gulf of Mannar in the east and Tirunelveli District in the south and west. The total geographical area of the district is 4707 sq. kms. and Constituting about 3.5 percent of the state. It has coastal line of 163.5 km. River Thamirabarani passes through the district. The major basin is Thamirabarani. The district is also an industrial oriented district among the southernmost districts.

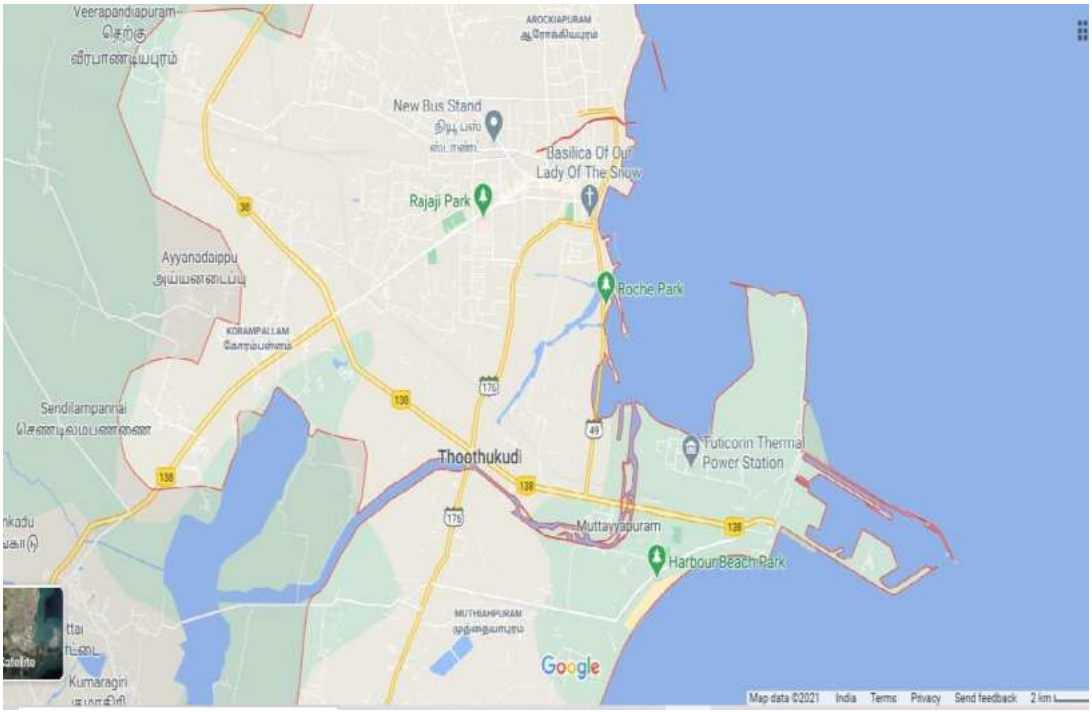
The district enjoys a hot tropical climate. The annual mean minimum and maximum temperature are 23°C and 29°C respectively. The climate is conducive for Agricultural and Horticultural crops. The average temperature of January is 27°C, February is 27°C, March is 29°C, April is 31°C, May is 31°C.

The predominant soil type is alkaline soil. Red loam was found in all taluks with more concentration in Udangudi, Kayathar, Sattankulam Taluk. Different types of soil, such as black soil and red sandy soil were seen extensively throughout the district and sandy coastal alluvium was found in coastal bed areas of Thiruchendur (District Statistical Handbook, 2017–18).

For the present study, nine representative soil samples are collected from nine different places namely Ananthamadan Patcheri, Thermal Nagar, Keela

Alagapuram, Krishnarajapuram, Madathur, Muthaiyapuram, St. Mary's College campus, T. Saveriarpuram and Thattar street in Thoothukudi.

Map showing study area*



(* google map)

REVIEW OF LITERATURE

Soil is the unconsolidated mineral on the surface of the earth that serves as a natural medium for the growth of plants. It is formed from rocks by physical, chemical and biological weathering (Roven *et al.,* 1998). Soil consists of various constituents such as minerals, organic matter, water and air (McBridge, 1994; Brady, 1988; Ahmed *et al.,* 1993). An average soil is 45 % mineral, 25 % water, 25 % air and 5 % organic matter (USDA). Soil is the basic medium providing majority of food items to the living organisms but its degradation has become a major global concern for the last few years as a result of increasing demands of land for food production.

Jayaraju *et al.* (2009) described acid extractable metals in the surface sediments of the coastal area of Tuticorin that revealed the contamination of the harbor sediments by Cd, Cu, Pb and Zn. Although concentrations were higher in the Silt + Clay fraction than in the sand fraction, percentages of total concentrations extracted from the coarse fraction, especially in the sandy harbor area showed that sand is also an important carrier of extractable metals.

Joel and Amajuoyi (2009) studied some selected physicochemical parameters and heavy metals in a drilling cutting dump site at Ezeogwu-Owaza, Nigeria. Test results indicated that some of the heavy metals like copper, ion and calcium showed a high level of contamination in most of the plots under the study area. Iron had a value as high as 880mg/kg, copper 84mg/kg and calcium 12560 mg/kg. These values were above target values as specified by the regulatory body,

Department of Petroleum Resources (DPR). Moreover, the oil and grease indicated a high level of contamination, with a concentration of up to 840mg/kg in one of the plots. This was evident in lack of plant growth noticed in the study area as a result of depletion of NPK values below to specify the value by USDA standards for the physiochemical parameters and heavy metals as seen in this project underscores the need for due diligence in managing drilling cutting discharges from drilling activities.

Concentration of heavy metals Cd, Cu, Fe, Mn, Ni, Pb and Zn in sea water, sediment and bivalve samples from three stations was studied for one year along Tuticorin coast. The concentration was in the order of Fe>Mn>Zn>Cu>Pb>Cd>Ni. High concentration of Fe, Mn, Cu, Pb and Zn was observed during monsoon season (Asha *et al.,* 2010).

The soil samples were taken from Shahpura Lake of Bhopal to assess the soil quality of Shahpura lake, Bhopal, Madhya Pradesh. During the study period physico-chemical parameters viz pH, moisture content, bulk density, chloride of soil was assessed as per the standard methods. High chloride value in the study area indicated pollution of soil sediment due to urbanization, industrialization and modernization in agricultural system results in extensive use of chemical fertilizers and pesticides (Anu *et al*., 2010).

The soil of different sites of the Harappa museum situated at Sahiwal district in Pakistan was collected and investigated for pH, electrical conductivity, organic matter, micronutrients and macronutrients. The soil is saline sodic. pH was found to be in the range of 7.62-8.27 which is slightly neutral to basic. Electrical conductivity was in the range 8.9-12.0 mS/cm while percentage of

organic matter was 0.276-1.035. The elements like Na, K, Ca, Mg, Zn, Ni, Cu, Mn, Mo, Se, B, P, N, S, Cl were found in the range, Na (1035-9050 mg/kg), K (3050-6000 mg/kg), Ca (153- 260 mg/kg), Mg (259-689 mg/kg), Zn (31-85 mg/kg), Ni (7.3-17.8 mg/kg), Cu (70-91 mg/kg), Mn (3.1-20.3 mg/kg), Mo (16.1-24.4 mg/kg), Se (0.01-0.062 mg/kg), B (6.7-16.6 mg/kg), P (164-696 mg/kg), N (5.76-13.38 mg/kg), S (62-281.3 mg/kg), Cl (102.3-200 mg/kg), respectively. The overall contents of the above parameters were found to be above permissible limits (Imran *et al.,* 2010). Magesh *et al.* (2011) evaluated the trace element contamination in sediments of the Tamiraparani estuary that is situated north of Thoothukudi city.

The nutrients quality present in the soil of Bhusawal, District Jalgaon (Maharashtra) was studied by Chaudhari (2013). They investigated various parameters like total Organic Carbon, Nitrogen (N), Phosphorus (P_2O_5), Potassium (K_2O), pH and Conductivity. Results showed that all the eight selected places of Bhusawal have medium or high mineral content. This information will help farmers to solve the problems related to soil nutrients, amount of fertilizers to be used to increase the yield of crops.

Ganorkar and Chinchmalatpure (2013) carried out work on the soils with physical properties, chemical properties and micronutrients. Soil samples were collected from six different locations covering Rajura Bazar, in Warud Tahsil in Amravati District, Maharashtra, India. The soil parameters like soil moisture, pH, EC, Carbon, Calcium carbonate, TDS, Magnesium, Calcium, Nitrogen, Copper, Potassium and Phosphorus content, were analysed in the month of February 2013.

The values of pH indicated that all samples of the soils are alkaline, all samples were containing moderate amounts of available micronutrients.

Raj and Bhagan (2013) analysed the fluoride concentration and some other important physiochemical parameters of 15 surface soil samples and 51 underground water samples of 10 fluoride areas of Agastheeswaram Union, South India. In all the fluoride areas, the surface soil samples were having fluoride levels greater than the soil was ranging between 2 to 3.5 ppm. Both the levels were found to be above the permissible limit. Other parameters such as pH, alkalinity, total hardness, calcium, magnesium, chloride, salinity and sodium were also measured. Alkalinity and pH were found to be higher than the permissible limit in all the soil and water samples at various seasons.

Wagh *et al.* (2013) dealt with the analysis of soil samples from sugarcane field which were collected in a period 2009 - 2010 from Manjari, Hadapsar and Phursungi villages situated towards Southern East of Pune city and this region is affected by the solid waste disposal as well as industrial effluents. This study was primarily focused on testing of soil quality from 12 representative sampling stations (numbered as 1 to 12). Physical parameters like pH, Electrical conductivity (EC), organic carbon (%) and chemical parameters like phosphorus, potassium, copper, iron, manganese, zinc and boron were analyzed. It has been revealed that there is excessive dose of phosphorous and potassium into the soil because most farmers are using excessive chemical fertilizers. Similarly, Cu, Fe, Mn and Zn concentration has also been seen higher than the normal range and due to poorer drainage conditions of this area making soil alkaline.

Karikalan *et al.* (2014) analysed the concentration of Fe, Mn, Cr, Cu, Ni, Co, Pb, Zn, and Cd of surface sediments of Thoothukudi. They found enrichment of these elements in the sediments.

Mahajan and Billore (2014) carried out physicochemical analysis like pH, specific conductivity, chloride, total alkalinity, calcium, magnesium, nitrate, sulphate, phosphate, sodium and potassium in soil of Nagchoon Pond Khandwa, MP, India from July 2008 to 2009. During the study year fluctuation was observed in several parameters. Some parameters were above permissible limit and some below the permissible limit which affects the quality and productivity of pond soil.

The physicochemical properties of soils from natural flood disaster affected areas of the Isoko Region of Delta States, Nigeria, were investigated by Osakwe (2014). The results indicated that there was an overall reduction in soil pH (5.425±0.313), phosphorus (7.47±6.34mgkg-1), and nitrate (0.34±0.07mgkg-1) contents as well as exchangeable calcium 1.97±0.31mgkg-1 potassium (0.09±0.01mgkg-1), and effective cation exchange capacity (5.076±1.532(cmolkg) and related parameters with 3.87±0.21,77.57±5.83 and 7.99±2.72 for base exchange capacity, base saturation and soil buffering capacity respectively. There was, however increased in the values of exchangeable magnesium (1.50±0.25mgkg-1), exchangeable sodium (0.28±0.004mgkg-1) and also the exchangeable acidity with the values 0.43+0.08 and 0.42±1.02mgkg-1 for hydrogen and aluminum respectively. There was no appreciate change in the values of total organic carbon (0.40±0.096%), Total nitrogen (0.025±0.035%) and sulphate (0.10±0.02mgkg-1). The overall results indicated that the flood increased

soil acidity and decreased the ability of the soils to adsorb metals but did not have an appreciable effect on the biodegradable and compostable materials.

Patel *et al*. (2014) correlated the chemical parameters of agricultural soil of different villages of Kutch district of Gujarat State in Western India. Their primary focus was to study mung bean crop based on randomly selected 30 medium black soil samples. Under the Soil Health Card Program of Government of Gujarat, Soil samples were collected by authorized locally trained farmers and brought for analysis to Soil Test Laboratory, Bhuj. Standard Methods were used for the soil quality analysis. They concluded that the statistical method 'correlation analysis' can provide a scientific basis for controlling and monitoring the agriculture soil fertility management.

Shivanna and Nagendrappa (2014) carried out work on the soil fertility status of selected command areas of three lakes-Eachanur, V. Mallenahalli and Halkurke in Tiptur Taluk . The variables tested included pH, EC, OC, N, P, and K. The study revealed that the pH of the soil samples ranged from 7.07 to 7.87 and was on slightly alkaline side but within the limit of 6.5-8.5 which is optimum for crops. EC values ranged from 0.26dSm-1 to 0.485dSm-1 and were within the limit of 0.8dSm-1 indicating low salinity status of the soils. OC content ranged from 0.50% to 0.67% and all the samples were of medium rating. Available nitrogen ranged from 54.825kg/ha to 85.72kg/ha; available phosphorus ranged from 54.33kg/ha to 10.79kg/ha and samples were nitrogen and phosphorus deficient. Potassium ranged from 156.18kg/ha to 434.38kg/ha and samples were of medium rating except one sample of high rating with respect to potassium.

Mlitan *et al*. (2015) investigated the effect of treated wastewater on soil chemical and physical properties. A field experiment was conducted in the Misurata region in central Libya with water treatment of wastewater. The soil physicochemical parameters such as pH, water content, total soluble salts, Cadmium, Zinc, Lead, Copper and Iron of soil added treat industrial wastewater. The results reveal that some sampling sites were affected by industrial wastewater pollution. Soil water content ranged from 7.68 tob19.56%. Total soluble salts ranged from 272.6 to 300 ppm and soil pH ranged from 7.7 to 8.0 and showed no appreciable differences within localities. The all-tested metals increased from first location to the third location expect iron. The irrigation system had a significant effect on Total soluble salts and microbial flora. Isolated microbial flora consists of 4 fungal genera belonging to, *Aspergillus, Penicillium, Rizopus* and *Fusarium*. The latter and one of the *Aspergillus* spices (*Aspergillus* sp3) may consider one of the resistance fungi in industrial wastewater due its large colony numbers isolated from the water contaminated metals area.

Muche *et al*. (2015) analysed physicochemical properties of soil under different land use types in Alket Wonzi watershed, Farta district, Northwest Ethiopia. Soil samples were taken at 0.25cm depth, on four land use types viz, natural forest, cultivated land, plantation forest and grazing land. The results obtained from the study indicated that soils of grazing, cultivated land and plantation forest are strongly acidic (pH<5.5). Therefore, appropriate reclamation method should be lunched to improve agricultural productivity and sustainability of the study area.

Mobar *et al.* (2015) carried out work on impacted and non-impacted soil of two areas i.e., Sanganer and Durgapura respectively, of Jaipur district. The soil quality was analysed by estimation of physicochemical parameters such as pH, electrical conductivity (EC), water holding capacity, texture analysis, organic matter, total hardness, sodium, potassium concentration, sodium adsorption ratio (SAR), cation exchange capacity (CEC) using standard protocols. The results showed a significant difference between pH, EC, water holding capacity, total hardness, SAR, CEC of both the soil, inferring the impact of industrial effluent on the quality, control of such industrial pollution assumes greater significance which can be assured by planned industrialization.

Ogundele *et al.* (2015) analyzed plant and soil samples to determine the heavy metals (Cd, Zn, Cu, Cr, Pb and Ni) along major roads in Kwara State, Nigeria. One sample each of soil and plant was collected from Kwara State University as the control sample. Three plant species *(Kyllinga pumila* michx, *Kyllinga squamulata* Thanm ex vahl, *Cenchrus biflorus* Roxb) on which animals feed were collected along major roadsides. The samples were digested using wet method and heavy metals were analyzed using Atomic Absorption Spectrophotometry Technique. Lead concentration of plants from the sites was found between 24-142 mg/kg and 24-157.667 mg/kg in soil samples. Copper was found between 28.55-115.2 mg/kg and 7.70-80.13 mg/kg in plant and soil samples respectively. Zinc ranges from 13.00-120.45 mg/kg and 30.8- 219.23 mg/kg in plants and soil respectively. Cadmium was between BDL-0.400 mg/kg and BDL-0.366 mg/kg in plants and soil. Chromium was detected between BDL-53.65 mg/kg and 10.57-77.10 mg/kg in plants and soil respectively. Nickel was between

1.65-11.85 mg/kg and 1.83-14.87 mg/kg in soil and plants samples. Heavy metals (Cd, Zn, Cu, Cr, Pb and Ni) in the control samples were found to be 0.35, 40.00, 88.55, 0.65, 238 and 0.65 mg/kg for Cadmium, Zinc, Copper, Chromium, Lead and Nickel in plants respectively. The soil samples were between 0.066, 9.50, 4.83, 55.63, 33.667, 4.33 mg/kg, Zinc, Copper, Chromium, Lead and Nickel respectively.

Sonikajha and Suneetha (2015) investigated different nutrients like total nitogen, phosphorus, potassium and exchangeable calcium and magnesium in soil samples of various places collected from horticulture spot, lakeside, agriculture area and mountain located in Vellore.

In surrounding areas north of Thoothukudi, in the Park Strait, Kasilingam *et al.* (2016) measured the concentrations of Fe, Mn, Cr, Cu, Ni, Cd, Pb, and Zn to decipher the trace element accumulation in surface sediments. The elemental concentration and correlation results suggest that fine fractions with CaCO3 content followed by organic matter (OM) of the surface sediments control the trace element accumulation in the study area. In addition, Fe and Mn concentration is chiefly contributed from riverine process and controlled by the mangrove ecosystem. The other elements are derived into marine environment through confluence of untreated industrial pollutants into the river system.

Soni (2016) conducted quality test survey of the soil in 2017. Fife representative samples were obtained from Abohar city, Punjab, India and analyzed for its alkalinity content, chloride, sulphate, pH, conductivity, sodium and potassium. The value of alkalinity was found to be from 20 to 64.0 meq/100 gm, chloride content was ranging from 1.23 to 1.98 g/100g, sulphate was found to

be between 0.063 to 0.742g, conductivity was ranging from 0.4 to 1.9 micro mohs, range of sodium was between 150.6 to 50 ppm and potassium from 100.9 to 135.5 ppm.

Twenty representative samples were collected from various parts of the Kadi taluka of Gujarat and its physico-chemical analysis have been performed to know its different parameters like pH, Electrical Conductivity, Phosphorous, Potassium, Sulfur, Carbon and Boron (Nisha *et al.,* 2017).

Makkar *et al.* (2018) investigated the physicochemical properties of soils collected from various areas of Punjab were studied viz. soil pH, Electrical Conductivity, Organic Carbon, organic matter and Nitrogen percentage. Most of soils were little alkaline having pH ranged from 7.0-8.2. The value of Electrical Conductivity ranged between 0.21-0.31 mmhos/cm indicating the normal nature of soil. Moreover, the soils were enriched with Organic Carbon ranging between 0.31%-0.80 %.

Rinni *et al.* (2018) evaluated the availability of nutrients that is present in the soil and fertility status of the soil. They collected a total of 1490 samples from the farmer's field of 9 districts of Western Uttar Pradesh (from February, 2012 to February, 2017) and analyzed for various soil fertility parameters such as pH, EC, organic carbon, macronutrients. They found that most of soil samples collected from study area was in the range of normal pH (6.5-7.8), 46% of soil samples showed medium nutrient index value for organic carbon. 84% of samples showed EC below 1. At the same time 43% of samples for phosphorous and 62% of samples for potassium showed the medium index.

Dandwate (2020) collected five representative soil samples from different places of Sangamner city and analyzed its alkalinity content, pH, electrical conductivity, organic carbon, sodium, potassium. A five soil samples were collected at a depth of 0–20 cm and analyzed for soils were neutral to slightly alkaline. The value of soil pH found to be 7.60 to 8.81, conductivity was ranging from 0.50 to 0.73 dSm−1, organic carbon was found to be 0.52 to 0.72%, range of sodium was 0.52 to 0.97meq% and potassium 125.31 to 630.15 kg/ha. Among the nutrients, available Nitrogen was found to be 140.01 to 252.68 kg/ha, Phosphorous was ranging from 15.11 to 54.13 kg/ha.

MATERIALS AND METHODS

Soil sampling and preparation

Nine different soil samples were collected from Ananthamadan Patcheri (sample I), Thermal Nagar (sample II), Keela Alagapuram (sample III), Krishnarajapuram (sample IV), Madathur (sample V), Muthaiyapuram (sample VI), St. Mary's College campus (sample VII), T. Saveriarpuram (sample VIII) and Thattar street (sample IX) in Thoothukudi. Samples are taken from 0 – 30 cm depth and sieved to remove foreign materials like roots, stones, pebbles and gravels and finally labeled.

Soil samples were collected to determine the Physical (electrical conductivity, soil moisture, specific gravity, bulk density and soil porosity) and chemical (soil pH, organic carbon, nitrogen, phosphorous, potassium, Sulphur, iron and copper).

Physical Parameters

Physical parameters of soil include pH, electrical conductivity (EC), moisture content, specific gravity, bulk density and porosity were done using the procedure of Murugesan and Rajakumari (2005).

a) Electrical Conductivity

1:5 soil: water suspension was prepared by weighing 10 g air-dry soil (<2 mm) and 50 mL deionized water into a bottle and mechanically shaked at 15 rpm for 1 hour to dissolve soluble salts. the conductivity meter was calibrated using the KCl reference solution. After rinsing the cell thoroughly, the electrical conductivity of the 0.01M KCl at the same temperature as the soil suspensions

was measured. The conductivity cell was rinsed with the soil suspension. The conductivity cell is refilled without disturbing the settled soil. The value indicated on the conductivity meter was recorded.

b) Moisture Content

10 gm of soil was dried in a hot–air oven at 105°C till constant weight is achieved. Final weight of the sample was recorded. Moisture content of the sample was determined using the formula.

$$\text{Moisture content of the soil sample (\%)} = \frac{(I\text{-}F) \times 100}{I}$$

Where,

I = Initial weight of the sample (in grams)

F = Final weight of the sample (in grams)

c) Specific Gravity

10g of sieved soil sample was dried in a hot air oven at 105°C. Two wide – mouthed glass bottles were taken and their initial weight was recorded. The dried soil sample was transferred to one of the bottles up to a fixed volume. The second bottle was filled with distilled water to the same volume. The weight of both the bottle with soil and distilled water was measured. Specific gravity of the soil sample was calculated by using the formula

$$\text{Specific gravity (mg/m}^3\text{)} = \frac{Y\text{-}Y_1}{Z\text{-}Z_1}$$

Y – Final weight of bottle with soil

Y_1 – Initial weight of the bottle used for soil

Z – Final weight of bottle with distilled water

Z_1 – Initial weight of bottle used for water

d) Determination of Bulk Density

10 g of sieved soil sample was dried in a hot-air oven at 105^0c until the constant weight was achieved. The dried soil sample was transferred to a measuring cylinder (volume taken as 100 cm^3) and the volume of the soil sample occupied in the measuring cylinder was recorded and the bulk density was calculated,

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of the soil taken (g)}}{\text{Volume of the soil in measuring cylinder (cm}^3\text{)}}$$

e) Determination of Porosity of Soil

Soil porosity was calculated from the results of specific gravity and bulk density.

$$\text{Soil porosity (\%)} = \frac{\text{Specific gravity – Bulk density}}{\text{Specific gravity}} \text{ X } 100$$

Chemical Parameters

Instrument:

Mridaparikshak is a minilab developed by ICAR-Indian Institute of Soil Science (IISS), Bhopal, an institute that comes under the Division of Natural Resource Management of Indian Council of Agricultural Research. Mridaparikshak has been developed in technical collaboration with M/s Nagarjuna Agrochemicals Pvt. Ltd., Bhopal. With Mridaparikshak one can determine the available quantities of soil nutrients and prescribe fertilizer doses for nitrogen (N), phosphorus (P), potassium (K), sulphur (S), Iron (Fe), zinc (Zn), boron (B), copper (Cu) and Manganese (Mn) based on the measures soil test values.

Macronutrient include phosphorus and micronutrient iron and copper in soil samples were estimated using this instrument. Other chemical parameters like pH, organic carbon, available nitrogen, sulphur (Murugesan and Rajakumari, 2005) (Abul-fadl, 1948) potassium and phosphorous (Olsen *et al*., 1954) were done using the standard procedure.

a) Soil pH

About 10 g of soil sample from various pots was placed in 100 ml of distilled water in a beaker and stirred well. The soil suspension was allowed to settle. After 24 hours, pH of the suspension was measured using pH meter (Model LI120) and the results were tabulated.

b) Estimation of Organic Carbon

1g soil sample was weighed in 500 ml conical flask. 10 ml of 1 N $K_2Cr_2O_7$ and 20 ml conc. H_2SO_4 (containing Ag_2SO_4) were added and mixed thoroughly and was allowed reaction to proceed for 30 minutes. The reaction mixture was diluted with 200 ml water and 10 ml of H_3PO_4, 10 ml of NaF solution and 2 ml of diphenylamine were added. The solution was titrated with standard FAS to a brilliant green colour. A blank without soil should be run simultaneously.

$$\text{Organic Carbon (\%)} = \frac{10}{\text{blank}} \quad (\text{Blank - Reading}) \quad X \quad \frac{0.003 \times 100}{\text{Wt. of soil}}$$

Where,

Weight of sample - 1 g

Normality of $K_2Cr_2O_7$ used - 1 N

Vol. of $K_2Cr_2O_7$ - 10 ml

Normality of FAS - 0.5 N

c) Estimation of Nitrogen

Procedure:

- Accurately 20 g of dried soil sample was weighed and transferred it into the distillation flask. 30 ml of distilled water and 1 ml of liquid paraffin were added to the soil sample.
- With this, 100 ml each of 25% NaOH and 0.32% KMnO4 were added to the soil in the distillation flask.
- To avoid bumping, pieces of glass beads was added.
- 20 ml of 2% boric acid with few drops of double indicator was taken in a 100 ml beaker. This beaker was kept at the outlet of the condenser.
- Now the contents of the flask were distilled till the distillation process liberates about 30 ml of distillate.
- The distillation process was continued till the liberated distillate is devoid of ammonia.
- This can be confirmed by keeping the moist red litmus paper near the delivery end of condenser. The litmus paper will appear blue as long as the ammonia is evolved.

Calculations:

$$\text{Nitrogen present in Kg/ha} = \frac{0.00028 \times A \times 2 \times 10^6}{20}$$

Where,

0.00028 = 1 ml of N/50 H_2SO_4

A = Volume of N/50 H_2SO_4 consumed

20 = Weight of the soil in grams

d) Estimation of Phosphorous

Reagents:

1. 0.5 M Sodium bicarbonate($NaHCO_3$) solution:
2. Activated Charcoal
3. 5 N Sulphuric acid (H_2SO_4) Solution
4. Reagent A
   - Dissolve 12.00 g of ammonium paramolybdate in 250 ml of distilled water.
   - Dissolve 0.2908 g of potassium antimony tartrate ($KSbO.C_4H_4O_6$) in 100 ml distilled water.
   - Above both solution mix thoroughly and made one litre in volumetric flask with the help of distilled water.
   - Add these dissolved reagents to one litre of 5N H_2SO_4.
5. Ascorbic acid working solution (Reagent B): Dissolve 1.056 g of ascorbic acid in 200 ml of reagent A and mix. This ascorbic acid (reagent B) should be prepared as required because it does not keep more than 24 hours.

Procedure:

- Take 2.5 g of soil sample in 150 ml conical flask and 0.5 g Darco G-60 activated charcoal.
- Then add 50 ml of 0.5 M $NaHCO_3$ solution and shake the solution for 30 minutes in a shaker. Similar processes run for a blank without soil.

- Filter the suspension through theWhatman no. 40 paper.
- Take 5 ml aliquot of the extract in a

  25 ml volumetric flask, and acidify with 5N H_2SO_4.
- Add small quantity of distilled water, and then add 4 ml of reagent B.

  The intensity of blue colour is read on spectrophotometer at 660 nm wavelengths after 10 minutes.

$$\text{Available P (kg ha}^{-1}) = \frac{\text{R x F x 50 x 2.24}}{\text{5 x 2.5}}$$

2.5 = Weight of soil sample (g)

50 = Volume of extractant used (ml)

5 = Volume of filtrate used (ml)

R = Absorbance

A = Absorbace of standard

B = Concentration of standard (ppm)

F= B/A

e) Estimation of Potassium

To 1 ml of digested soil solution 2 ml of sodium cobalt-nitrite reagent was added slowly with constant agitation. After 45 minutes, 2 ml of double distilled water was added, the contents were mixed and centrifuged at 2000 rpm for 15 minutes. The tube was then inverted and immediately drained on filter paper; 2 ml. of water are added down the side of the tube without disturbing the precipitate. The tube was again centrifuged for 5 min., inverted and thoroughly drained. The precipitates were washed with 5 ml 70% ethanol, which was blown into the tube

so as to stir up the precipitates. After centrifuging and draining thoroughly, 2 ml. of water was added to the tube, and the tube placed in a boiling water bath until dissolution was complete. While still hot, 1 ml glycine solution (7.5%) and 1 ml Na_2CO_3 solution (25%) were added and thoroughly mixed. 1 ml diluted Folin-Ciocalteu reagent was then added, the contents were mixed again, and the tube was allowed to stand in a water bath at 370 C for 10-15 min. After cooling to room temperature, the volume is accurately adjusted to 6 ml and the absorbance was read in a photoelectric colorimeter, using red filter. Distilled water was used instead of the sample for the blank.

f) Determination of Sulphur

Procedure:

- 5 g of soil was transferred into a silica basin containing 20 ml distilled water and was kept it in boiling water bath till all its water content evaporates.
- Following this, it was heated in a hot-air oven at 102°C for 1 hour and was allowed it to cool.
- The crucible content was transferred into a centrifuge tube containing 33 ml of 1% sodium chloride and centrifuged at 2000 rpm for about 5 minutes and the clear liquid was collected in a silica crucible.
- The procedure was repeated till 25 ml of the extract ½ is collected.
- 2 ml of 3% H2O2 was added to the silica crucible and was heated in a boiling water bath to eliminate the organic matter.
- Again, to remove the excess water, it was placed in a hot-air oven at 102°C for 1 hour.

- The residue was transferred to a centrifuge tube containing 25 ml water and it was rotated at 2000 rpm and collect the supernatant.
- 10 ml of this extract was poured into conical flask and was made up the volume to 30 ml using distilled water.
- Similarly, several dilutions of standard sulphate solution were prepared into a series of beakers with their concentrations ranging from 5 ml to 50 ml at 5 ml interval. The volume of each beaker was made up to 30 ml using distilled water.
- 2.5 ml of stabilized solution and 0.3 g of barium crystals were added to the standards and soil extract solution.
- The contents of each flask were mixed well and observe for the turbid formation.
- The absorbance of both the standard and extract solution was measured in a spectrophotometer at 3340nm.
- A standard curve was prepared by plotting the concentration of standards against their absorbance values.
- From the calibration curve, the sulphur content in the extract solution was evaluated. Let this be α.

Calculations:

$$\text{Amount of Sulphur (mg/Kg)} = \frac{\alpha \times V1}{V2 \times W}$$

Where,

α = Amount of sulphate (mg/I) in the soil extract

V1= Volume of extract (10ml)

V2= Volume of aliquot (25ml)

W = Weight of soil

RESULT AND DISCUSSION

The study of physicochemical parameters is vital for preserving plants growth and soil management. Soil test based nutrient management has emerged as a key issue in efforts to increase agricultural productivity and production since optimal use of nutrients, based on soil analysis can improve crop productivity and minimize wastage of these nutrients (Soni, 2016). In this view, a physicochemical study of nine representative soil samples from Thoothukudi is conducted based on various parameters like soil pH, electrical conductivity (EC), moisture content, specific gravity (Gs), bulk density, porosity, organic carbon (OC), available nitrogen (N), phosphorus (P), potassium (K), sulphur (S) and micronutrients (Fe, and Cu).

Physical Parameters

Electrical conductivity (EC)

Electrical conductivity is very important property of the Soil. It indicates total soluble salts content of the soils. The value of conductivity is the measure of ions present in the soil sample (Nisha et al., 2017).

Figure 1 portrays EC values of soil samples ranges from 0.21 to 2.83 dSm^{-1}. The electrical conductivity of a soil solution increases with the increased concentration of ions. During this process the cations of the clay/colloidal matter are exchanged in equivalent quantities with the cations of soil and salt solutions. This process of exchanges of cation of soil and salt solution is known as cation exchange. Cations like Ca, Mg, Na, K and anions such as CO_3, HCO_3, PO_4. The

conductivity values can be varying with chemical properties of soil. If EC is less than 4 soil type is normal (Dandwate, 2020). All soil samples had lower EC values. According to USDA (United States Department of Agriculture) degree of salinity, sample V is slightly saline soil and others are non-saline soil (Table 1). Optimal electrical conductivity levels in the soil usually range from 1.09-5.7 dS/m. Too low electrical conductivity indicate low availability of nutrients, and too high electrical conductivity indicate an excess of nutrients. Among all the samples, sample V is rated good for growth of plants. All other soil samples have low available nutrients

Moisture content

Soil moisture is the water that is held in the spaces between soil particles. The moisture content ranged from 1.64% to 12.15% (Table 2). The moisture content was found to be maximum in sample IV (12.15%) which is followed by sample VIII (4.65 %), sample II (4.00 %), sample V (3.57 %), sample VI (2.26 %), sample IX (2.08 %), sample VII (2.04 %), sample III (1.79 %) and sample I (1.64 %).

The water is held on the surface of the colloids and other particles and in the pores. The fact that soils hold water (moisture) is due to their colloidal properties and aggregation qualities. If the moisture content of a soil is optimum for plant growth, plants can readily absorb soil water. Not all the water, held in soil, is available to plants. Much of water remains in the soil as a thin film. Soil water dissolves salts and makes up the soil solution, which is important as medium for supply of nutrients to growing plants.

Specific gravity (Gs)

The soil specific gravity is defined as the ratio of the weight of a given volume of the material to the weight of an equal volume of distilled water. Table 3 indicates that specific gravity of soil samples ranged between 1.36 and 2.58 mg/m^3. The Specific gravity of soil generally ranges from 2.60 to 2.90 mg/m^3. The result revealed that sample IV has highest Gs (2.58 mg/m^3). Organic matter and porous particles may have specific gravity values below 2.0 and soil which has heavy substance or particles may have values above 3.0. specific gravity of other soil samples except sample IV is less than 2 indicating their high organic matter.

Bulk Density

Soil bulk density is the mass of dry soil per unit of bulk volume, including the air space. The bulk density values varied from 0.86 g/cm^3 to 1.81 g/cm^3 (Table 4). The bulk density of soil depends greatly on the mineral make up of soil and the degree of compaction. Factor such as organic matter, soil structure and porosity influence the level of bulk density. Bulk density value is minimum in pre-monsoon may be due to high rate of evaporation, deep percolation and moist extraction by cultivated plants and trees which ultimately lost from transpiration. (Jat, 2002). Soil bulk density can vary substantially among different soil types and is affected by management practices (e.g. tillage, livestock grazing, timber harvesting). Incorporation of large amounts of organic matter into the soil will lower the bulk density, while processes that compact the soil will increase bulk density. High bulk density is an indicator of low soil porosity and soil compaction.

High bulk density impacts available water capacity, root growth, and movement of air and water through soil. Compaction increases bulk density and reduces crop yields and vegetative cover available to protect soil from erosion (USDA). Generally, the bulk density of mineral soils ranges from 1.0 to 1.8 g/cm^3 (Carter, 1990). All the selected soil samples, except sample IX have ideal bulk density for plant growth.

Soil porosity

Soil porosity is the amount of pore volume (%age of pore space). Result showed that soil porosity of selected soil samples varied from 5.45 to 51.68% (Table 5). A medium textured, well-aggregated soil contains about 50% pore space and is in good condition for plant growth when the pores hold an equal distribution of air and water. Pore size affects pore activity. Sample II and V have no porosity. Sample IX has high percentage of porosity (51.68 %) followed by sample IV (41.47 %), sample III (40.56 %), sample VII (30.92 %), Sample VI (26.92 %) and sample I (25.69 %).

Chemical Parameters

pH

pH measures the relative conc. of hydrogen ion in the solution. The pH of soil is one of the most important physicochemical parameters. The soil pH measures very useful for identification active soil acidity or alkalinity nature. If pH of soil is 6.9 or less, the soil is acidic. Soils with a pH of 7.0 are neutral, values higher than 7.0 are alkaline. An examination of soil samples (Figure 2) showed that the values for pH range from 7.65 to 8.32 indicating that the soils are alkaline

(Table 6) and under such conditions the solubility of minerals decreases creating nutrient deficiencies in the soils. pH plays an important role in plant metabolism (crop nutrient acquisition), microbial metabolism, irrigation, soil temperature, and amendments, including fertilizers, lime, sulfur, etc. Soil pH exists in dynamic equilibrium within a crop production system and will fluctuate over time as changes in crop production methods, inputs and seasonal shifts occur. It affects mineral nutrient soil quality and much microorganism activity (Chaudhari, 2013).

Soil organic carbon (SOC)

Soil organic carbon (SOC) refers only to the carbon component of organic compounds. Soil organic matter (SOM) is difficult to measure directly, so laboratories tend to measure and report SOC. The result indicated that the organic carbon (%) ranges from 0.01 to 1.29 % (Figure. 3). From the result, it is clear that organic carbon is the medium level in samples II (1.29%), IV (0.56%) and V (0.64). Samples III, VIII and I have least amount of organic carbon (0.01%).

Soil organic carbon is the basis of soil fertility. It releases nutrient for plant growth, promotes the structure, biological and physical health of soil, and is buffer against harmful substances. Increasing soil organic carbon has two benefits- as well as helping to mitigate climate change, it improves soil health and fertility (Soni, 2016). SOC is one of the most important constituents of the soil due to its capacity to affect plant growth as both a source of energy and a trigger for nutrient availability through mineralization. SOC fractions in the active pool, previously described, are the main source of energy and nutrients for soil microorganisms. Humus participates in aggregate stability, and nutrient and water holding capacity.

OC compounds, such as polysaccharides (sugars) bind mineral particles together into microaggregates. Glomalin, a SOM substance that may account for 20% of soil carbon, glues aggregate together and stabilizes soil structure making soil resistant to erosion, but porous enough to allow air, water and plant roots to move through the soil. Organic acids (e.g., oxalic acid), commonly released from decomposing organic residues and manures, prevents phosphorus fixation by clay minerals and improve its plant availability, especially in subtropical and tropical soils. An increase in soil organic matter, and therefore total C, leads to greater biological diversity in the soil, thus increasing biological control of plant diseases and pests. (Edwards *et al.,* 1999).

Nitrogen, Phosphorus and Potassium content

Table 7 shows that nitrogen content in the selected soil samples ranged from 118.0 to 302.9 Kg/ha. Among the samples tested, Sample II has maximum level of nitrogen (302.9 Kg/ha) followed by sample V (210.8 Kg/ha), sample IV (198 Kg/ha), sample I (150.4 Kg/ha), sample VII (140.0). Sample III, VI, VIII and IX have least amount of nitrogen (118.0 Kg/ha). According to Tamil Nadu Agriculture Department soil rating chart (https://agritech.tnau.ac.In/agriculture/agri_soil_soilratingchart.html), sample II has medium level of nitrogen. This may be due to increase in soil water content due to rainfall and also the favorable temperature and humidity together which favours the luxuriant growth of nitrogen fixing bacteria, blue green soil algae which are ultimately responsible for nitrogen content in soil. Other soil samples have low level of nitrogen. This may be due to loss of nitrate by leaching. Leaching is the loss of soluble NO_3^- as it moves with soil water, generally excess water, below the root zone. Nitrate that moves below

the root zone has potential to enter either groundwater or surface water through tile drainage systems. Coarse-textured soils have a lower water-holding capacity and, therefore, a higher potential to lose nitrate from leaching when compared with fine-textured soils. Some sandy soils, for instance, may retain only 1/2 inch of water per foot of soil while some silt loam or clay loam soils may retain up to 2 inches of water per foot. Nitrate can be leached from any soil if rainfall or irrigation moves water through the root zone (Solanki and Chavda, 2012).

Phosphorus content of selected soil samples ranged from 25.02 to 111.7 Kg/ha (Table 7). According to Tamil Nadu Agriculture Department soil rating chart (https://agritech.tnau.ac.in/agriculture/agri_soil_soilratingchart.html), all the soil samples have high amount of phosphorus. Among the soil samples tested, sample II (111.7 Kg/ha) has maximum level of phosphorus followed by sample IV (69.71 Kg/ha), sample V (66.13 Kg/ha), sample VII (65.24 Kg/ha), sample III (52.73 Kg/ha), sample IX (48.26 Kg/ha), sample I (45.58 Kg/ha), sample VIII (26.8 Kg/ha) and sample VI (25.02 Kg/ha). Phosphorus considered as micro nutrient, is utilized by plant in the form of H2PO4- & HPO4-2 species. Appropriate concentration of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop (Iram and Khan, 2018). . Phosphorus (P) is an essential element classified as a macronutrient because of the relatively large amounts of P required by plants. Phosphorus is one of the three nutrients generally added to soils in fertilizers. One of the main roles of P in living organisms is in the transfer of energy. Organic compounds that contain P are used to transfer energy from one reaction to drive another reaction within cells. Adequate P availability for plants stimulates early

plant growth and hastens maturity. The increase in phosphorus might be due to the absorption of phosphate ions in to suspended particles and sediments. The decrease might be due to assimilation of phosphorus from the water by phytoplankton would find to release more phosphorus from the sediments. According to McAuliffe *et al.* (1948), phosphate ions are very strongly absorbed by solid phase of soil; the result is very low concentration of phosphate in soil solution.

In selected soil samples, available potassium level ranged between 175.5 and 352.4 Kg/ha (Table 7). Sample IX (352.4 Kg/ha), VI (348.2 Kg/ha), VII (305.1 Kg/ha) and Sample V (299.5 Kg/ha) have maximum level of potassium as per the Tamil Nadu Agriculture Department soil rating chart (https://agritech.tnau .ac.in/agriculture/agri_soil_soilratingchart.html) whereas sample I (230.2 Kg/ha), III (208.9 Kg/ha), II (203.4 Kg/ha), VIII (185.2 Kg/ha) and IV (175.5 Kg/ha) have medium level of potassium. Potassium is used for flowering purpose, it is also required for building of protein, photosynthesis, fruit quality and reduction of diseases and phosphate is used for growth of roots in plants. Potassium is an essential nutrient for plant growth. Potassium is involved in many plant metabolism reactions, ranging from lignin and cellulose used for formation of cellular structural components, to regulation of photosynthesis and production of plant sugars that are used for various plant metabolic needs. It controls water loss from plants and is involved in overall plant health. Soils that have adequate potassium allow plants to develop rapidly and outgrow plant disease, insect damage and protect against winter freeze damage. Potassium is an element that contains a positive electrical charge known as a cation. The soil clay particles

contain a negative charge so, opposites attract. This feature prevents or limits the loss of potassium by leaching. The behavior of K in soil, release, absorption, fixation and leaching, is strongly dependent on the clay content and types of clay minerals present (Mengel and Kirkby, 1987). Soils with high clay content have both a greater amount of mineral potassium and more negative charges and therefore have more potassium. A sandy soil conversely has less mineral content and fewer negative charges and usually contains lower levels of plant available potassium. As result sandy soils will most likely have a greater need for potassium supplementation than clay soils.

Sulphur content

Table 8 displays that sulphur content of selected soil samples ranged from 3.0 to 17.0 mg/Kg. Considering the 10 mg kg^{-1} available sulphur as the critical level of suggested by Mehta et al. (1988), the deficiency of sulphur is noted in all the screened soil samples except sample V which has maximum level (17.0 mg/Kg). Sulphur is as necessary as phosphorus and is considered an essential mineral. Sulphur in plants helps form important enzymes and assists in the formation of plant proteins. It is needed in very low amount, but deficiency can cause serious plant health problems and loss of vitality (Nisha *et al.,* 2017). The deficiency of sulphur in soils of Red and Lateritic zone may be attributed to several factors. Among these some of the factors causing sulphur deficiency in the Red and Lateritic soils are inherent to soil properties and others are induced by manmade activities. Among these low native sulphur content, coarse texture, inherent low organic matter content and soil conditions that favour sulphur leaching losses. Although sulphur is one of the essential plant nutrients for plant

growth with crop requirement similar to phosphorus, it has not received as much attention as P until recently. This lack of attention in the past may be attributed to subsistence farming, low crop yields, sulphur non-responsive varieties, incidental sulphur returns to soil through farmyard manure, and the use of conventional sulphur containing fertilizers, such as single superphosphate (SSP) and ammonium sulphate (Patra *et al.,* 2012).

Iron content

Iron content of selected soil samples varied from 0.39 to 18.0 mg/Kg (Table 9). Some estimates suggest that soil should have at least 10 mg/kg of iron in soil. Soil samples II (18.0 mg/Kg) and V (17.0 mg/Kg) contain higher than the minimum amount of iron required. Other tested soil samples showed iron deficiency. Iron is an essential constituent for all plants and animals. On the other hand, at high concentration, it causes tissues damage and some other diseases in humans. It is also responsible for anemia and neurodegenerative conditions in human being (Fuorte and Schenck, 2000). The amount of iron and its availability in soil is increased by alkaline pH, less availability of organic matter, high moisture content, compacted and/or poorly aerated soils. When all of these issues are either excluded or remedied then organic sources of iron include some iron chelates and synthetic sources include ferrous and ferric sulfate can be added to treat the iron deficiency in soil.

Copper content

Table 10 indicates that copper content ranged from 0.09 to 27.5 mg/Kg in selected soil samples. Preferably, the healthy and productive soil should contain 2-50 mg/kg of copper. Amongst all the soil samples, sample V (27.5 mg/Kg)

showed maximum level of copper followed by sample II (10.4 mg/Kg), sample VIII (6.74 mg/Kg), sample IX (2.71 mg/Kg) and sample IV (2.62 mg/Kg). In contrast, soil sample I (1.21 mg/Kg), sample VI (0.93 mg/Kg), sample III (0.20 mg/Kg) and sample VII (0.09 mg/Kg) showed copper deficiency. The permissible limit for copper in soil according to WHO standard (1996) is 10 mg/kg. Fortunately, no one soil sample crossed this limit. Copper is a micronutrient in plants and an important constituent, in small amounts, of the human diet. It is a naturally occurring element in the soil and it can be found as a metal or in a variety of ores. It is a constituent of many man made alloys and is used in wire and some coins. It facilitates respiration and photosynthesis and is important for plant metabolism. It is a component of a variety of enzymes and plant cell walls so it is important for plant strength. Copper also affects the flavour, sugar content and storage life of fruit. A variety of factors can affect the availability of copper including: poor root growth, alkaline soils, high organic matter, high moisture content, excess amount of Zn, N and P (https://plantprobs.Net/plant/nutrientImbalances/copper.html).

The physicochemical study of parameters is important to agricultural chemists for plants growth and soil management. A physicochemical study of nine soil samples from Thoothukudi, shows that all the soil parameters are normal range. This study gives information about nature of soil, present nutrient in soil, according to this information farmer could arrange the amount of which fertilizers and nutrients needed to soil for increase the percentage yield of crops.

SUMMARY AND CONCLUSION

Nine different soil samples were collected from Ananthamadan Patcheri (sample I), Thermal Nagar (sample II), Keela Alagapuram (sample III), Krishnarajapuram (sample IV), Madathur (sample V), Muthaiyapuram (sample VI), St. Mary's College campus (sample VII), T. Saveriarpuram (sample VIII) and Thattar street (sample IX) in Thoothukudi. In order to study the nature of the soil, various physicochemical parameters like soil pH, electrical conductivity (EC), moisture content, specific gravity (Gs), bulk density, porosity, organic carbon (OC), available nitrogen (N), phosphorus (P), potassium (K), sulphur (S) and micronutrients (Fe, and Cu) were analyzed.

Electrical conductivity (EC) values of soil samples range from 0.21 to 2.83 dSm^{-1}. According to USDA (United States Department of Agriculture) degree of salinity, sample V is slightly saline soil and others are non-saline soil. The moisture content ranged from 1.64% to 12.15% (Table 2). The moisture content was found to be maximum in sample IV and lower in sample I (1.64 %). The specific gravity of soil samples ranged between 1.36 and 2.58 mg/m^3. specific gravity (Gs) of soil samples ranged between 1.36 and 2.58 mg/m^3. Among the samples tested, sample IV had highest Gs (2.58 mg/m^3) whereas other soil samples except had less than 2 mg/m^3 indicating their high organic matter. The bulk density values varied from 0.86 g/cm^3 to 1.81 g/cm^3. All the selected soil samples, except sample IX have ideal bulk density for plant growth. Soil porosity of selected soil samples varied from 5.45 to 51.68 %. Sample IX had high percentage of porosity (51.68 %) whereas sample I showed low percentage of porosity (25.69 %).

The pH range of soil samples from 7.65 to 8.32 indicating that the soils are alkaline. The organic carbon (%) ranged from 0.01 to 1.29 %. From the result, it was clear that organic carbon is the medium level in samples II (1.29%), IV (0.56%) and V (0.64). Samples III, VIII and I have least amount of organic carbon (0.01%).

As far as nitrogen is concerned, Sample II has maximum level of nitrogen (302.9 Kg/ha) followed by sample V (210.8 Kg/ha), sample IV (198 Kg/ha), sample I (150.4 Kg/ha), sample VII (140.0). Sample III, VI, VIII and IX have least amount of nitrogen (118.0 Kg/ha). According to Tamil Nadu Agriculture Department soil rating chart, sample II has medium level of nitrogen. Phosphorus content of selected soil samples ranged from 25.02 to 111.7 Kg/ha. According to Tamil Nadu Agriculture Department soil rating chart, all the soil samples have high amount of phosphorus. In selected soil samples, available potassium level ranged between 175.5 and 352.4 Kg/ha. Sample IX (352.4 Kg/ha), VI (348.2 Kg/ha), VII (305.1 Kg/ha) and Sample V (299.5 Kg/ha) have maximum level of potassium as per the Tamil Nadu Agriculture Department soil rating chart whereas sample I (230.2 Kg/ha), III (208.9 Kg/ha), II (203.4 Kg/ha), VIII (185.2 Kg/ha) and IV (175.5 Kg/ha) have medium level of potassium. Sulphur content of selected soil samples ranged from 3.0 to 17.0 mg/Kg. The deficiency of sulphur is noted in all the screened soil samples except sample V when considering the 10 mg kg^{-1} available sulphur as the critical level.

As far as micronutrients was concerned, Soil samples II (18.0 mg/Kg) and V (17.0 mg/Kg) contain higher than the minimum amount of iron required. Other tested soil samples showed iron deficiency. Amongst all the soil samples, sample V

(27.5 mg/Kg) showed maximum level of copper followed by sample II (10.4 mg/Kg). On the other hand, soil sample I (1.21 mg/Kg), sample VI (0.93 mg/Kg), sample III (0.20 mg/Kg) and sample VII (0.09 mg/Kg) showed copper deficiency.

The conclusion can be drawn that this study of physicochemical parameters of selected soil samples showed different values at different places. This can be due to the unequal distribution of dissimilar parameters present in soil. Such type of monitoring of soil samples is beneficial to know the concentrations of various parameters present in soil samples. From the analysis of the collected samples, it is clear that all the soil will be quite healthy for plant growth, if it is treated with proper fertilizer for its respective deficiency of nutrients. The highest fertile soils are vital for commercial points of view for farmers resulting in increase in economy of the particular region.

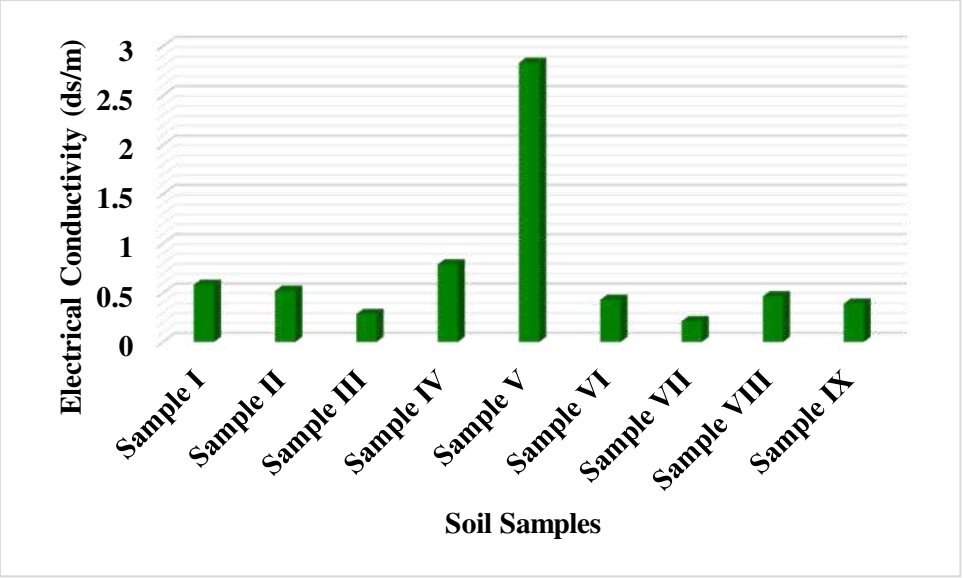


Figure 1: Electrical conductivity of the soil samples

Table 1: Salinity classes of the soil samples

Soil Samples	Degree of Salinity (Salinity classes)
Sample I	Non -Saline
Sample II	Non -Saline
Sample III	Non -Saline
Sample IV	Non -Saline
Sample V	Slightly Saline
Sample VI	Non -Saline
Sample VII	Non -Saline
Sample VIII	Non -Saline
Sample IX	Non -Saline

Table 2: Moisture Content of soil samples

Soil Samples	Moisture Content (%)
Sample I	1.64
Sample II	4.00
Sample III	1.79
Sample IV	12.15
Sample V	3.57
Sample VI	2.26
Sample VII	2.04
Sample VIII	4.65
Sample IX	2.08

Table 3: Specific gravity of soil samples

Soil Samples	Specific gravity (mg/m^3)
Sample I	1.44
Sample II	1.59
Sample III	1.60
Sample IV	2.58
Sample V	1.36
Sample VI	1.56
Sample VII	1.94
Sample VIII	1.65
Sample IX	1.78

Table 4: Bulk density of the soil samples

Soil Samples	Bulk density (g/cm^3)
Sample I	1.81
Sample II	1.59
Sample III	1.49
Sample IV	1.51
Sample V	1.36
Sample VI	1.14
Sample VII	1.34
Sample VIII	1.56
Sample IX	0.86

Table 5: Porosity of soil samples

Soil Samples	Porosity (%)
Sample I	25.69
Sample II	0.0
Sample III	40.56
Sample IV	41.47
Sample V	0.0
Sample VI	26.92
Sample VII	30.92
Sample VIII	5.45
Sample IX	51.68

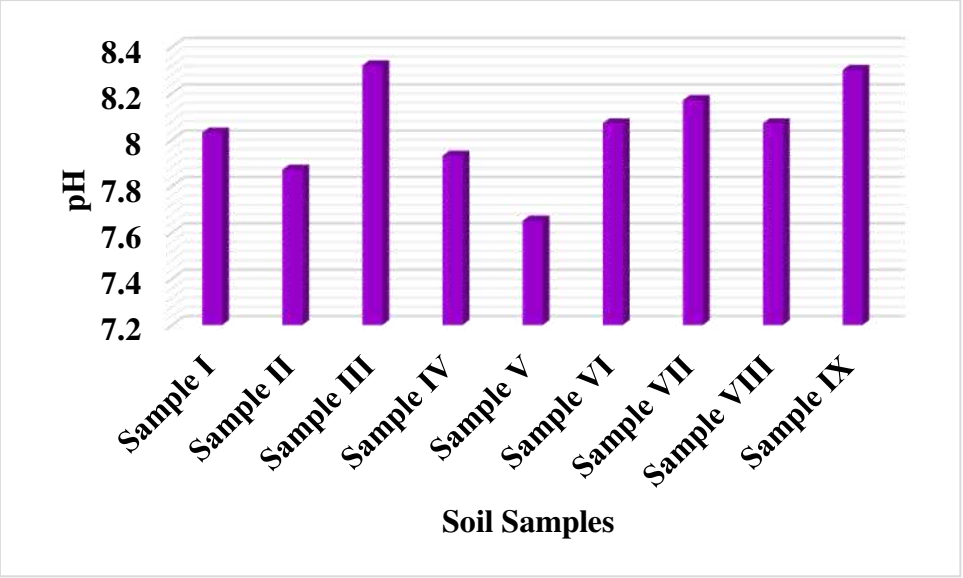


Figure 2: pH values of the soil samples

Table 6: pH values of the soil samples

Soil Samples	pH
Sample I	Alkaline
Sample II	Alkaline
Sample III	Alkaline
Sample IV	Alkaline
Sample V	Alkaline
Sample VI	Alkaline
Sample VII	Alkaline
Sample VIII	Alkaline
Sample IX	Alkaline

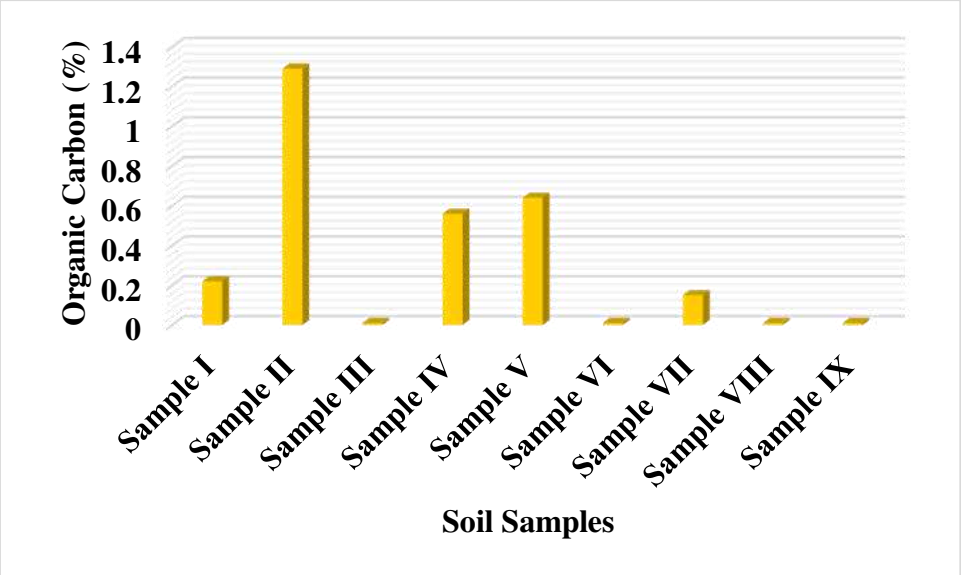


Figure 3: Organic carbon content in the soil samples

Table 7: Nitrogen, Phosphorus and Potassium content in the soil samples

Soil Samples	Nitrogen (Kg/ha)	Phosphorus (Kg/ha)	Potassium (Kg/ha)
Sample I	150.4	45.58	230.2
Sample II	302.9	111.7	203.4
Sample III	118.0	52.73	208.9
Sample IV	198.1	69.71	175.5
Sample V	210.8	66.13	299.5
Sample VI	118.0	25.02	348.2
Sample VII	140.0	65.24	305.1
Sample VIII	118.0	26.81	185.2
Sample IX	118.0	48.26	352.4

Table 8: Sulphur content in the soil samples

Soil Samples	Sulphur (mg/Kg)
Sample I	6
Sample II	9
Sample III	7
Sample IV	5
Sample V	17
Sample VI	3
Sample VII	5
Sample VIII	4
Sample IX	3.0

Table 9: Iron content in the soil samples

Soil Samples	Iron (mg/Kg)
Sample I	0.39
Sample II	18.0
Sample III	5.59
Sample IV	8.73
Sample V	17.0
Sample VI	4.25
Sample VII	6.49
Sample VIII	5.37
Sample IX	7.72

Table 10: Copper content in the soil samples

Soil Samples	Copper (mg/Kg)
Sample I	1.21
Sample II	10.4
Sample III	0.20
Sample IV	2.62
Sample V	27.5
Sample VI	0.93
Sample VII	0.09
Sample VIII	6.74
Sample IX	2.71

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Phytochemical Analysis of *Syringodium isoetifolium* And *Cymodocea serrulata*

Short term project work submitted toSt. Mary's College (Autonomous), Thoothukudi, affiliated to Manonmaniam Sundaranar University, Tirunelveli in partial fulfilment for the award of degree of

Bachelor of Science in Botany

by

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2020-2021

CERTIFICATE

This is to certify that this short term project work entitled **Phytochemical Analysis of *Syringodium isoetifolium* and *Cymodocea serrulata*** submitted to **St. Mary's College (Autonomous), Thoothukudi,** affiliated to **Manonmaniam Sundaranar University, Tirunelveli** in partial fulfilment for the award of the degree of Bachelor of Science in Botany was done under my supervision by the following students at the Department of Botany, St. Mary's College (Autononous),Thoothukudi during the year 2020-2021.

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INTRODUCTION

INTRODUCTION

Seagrasses are one of the most productive and dynamic ecosystem on earth. Seagrass is an angiosperm that lives in marine or brackish environment. Unlike the flowering land plants, sea grass flowers are submerged. They represent a unique flora adapted to rigorous salinity, immersion, occasional desiccation, anchorage on the sea bed and hydrophilic pollination. Sea grasses are widely distributed along the coasts of temperate and tropical seas, and may be considered a conspicuous feature of the shore. They are found along the coasts having clear, shallow water which allows light penetration. The sea grass meadows play an important ecological role as they provide food source and shelter for many organisms such as crustaceans like shrimp, fish, sea turtles, sea horses and sea cow. They play important role in recycling of nutrients. Their extensive root system minimises the benthic erosion. Sea grasses are one of the prominent and specialized groups of marine flora, but are poorly explored and tapped in India compared. They are not only known for their ecological significance but also medicinal use which is much less explored area when compare to the amount of research available related to their ecological significance.

Sea grasses are monocotyledons that are not true grasses, but are rather more closely related to the Lily family. Sea grasses evolved approximately 100 million years ago from land plants that return to the sea. The vegetative plant body consists of roots, rhizome, stem and leaves. Vegetative mode is the most common mode of reproduction for sea grasses. Most sea grasses can reproduce by pollination while submerged and complete their entire life cycle under water. Pollination in sea grasses is hydrophilic. Sea grass pollen grains are elongated into a filamentous shape. The filamentous nature of pollen grains helps transport within water. (Len McKenzie, 2008)

Sea grass requires two key nutrients, nitrogen and phosphorus, for growth. Sea grasses themselves are food for a large number of herbivores including urchins, manatees and sea turtles. Green sea turtles are a particularly important consumer of sea grasses. Sea grasses may significantly influence the physical, chemical and biological environments in which they grow by acting as 'ecological

engineers or foundation plant species'. Sea grass beds absorb and transform nutrients in the marine environment. Sea grasses can improve water quality. Fast moving water stirs up the sediment on the bottom, which makes the water cloudy. Sea grasses can also help by acting like a filter if there are too many nutrients in the water or sediment.

The seagrass community needs a delicate balance to survive. Large-scale threats to seagrass survival include global climate change, storms and mass removal for coastal development. The diving and snorkelling tourism industry can also pose a significant risk. Operators often ground or anchor boats in shallow seagrass areas for loading and unloading. Divers and snorkelers also often accessed sites via a long trudge from shore over this delicate habitat. This leaves large scars in the seagrass, removing essential habitat and food for many species. If this disturbance is repeated regularly, the meadow may never recover.

Sea grasses have been used to fertilize fields, insulates house, weave furniture, thatch roofs, make bandages, and fill mattresses and even car seats. Sea grasses support commercial fisheries and biodiversity, clean the surrounding water and help take carbon dioxide out of the atmosphere. Sea grasses are known as the "lungs of the sea" because one square meter of sea grass can generate huge amount of oxygen everyday through photosynthesis. In response to physical, chemical and biological changes in the environment, Sea grass produces the bioactive compound, which can be used in the treatment of fever, skin diseases, muscle pain and wound healing. Under stress conditions, sea grass acts as a defence mechanism due to the production of secondary metabolites. Sea grass also provides dietary fibre, minerals, vitamins, amino acids and fatty acids to the marine species. Sea grasses were used as a livestock feed, fertilizer, also used as a food for marine organisms. They also rich in protein, fibre, and lipid and the beneficial effects of this is, it will cures obesity and diabetes. Sea grasses have anti fungal activity, anti microbial activity, anti cancer, anti oxidant and anti viral agents. (Kavitha*et al.*, 2020)

The diverse seagrass species are getting differ from the others by not only it's appearances but also in the primary, secondary metabolites present in it and

their quantities. These constituents are phenolic compounds, flavonoid, tannin, proteins, vitamins, sugars, steroids, alkaloids, saponin, amino acids etc., and all of them were confirmed by appropriate methods. The amount of amino acids in the sea grasses was found to be very high compared to sea weed but the level of carbohydrates, lipid is very low. Presence of these components in the sea grass offers different therapeutic activities such as antioxidants, anti-viral, anti cancer, anti inflammatory, pyretic muscle pain, skin diseases, stomach problems anti diabetic wounds tranquillizer and protection of plant proteins from ruminants. (Trease GE *et al.*, 1978)

The importance of seagrasses has been documented in many coastal communities around the world, including India, Africa, Canada, Mexico and Sweden. Some of these studies describe the role of seagrasses in ecosystem function, while others highlight their economic and traditional value, such as the research of Wyllie-Echeverria and Cox concerning wild and commercial gathering of seagrass by North American fishing communities during the early to mid 1900s for use as green manure and insulating products. Seagrasses have also been studied for their potential use in modern medicine. Recent research on seagrassphyochemistry has shown that they are an important source of antioxidants, antibacterial agents, vitamins, minerals and anticancer compounds (Trease GE *et al.*, 1978; Bharathi NP *et al.*, 2011).Newmaster AF *et al.*,(2011) provide survey based detailed record of 12 ailments such as wounds, sea sickness, low blood pressure, heart disease, indigestion, hangover, iron deficiency, skin disease, burns, boils, acid reflux and mental disorders for which fifty ethnotaxa serve as treatment. (Newmaster AF *et al.*,2011)

Compared to algae, seagrasses remain less exploited despite the fact that they offer tremendous opportunities to find new commercially valuable phytochemicals. The present study aimed to analyse the phytochemicals in two sea grass species such as *Cymodoceaspp, Syringodium spp.* The study brings out the medicinal value of *Cymodoceaserrulata* and *Syringodiumisoetifolium* which can be used as a nutraceutical preparation in various food and pharmaceutical industries.

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIVES

In the present study we aimed to

1. sequentially cold extract the phytochemicals using organic solvents in the increasing order of their polarity
2. cold extract phytochemicals from S*yringodiumisoetifolium* by passing it through hexane, chloroform, acetone, ethyl acetate and methanol
3. cold extract phytochemicals from *Cymodoceaserrulata* using petroleum ether, chloroform, acetone, ethyl acetate and methanol
4. test all the samples for the presence of flavanoids, alkaloids, quinones and terpenoids
5. analysethe biological significance of the results by text mining.

The search for new drugs is progressing rapidly. We expect our humble effort using two popular seagrass of Tuticorin coast may provide some preliminary information to the search data pool, and add up to the great efforts.

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS

Chemicals: Hexane, petroleum ether, chloroform, acetone, ethyl acetate, methanol, sodium hydroxide, hydrochloric acid, Meyer's reagent, sulphuric acid

Glass wares and equipments: test tubes, petridish, funnel, beaker, measuring cylinder, pipette, dropper, test tube stands, electronic weighing machine

Biological Samples: Shade dried leaves of *Syringodium isoetifolium* and *Cymodocea serrulata*

METHODS

Seagrass Collection

Coastal region of Hare Island was surveyed to collect the dominant sea grass species in the area during low tide. Time of visit was fixed by using online tidal chart. The fresh plant materials of *Cymodocea serrulata* and *Syringodium isoetifolium* were collected from the Hare Island . Sea grasses were collected in bulk and separated at lab. The plants were identified with the help of local floras. The collected plants were preserved as per the standard procedure (Jain and Rao, 1977). Voucher specimens of all the selected taxa were deposited and preserved at Dpt of Botany, St. Mary's college, Thoothukudi, Tamil Nadu, India.

The collected sample were washed with sea water to remove epiphytes and sand particles and then with fresh water. The clean plant materials were shade dried and saved for processing.

Preparation of plant extracts

10 grams of each sample was weighed. The *Syringodium* sample was first immersed in the 50ml Hexane solution and left it for three days. After three days the extract was filtered through muslin cloth and stored it in brown bottle. The remaining material was dried and passed sequentially through chloroform,

acetone, ethyl acetate and methanol. Before each passing, the samples were air dried for in a clean petridish to make sure that the solvent was completely evaporated off. All the extracts were collected in reagent bottles and saved for further processing. The *Cymodocea* sample was first immersed in petroleum ether and then passed through chloroform, acetone, ethyl acetate and methanol. All other processing is same as that of *Syringodium*.

Qualitative analysis

Test for flavonoids

2ml of plant extract is transferred to the test tubes and then the 1ml of 2N sodium hydroxide was added. Presence of yellow colour indicates the presence of flavonoids.

Test for alkaloids

2ml of plant extract was taken in the clean test tube and then added 2ml of concentrated hydrochloric acid. Then few drops of Meyer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

Take 1ml of plant extract is taken in the test tube and then 1ml of concentrated sulphuric acid is added. Formation of red color indicates the presence of quinone.

Test for terpenoids

To 0.5ml of plant extract is taken in the clean test tube. Then add 2ml of chloroform and concentrated sulphuric acid carefully. Formation of red brown color at the interface indicates the presence of terpenoids.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Plant based compositions were widely utilized by the oldest medical system, the Chinese and the Indian Ayurvedic medicine.Phytochemistry is the study of the chemicals produced by plants, specially the secondary metabolites, synthesized for their self-defence. Phytochemicals are simply plant-derived chemicals. The word "phyto" comes from the Greek word'phyton',it means plant. It is used to refer to the secondary metabolites produced by plants. Phytochemicals are used in the treatment of various diseases such as infectious diseases, neurological diseases, cancer, systemic diseases, cardiovascular diseases etc. The knowledge became prominent in the 19th and 20th century due to the extensive research using sophisticated hybrid chromatography and spectroscopy for the extraction, isolation, characterization, and purification of phytochemicals. This time around, research is ongoing and individual molecules are been constantly discovered. The search for the discovery of new drugs and repurposing of existing ones have driven the study of phytochemistry to a new era employing *in silico* study techniques, applying simulation, and molecular docking procedures of bioinformatics and cheminformatics.

It is used to refer to the secondary metabolites produced by plants. Phytochemistry is important in search for the discovery of new drugs and repurposing of existing ones. Characterization and standardization of traditional herbal drugs in the crude form can also be done. Assessment of the toxicity levels of plants(Mohammad *et al.*, 2019; Ragunath*et al* ., 2015)and understanding of plant physiology biosynthetic pathways, and metabolomics is another aspect of phytochemistry(Adeyemi*et al.*, 2013).Phytochemistry can be applied in the identification and classification of plants and study of inter and intraspecific chemical variability within plants (Andres *et al.*, 2019).

Biotechnology and genetic engineering for the optimization and synthesis of classic compounds and the development of environmentally friendly, biofungicides, insecticides, pesticides, and herbicides, food preservations, phytoremediation of toxic substances such as poisons and heavy metals etc are also fall under the applications of phytochemicals: A vast number of

phytochemicals have been reported. A simple classification system divides phytochemicals into three chemically distinct groups. They are the phenolics, terpenes, N, and S containing compounds. (Deepak *et al.*, 2018)

Phytochemicals are metabolites, usually synthesized as a measure for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure, and environmental hazards. Phytochemicals differ from the essential nutrients (primary metabolites) such as the carbohydrates, proteins, fats, minerals, and vitamins that are needed for the day to day maintenance of the plants. Sometimes, phytochemicals are used to refer to functional foods with antioxidant properties, nutraceuticals, phytonutrients, antinutrients, phytotoxins, and so forth.(Farzana*et al.*, 2019)

Phytochemicals perform quite a number of roles in the living organisms, and the mechanism by which they accomplish it has not been fully understood. However, phytochemical functions as: antioxidants by preventing oxidative damage of important biomolecules such as nucleic acids, proteins, and fats; antimicrobial agents: antibacterial, antifungal, antiviral, anti-trypanocidal agents, stimulation of immune system, modulation of detoxifying enzymes, anti-inflammatory functions, reduction of platelet aggregations, physiological activities such as interfering with the binding of pathogens to cell receptors etc.(Kyung and Robert, 2014)

Others include antimalarial activity, antidiarrheal, antihelminthic, hepatoprotective, anti-atherosclerosis, anti-allergic, antidiabetic, antimutagenic, wound healing, pain relief, and antihypertension. Phytochemicals are also used in the treatment of a sore throat, cough, toothache, ulcers, menstrual bleeding, improving sperm count, dysentery treatment, stomach upset, vertigo, and appetite enhancing. Many other functions of phytochemicals exist depending on the plant. About 80% of the world most useful drugs are from plants.

Plants synthesize a large number of secondary metabolites that do not play a direct role in their growth but help them to survive in the environment especially by providing defence against diseases and pests. The wide variety of

secondary compounds is synthesized mainly by the isoprenoid, phenylpropanoid, alkaloid or fatty acid, or polyketide pathways. A lot of essential oils in plants have shown a high potential for getting rid of insects. A range of essential oils such as cinnamaldehyde, α-pinene, extracts from clove (*Syzygium aromaticum*, major oil being eugenol) and star anise (*Illicium verum*) has been shown to have fumigant and antifeedant. Plant-derived aldehydes and ketones play key roles against pathogenic fungi. Among aliphatic aldehydes and ketones, cinnamaldehyde has been shown to have the most potent activity against fungi especially two species of *Penicillium* that causes disease in humans(*P. cyclopium* and *P. frequentans*). The effects of perillaldehyde and citral were slightly weaker but potent enough. *Penicillium italiense*, an important pathogen causing molds in citrus, and other *Penicillium spp.* causing molds in apple and pear can be targeted using these aliphatic aldehydes that have one or more double bonds.(Kurita N *et al.*,1981;Chukwuebuka *et al.*, 2018)

Among aromatic aldehydes, cuminaldehyde had been shown to have fairly potent antifungal activity (Kurita N *et al.*, 1981)). The essential oils of *Thymbra spicata* and *Satureja thymbra* plants used as spices in Mediterranean cuisine have been shown to inhibit phytopathogenic fungi such as *Fusarium moniliforme*, *Rhizoctonia solani*, and *Phytophthora capsici* at a concentration of 400–800 µg/mL.Thymol and carvacrol have been identified as the major constituents in the essential oils involved in the fungicidal property, followed by monoterpenes γ-terpenin and p-cymene.(Chukwuebuka *et al.*, 2018)

Phenolic compounds play a signicant role in plant defense against bacteria and fungi. One important phenolic compound is coumarin. Halogenated coumarin, often brominated, chlorinated, or iodinated, is more stable than coumarin. It has been shown to be particularly effective against plant pathogenic fungi such as *Macrophomina phaseolina* (charcoal rot), *Phytophthora spp.* (damping off and seedling rot), Rhizoctonia spp.(damping off and root rot), and *Pythium spp.* (seedling blight). These four fungi are from different families, showing the broad spectrum activity of halogenated coumarins. In addition, halogenated coumarins have polymer seed coating abilities and less phytotoxicity, making them good candidates for natural pesticide development.(Chukwuebuka *et al.*, 2018).In

another study, 7-hydroxylated coumarin has been shown to be effective against parasitism of *Orobanchecernua*in sunflower. (Serghini*et al.*, 2001)

Terpenes are a big and diverse class of phytochemicals, also known as terpenoids. They are mostly found in plants and form the major constituent of essential oils from plants. Among the natural products that provide medical benefits for an organism, terpenes play a major and variety of roles. The common plant sources of terpenes are tea, thyme, cannabis, Spanish sage, and citrus fruits (e.g., lemon, orange, mandarin). Terpenes have a wide range of medicinal uses among which antiplasmodial activity is notable as its mechanism of action is similar to the popular antimalarial drug in use—chloroquine. Monoterpenes specifically are widely studied for their antiviral property. With growing incidents of cancer and diabetes in modern world, terpenes also have the potential to serve as anticancer and antidiabetic reagents. Along with these properties, terpenes also allow for flexibility in route of administration and suppression of side effects. Certain terpenes were widely used in natural folk medicine. One such terpene is curcumin which holds anti-inflammatory, antioxidant, anticancer, antiseptic, antiplasmodial, astringent, digestive, diuretic, and many other properties. Curcumin has also become a recent trend in healthy foods and open doors for several medical research works. (Nivedida*et al.*, 2019)

Alkaloids are a huge group of naturally occurring organic compounds which contain nitrogen atom or atoms (amino or amido in some cases) in their structures. Generally based on structures, alkaloids can be divided into classes like indoles, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, tropanes, and terpenoids and steroids. Alkaloids because of their bitter taste are natural compound to deter herbivorous organisms. In some plants they are used as natural pesticides. It was suggested that alkaloids in plants have a function to protect them from destructive activity of some insect species. Alkaloids have diverse physiological effects: antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and antitumor activity and many others. Nowadays, alkaloids from plants are of great interest to organic chemists, biologists, biochemists, pharmacologists, and pharmacists. Well-known

alkaloids include morphine, strychnine, quinine, atropine, caffeine, ephedrine, and nicotine (George R *et al.*,1978).

Tannins are another class of phenolic compounds that provide defensive properties. Though tannins are mostly known to provide defense against herbivores due to their astringent properties, they also play some fungicidal roles. They are active against *Colletotrichumcircinans*, a fungus that causes smudge in onions. Tannins are also known to be inhibitory for fungal spore germination (Mazid S *et al.*, 2011).

Sources of phytochemicals

Phytochemicals are found in fruits, vegetables, whole grains, spices, legumes, herbs, shrubs, and trees. They get accumulated in plant parts at different concentrations such as in the leaves, fruit, bark, stem, roots, seeds, and flowers. Some phytochemicals are also synthesized by other living organisms such as fungi, although the mechanism by which they synthesize it might differ. However, many foods containing phytochemicals are already part of our daily diet except for some refined foods such as sugar or alcohol. The easiest way to get more phytochemicals is to eat varieties of at least five to nine servings of fruits or vegetable per day representing colors of rainbows. (Jonathan C *et al.*, Kurita N *et al.*,2018)

Interest in the importance of marine plants as a source of new substances is growing. With marine species comprising approximately half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity (GerwickWH, 1993). Marine and estuarine submersed aquatic angiosperms, or seagrasses, produce antimicrobial compounds that may act to reduce or control microbial growth. Researchers have described antibacterial.(Harrison 1982; Ballesteros *et al.*, 1992; Jensen *etal.*, 1998)

Seagrass

Seagrasses are marine flowering plants falls under in monocotyledonae. They are adapted to the marine environment and complete their life cycle under water In contrast to other submerged marine plants (e.g. seaweeds), sea grasses flower, fruit and produce seeds. They also have true roots and internal system for the transport of gases and nutrient. They are classified in three separate families. Worldwide there are about 12 major divisions, consisting of approximately 60 species of sea grass. The 72 species of sea grasses are commonly divided into four main groups: Zosteraceae, Hydrocharitaceae, Posidoniaceae andCymodoceae. Most common names are applied to sea grass species, such as turtle grass, eel grass, tape grass, spoon grass, and shoal grass. Sea grasses are often confused with sea weeds, but it is not sea weeds.

Distribution of Seagrass

They generally grow in shallow coastal water from the intertidal zone to depths up to 10m. In turbidestuahne environment, such as the Indian coast, where there is an enormous deposition of silt into the sea by major rivers, sea grasses are rarely encountered at depths below 10m. In less turbid areas, such as the Caribbean sea and Australian coast, sea grasses can be found at depths of 50m or more. Seagrasses are widely distributed along temperate and tropical coastlines of the world. (Len McKenzie, 2008; Carmine *et al.*, 2018).

Seagrasses have key ecological roles in coastal ecosystems and can form extensive meadows supporting high biodiversity. The global species diversity of seagrasses is low, but species can have ranges that extend for thousands of kilometers of coastline. The Temperate North Atlantic has low seagrass diversity, the major species being *Zostera marina,* typically occurring in estuaries and lagoons. The Temperate North Pacific has high seagrass diversity with *Zostera* spp. in estuaries and lagoons as well as *Phyllospadix spp.* in the surf zone. The Temperate Southern Oceans bioregion includes the temperate southern coastlines of Australia, Africa and South America. Extensive meadows of low-to-high diversity temperate

seagrasses are found in this bioregion, dominated by various species of *Posidonia* and *Zostera*. The tropical bioregions are the Tropical Atlantic and the Tropical Indo-Pacific, both supporting mega-herbivore grazers, including sea turtles and sirenia. The Tropical Atlantic bioregion has clear water with a high diversity of seagrasses on reefs and shallow banks, dominated by *Thalassiatestudinum*. The vast Tropical Indo-Pacific has the highest seagrass diversity in the world, with as many as 14 species growing together on reef flats although seagrasses also occur in very deep waters. The global distribution of seagrass genera is remarkably consistent north and south of the equator; the northern and southern hemispheres share ten seagrass genera and only have one unique genus each. Some genera are much more speciose than others, with the genus Halophila having the most seagrass species. The most widely distributed seagrass is *Ruppiamaritima*, which occurs in tropical and temperate zones in a wide variety of habitats. Seagrass bioregions at the scale of ocean basins are identified based on species distributions which are supported by genetic patterns of diversity. Seagrass bioregions provide a useful framework for interpreting ecological, physiological and genetic results collected in specific locations or from particular species. (W.Dennison*et al.*, 2007).

Six genera and thirteen species of sea grasses were seen in India, in which maximum number of species was identified in the Gulf of Mannar, palle bay harbor than Andamon and Nicobar Lakshadweep islands.

Features of seagrass

Most of the sea grasses having long, ribbon-like grassy leaves, and are named on as so. But some other forms have ovoid and slightly long leaves (*Halophilis sp.*); those don't have the expected grass-like appearance. They have roots, rhizome, and leaves, and produce flowers and seeds. They have chloroplasts, and there for photosynthetic ability. They capture carbon dioxide 35% faster than the tropical rain forests. The roots absorb nutrients. The water and nutrients are transported throughout the plant body via veins. The air pockets called lacunae help keeping the leaves buoyant and exchange oxygen and carbon dioxide. Seagrass ranges

from the size of your fingernail to the plant with leaves as long as 7 metres. Some of the shapes and sizes of leaves of different species of sea grasses include an oval shape, a fern shape, a long spaghetti like leaf and a ribbon shape. Ones that have a ribbon shaped leaf are the *Cymodocea, Thalassia, Thalassodendron, Halodule* and *Zostera*. Spaghetti like sea grass is called *Syringodium*. At the base of a leaf is a sheaths, which protects young leaves. At the other end of the leaf is the tip, which can be rounded or pointed. Sea grass leaves lack stomata but have thin cuticle to allow gas and nutrient exchange. The roots and horizontal stems (rhizomes) of sea grass are often buried in sand or mud. They anchor the plant, store carbohydrates and absorb nutrients.

Ecological significance of seagrass

A vital part of the marine ecosystem due to their productivity level, sea grasses provide food, habitat, and nursery areas for numerous vertebrate and invertebrate species. Seagrasses perform numerous functions such as stabilizing the sea bottom, providing food and habitat for other marine organisms, maintaining water quality, supporting local economies and stabilizing the sea bottom. Seagrasses are a vital part of the marine ecosystem. Ocean bottom areas that are devoid of seagrass are vulnerable to intense wave action from currents and storms. The extensive root system in seagrasses, which extends both vertically and horizontally, helps stabilize the sea bottom in a manner similar to the way land grasses prevent soil erosion. With no seagrasses to diminish the force of the currents along the bottom, Florida's beaches, businesses, and homes can be subject to greater damage from storms. The relative safety of seagrass meadows provides an ideal environment for juvenile fish and invertebrates to conceal themselves from predators. Seagrass leaves are also ideal for the attachment of larvae and eggs, including those of the sea squirt and mollusk.

Seagrasses strongly impact their physical and biological surroundings and are therefore frequently referred to as ecological engineers. The effect of sea grasses on coastal bay resilience and sediment transport dynamics was studied by Donatelli *et al*,2018. The role of these vegetated surfaces on the sediment storage capacity of shallow bays is investigated using six historical maps of seagrass

distribution in Barnegat Bay, USA. Analyses are carried out by means of the Coupled-Ocean-Atmosphere-Wave-Sediment Transport (COAWST) numerical modeling framework. Results show that a decline in the extent of seagrass meadows reduces the sediment mass potentially stored within bay systems. The presence of seagrass reduces shear stress values across the entire bay, including unvegetated areas, and promotes sediment deposition on tidal flats. On the other hand, the presence of seagrasses decreases suspended sediment concentrations, which in turn reduces the delivery of sediment to marsh platforms. Results highlight the relevance of seagrasses for the long-term survival of coastal ecosystems, and the complex dynamics regulating the interaction between subtidal and intertidal landscapes .(Len McKenzie, 2008; Carmine *et al.*, 2018).

The seagrass ecosystem is defined as a unit of biological organization comprised of interacting biotic and abiotic components. The structural components are shelter and food and feeding pathways and biodiversity. Functional components include the rate of nutrient cycling, the rate of energy flow, and biological regulation. Healthy intact seagrass ecosystems provide services since they relate to the health, stability and well-being of the environment in which they live, but also to that of human populations. (Phillips RC *et al.*,2003).

Gulf of mannar marine biosphere reserve is the first of its kind in India and also in south east Asia. It extends from Rameswaram in the north to Tuticorin in the south. GOM is having a chain of 21 islands running almost parallel to the mainland. These areas are endowed with a combination of ecosystems including mangroves, seagrasses and coral reefs. Remote sensing techniques offer a wide range of possibilities in the study of various ocean related parameters. During the present survey, the occurrence of 12 seagrass species in the islands was verified and is mapped using satellite imagery. The study reported a total of 85.5 sq km area covered by seagrass beds in Gulf of mannar (GOM) based on IRS- 1D 1998 satellite data and there is a need for the continuous monitoring of the seagrass resources because of its importance to the marine environment. (Ramaswamy*et al.*,2009)

The marine sea grasses form an ecological and therefore paraphyletic group of marine hydrophobia angiosperms which evolved three to four times from land plants towards an aquatic and marine existence. Their taxonomy is not yet solved on the species level and below due to their reduced morphology. So far also molecular data did not completely solve the phylogenetic relationships. Thus, this group challenges a new definition for what a species is also their physiology is not well understood due to difficult experimental in situ and in vitro conditions. These remain several open questions concerning how sea grasses adapted secondarily to the marine environment. Here probably exciting adaptations solution will be detected. Physiological adaptations seem to be more important than morphological ones. Sea grasses contain several compounds in their secondary metabolism in which they differ from terrestrial plants and also not known from other taxonomic groups. Some of these compounds might be of interest for commercial purposes. Therefore their metabolite contents constitute another treasure of the some of the most interesting aspects from phylogenetical, physiological and metabolic points of view . (M Kwaaltaal and I. Zarra , 2012)

Seagrasses, one of the most threatened yet overlooked ecosystems on Earth, are the only flowering plants to recolonising the seabed. Apart from their critical ecological prominence on the life of many marine organisms, seagrasses are also used as an alternative or complementary medicine to manage an array of pathological disorders such as muscle aches, wounds, abdominal pain, indigestion, hangover, and mental disorders. However, a compilation of existing work on their ethnopharmacological uses, nutritional values, pharmacological propensities and bioactive compounds is lacking. Thus, this review aims at elaborating on the biochemical composition, phytochemical analysis, and biological properties including antioxidant, antimicrobial activities of various species of seagrasses. Seagrassesharbour several metabolites with multiple bioactivities. The phytochemical compounds isolated from *Zostera marina L.*, *Thalassiatestudinum* and *Thalassodendronciliatum* (Forssk.) exhibit a plethora of biological activities, including cytotoxicity against cancer cell lines, anti-human immunodeficiency virus (HIV), antimicrobial, and skin regenerating properties. This review also identifies vital lacuna in seagrass research. For instance, the

mechanism and site of action of compounds displaying potent biological activities has not been adequately addressed together with optimisation of extraction methods to isolate minor metabolites and applying technological advancements in biological assays. In conclusion, this review provides a synthesis of current knowledge and highlights future work that needs to be undertaken for the biomedical application of such natural resources.(Nabeelah*et al.*, 2021)

Of the six genera of sea grasses recorded in India, four were present throughout a one year study period at a station of Hare Island, Tuticorin (18 degrees 45'N 78 degrees 12'E) in India Gulf of mannar region. Phytochemical analysis of these sea grass extracts revealed the presence of pharmaceutically potent secondary metabolites and appreciable quantities of primary and some secondary metabolites,(Thirumalai*et al.*, 2008). Seagrasses are the residents of the coastal waters, globally which are rated as one of the most valuable ecosystems. During photosynthesis, they release oxygen to the water column and also pumps oxygen into the sediments through their roots to create an anoxic environment around roots to support extensive nutrient uptake. They are represented as one of the highly productive coastal ecosystem as well as of protects the shorelines against the erosion in the middle, lower intertidal and subtidal zones of the world. In folk medicine, seagrasses have been used for a variety of remedial purposes. But there is no review concerning various uses of seagrasses. In the presented study, an attempt had been made on various species of seagrass and find out the different phytochemicals and pharmacological uses.(Kavitha*et al.*, 2020)

Seagrass meadows are among the most diverse coastal. Erik *etal.*,2000test the importance of seagrass density and morphology for benthic infaunal recruitment in a 2 mo (June/July 1997) field-experiment with both bare and vegetated (3 densities of artificial Ruppiamaritima and Zostera marina) colonization trays with azoic sediment. These artificial seagrass patches were placed at 3 m depth in an unvegetated area of a sandy bottom seagrass site on the Åland Islands, northern Baltic Sea. The data shows strong effects of seagrass complexity and wind disturbance on (1) physical processes such as accumulation of drifting algae, particle trapping and sediment binding, (2) development of community

parameters (abundance, species richness, diversity) and (3) species-specific colonization patterns. Their data further demonstrate the importance of post settlement events for distribution of juvenile macrofauna (e.g. resuspension or transport by means of drifting algae), and show negative and positive effects of wind-mediated disturbance in low- and high-complexity habitats, respectively. It is concluded that wind disturbance may act as a mechanism creating and maintaining high animal diversity in seagrass meadows.(Erik *et al*, 2000)

Seagrass beds are thought to have a fundamental role in maintaining populations of commercially exploited fish and invertebrate species by providing one or more of the following: (1) a permanent habitat, allowing completion of the full life cycle, (2) a temporary nursery area for the successful development of the juvenile stages, (3) a feeding area for various life-history stages and (4) a refuge from predation. In addition to these primary roles, seagrass beds are thought to maintain fisheries indirectly by providing organic matter which is incorporated into coastal nutrient cycles and which supports secondary production, including fisheries species. Unfortunately, these roles have been distilled from a disparate literature that often reports results using different sampling methods, seagrass species, geographical locations and temporal or spatial scales. The aims of this review are to summarise the literature assessing the importance of seagrass habitats for fishery species, to highlight possible confounding factors that may help to explain some of the contradictory statements in the literature and to identify areas of seagrass ecology that require further investigation.(Ashley A *etal*, 2001). Multiple studies have documented the ecologically important role that seagrasses play in estuarine and marine ecosystems. Unfortunately, economic valuations of these systems have not been as widespread. To date, most techniques rely on mechanisms that do not incorporate the actual ecological drivers behind the economic service, but rather rely on proxy measures to derive value. In this manuscript we review the many values that seagrasses have that result in economic services, and the valuation techniques used to estimate their monetary value. We present a conceptual framework linking seagrass ecosystems to the economic services they provide, showing the areas where novel valuation approaches are most lacking. We conclude that indirect methods used to valuate seagrass ecosystems underestimate the

economic value of their services, and that more derivative-based models linking ecological structure and function to all associated economic services are essential for accurate estimations of their dollar value.(Bryan *et al.*, 2016)

Sea grasses are the excellent and potential bioresource to discover new natural bioactive compounds such as antioxidants that have beneficial effects on health, natural antioxidants have many functions in biological systems, primarily for defense against oxidation which produces free radicals in food, chemicals and living systems. This study aimed to discover new natural antioxidants agents, *Enhalusacoroides* (L.f). Royal was evaluated for phytochemical constituents and the antioxidant activity against superoxide dismutase (SOD) was assessed. Extracts of the sea grass *E.acoroides* (L.f) Royal activated in different areas have different phytochemical constituents and SOD activities. The secondary metabolites of phenols, flavonoids and steroids contained in the ethyl acetic extracts of *E.acoroides* were in early correlated with their antioxidant activity, which exhibited an IC50 of Fppm.*E.coroides*(L.f) Royal samples cultivated in the two areas contained different phytochemical constituent profiles, indicating an effect of environmental factors, and both can be used as potential natural sources of antioxidant compounds. (Ferry *et al.*, 2020)

Phenolic compounds in plant plays important role in pigmentation, growth, reproduction, resistance against pathogens, defense mechanism as well as protecting plants from electerious effects of ultra violet radiation and oxidants. Comparatively higher contents of phenolics and flavonoids is noticed in sea grasses from Palk Bay than Gulf of mannar respective body parts. The leaves of *C.serrulata* contained phenolics ranging from 41 to 298 mg/100g Gallic acid equivalents and flavonoids 13 to 140 mg/100g quercetin equivalents where as in the roots and rhizomes phenolics varied from 29 to 292 mg/100g and flavonoids 9.1 to 75 mg/100g. The tannins varies from 0.20 to 5.02 mg/100g chlorophyll A in sea grasses ranged from 11.5 to 850 mew g/g chlorophyll B from 11 to 1029 mew g/g and pheophytin from 41 to 204 mew g/g. These results indicate variations in the phenomenon compounds with references to the ecosystem and the presence of other plants in the meadows. (Libin*et al.*, 2016)

Seas and oceans represent a big store of numerous marine organisms and offer an enormous source of many novel compounds. With the increasing trend in antimicrobial resistance, nowadays the uses of marine organisms for the production of pharmaceuticals are becoming interesting. In comparison to terrestrial, marine organisms possess very exigent competitive and aggressive surrounding, which is very different in many aspects from the terrestrial environment. Such a situation demands them to produce quite specific and potent active molecules. Marine angiosperms especially sea grass are one of the shelters for a wide array of secondary metabolites like saponins, phlobatannins, phenols, amino acids, protein, tannins, flavonoids, terpenoids, cardiac glycosides and steroids. Naturally, these sea grass are also used to produce compounds for a defense mechanism which are found to be antioxidative in nature. The present review is thus focusing to present the pharmacological activities of eight marine sea grass species found in the Eritrean Red Sea for their antimicrobial and antioxidant properties.(Mehari *et al*, 2019)

Antimicrobial activities of the three sea grass *Cymodoceaserrulata*, *Halophilaovalis*and*Halodulepinifolia* were tested against ocular pathogens *E.coli*, *Enterococcus faecalis*, *Coreynebacterium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiellapnemonia*, *Methicillin sensitive*, *Staphylococcus saphrophyticus*and *Staphylococcus epidermidis* using different solvents hexane, ethyl acetate, chloroform and ethanol namely were investigated. The chloroform and ethyl acetate extract showed maximum activity. In the case of phytochemical analysis the ethyl acetate, ethanol and chloroform extracts showed positive activity with phytoconstituent such as phenols, steroids, terpinoids, flavanoids, alkaloids, glycosides, saponins and tannins but sugars and quinine show negative activity. Further experiments are underway to isolate active compounds in controlling the growth of pathogens. (Girija K *et al*, 2013)

The coast of southest Indian and the tropical part of the western pacific is a habitat for the tropical sea grass *Enhalusacoroides*. In Ekas Bay, East London, *E.acoroides*grows well in seashores which is important to tropical marine ecosystem. Previous research reported the pharmacological activity of *E.acoroides* such as antioxidant and antimicrobial. *E.acoroides*was tested to

identify their secondary metabolites using phytochemical screening. The secondary metabolites were further characterized using thin layer chromatography with specific spray reagent. The phytochemical screening identified primary metabolites such as carbohydrates and proteins. On the other hand, the phytochemical screening also detected secondary metabolites such as alkaloid, phenolic, tannin, saponins, flavonoids, monoterpenes and sesquiterpenes. The TLC profile with specific spray reagent confirmed the bioactive components such as phenolic, flavonoid and terpenes in methanol extract of *E.acoroides*. These results had significant impact on profiling the marine plant as a new drug candidate. The information of secondary metabolites from *E.acoroides* will contributed to further research in determination of antioxidant and antimicrobial activity of *E.acoroides* as a promising marine drug candidate from Indonesia.(J P Purba*et al*, 2019)

It is essential to study the phytochemical constituents and toxicological properties of seagrasses when considering their food applications. Aqueous methanolic extracts of six seagrassesare evaluated for their antibacterial, cytotoxic (brine shrimp leathality assay) and haemolytic activity. Thin layer chromatography (TLC) and phytochemical analysis were used to compare the phytochemical profiles of six seagrasses. Among the six seagrasses examined, *Halodulepinifolia* and *Cymodocearotundata* showed predominant growth inhibitory activity against all the tested human pathogens. Cytotoxicity of seagrass extracts against nauplii of Artemiasalina revealed that *Syringodiumisoetifolium*exhibited lesser toxicity with LC50 value of 699.096 lg/ml. Of all the seagrasses tested, *H. pinifolia* recorded the minimum haemolytic activity of 2.07 ± 0.63% at 1000 lg/ml concentration. Phytochemical analysis showed the presence of common plant chemical constituents which varied with respect to species. These findings suggest the possible pharmacological applications of selected seagrasses that can be used as food ingredients. (Rengasamy*et al*., 2012)

*Thalassiahemprichii*s one type of sea grass that grows in Indonesia water areas. Sea grass is often found in the water areas of Lampung, Bali, Kepulauanseribu, Manado and Wakatobi. *Thalassiahemprichii*s not used only to maintain the sea

ecosystem, especially the shallow sea, but also to be processed as medicines and cosmetics. *Thalassiahemprichii* can be used as medicines because it contains secondary metabolite compound which has the potential to be an antimicrobial that fights against agents of pathogenic diseases on human. As the basic material of cosmetics, *Thalassiahemprichii* contains high antioxidants which can counteract free radicals. It is an added values that *Thalassiahemprichii* has to be the basic material for cosmetic products. Besides those some functions, *Thalassiahemprichii* extract has the potential as antifungal, antivirus, antifertility, anticancer and antidiabetes. (Jafriati *et al.*, 2019)

The antioxidant potential of methanol, acetone and hexane extract of three seagrasses collected from Tuticorin coast are determined using total antioxidant activity, total phenolic compound, DPPH radical scavenging activity, hydrogen peroxide radical scavenging assay, nitricoxide radical scavenging assay and reducing power. The methanolic extract of *Halodulepinifolia*, shows higher phenolic content than other seagrasses. Higher antioxidant activity is observed in methanol and acetone extract of *H. pinifolia*. Higher DPPH radical scavenging activity is also observed in the methanol extracts of *H. pinifolia* (87.81%). Higher hydrogen peroxide radical scavenging activity observed in the hexane extract of *H. pinifolia* (71.49%). Nitricoxide radical scavenging activity is observed in the hexane extract of *Syringodiumisoetifolium*(51.49%). The maximum reducing power is observed in methanolic extract of *H. Pinifolia*. In the present study, the extract of *H. pinifolia* is found to possess strong antioxidant activity. The antioxidant mechanisms of seagrass extracts may be attributed to their free radical-scavenging ability. In addition, phenolic compounds appear to be responsible for the antioxidant activity of seagrass extracts. (J. Sangeetha and S. Ashokan, 2016)

Cymodaceaceae is a family of flowering plants, sometimes known as the "manatee – grass family", the family cymodaceaceae includes only marine species. The angiosperm phylogeny II systems, of 2003 (unchanged from the APG system, of 1998), does recognize cymodaceaceae and places it in the order Alismatales, in the clade monocots. They are marine hydrophytes that grow and complete their life cycle is a submerged condition in a saline environment. Like

terrestrial plant they obtain their energy from light through photosynthesis thus, they grow only in clear and shallow water, and at the suitable condition, they form beds or meadows. The family includes five genera, totalling 10 species of marine plants occurring in tropical seas and oceans. Cymodaceaceae consist of five genera such as *Amphibolis, Cymodacea, Halodule, Syringodium* and *Thalassodentron*. These sea grass species have unique nature and wide application to the environment including human being. In this article botanical aspects, phytochemistry and ethanopharmacology of these five sea grass species belong to cymodaceaceae family will be discussed. (Pushpa*et al*., 2017)

Antibacterial activities of the solvent extracts of sea grass *Cymodocearotundata* against 10 human pathogens were investigated. The ethanol extract showed best activity. In the case of phytochemical analysis the ethanol and FYImethanol extracts show positive activity with phytoconstituents such as tannins, saponins, resins, proteins, acidic compounds, reducing sugars, terpenoids, cardiac glycosides and alkaloids but phenols, steroids, catachols and flavanoids showed negative activity. (Mani AE *et al*., 2012)

Syringodiumisoetifolium comes under the family Cymodoceaceae, this grass is otherwise called as noodle grass. Phytochemical compounds such as flavonoids, terpenoidscoumarins, xanthoproteins, sugars, carboxylic acids can be extracted by the solvents such as methanol, hexane, and acetone.*Syringodiumisoetifolium* has pharmacological activities such as antioxidant, antihemolytic, antibacterial cytotoxicity, and antifungal activity. During photosynthesis, lacunal gas is discharged from lacunae, and it is composed of oxygen and nitrogen. Pheophytin a compound was isolated from the crude extract of *Syringodiumisoetifolium* by treating it with human pathogens. Binding sites of the pathogen can be found by the Molecular docking study.(Kalaivani P *et al*., 2019)

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Plant derived substances have recently become of great interest owing to their versatile applications. Phytochemicals are non-essential nutrients and mainly produced by plants for defense. 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 contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. They offer protection against numerous diseases and disorders such as diabetes, high blood pressure, coronary heart disease, osteoporosis, microbial, viral and parasitic infection, psychotic disease, ulcers, cancer, neurologic disorders, inflammation and allied disorders (Kavitha D *et al*, 2020). Phytochemistry research is actively growing around the globe.

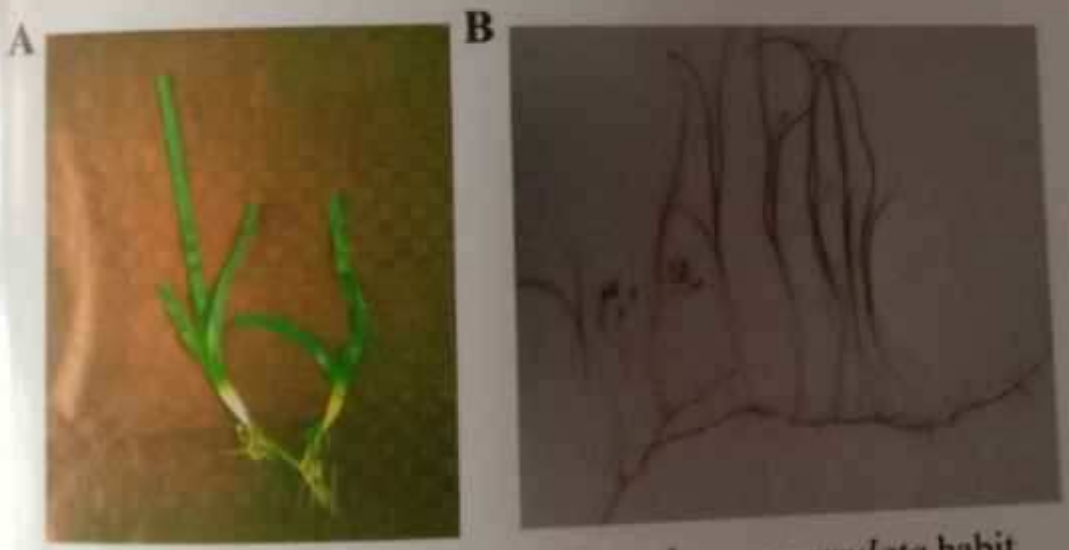

Figure 1: Seagrass habit. A. *Cymodoceae serrulata* habit B. *Syringodium isoetifolium* habit

Researchers explore new disease targets and ligands since the success rate of various drugs using currently is not up to the expectation. Seagrass recently became one of the interesting target plant groups of phytochemical studies.

Though their ecological relevance has been studied widely their phytochemicals are not tapped out much.

The present study aimed to reveal the presence of flavanoids, alkaloids, quinones and terpenoids in (secondary metabolites) in hexane, chloroform, acetone, ethyl acetate and methanol of leaves of *Syringodiumisoetifolim*andpetroleum ether, chloroform, acetone, ethyl acetate and methanol of leaves of *Cymodoceaserrulata*. The solvent extraction was done using all the five different solvents successively in increasing order of their polarity at room temperature under an incubation period of 3 days each. The results are depicted in Table 1-2 and Figure 1-2.

Seagrass meadows are declining in a faster rate because of various reasons (Suzanna M *et al.*, 2018). Therefore the collection of leaf for the study is a justifiable mean to cope up with seagrass conservation view. To make use the collected sample maximum a sequential cold extraction method was adopted in extracting the phytochemicals.

The result of preliminary screening reveals the presence of alkaloids, flavonoids, quinones, and terpenoids in hexane, chloroform, acetone, ethyl acetate and methanol of leaves of *Syringodiumisoetifolim*andpetroleum ether, chloroform, acetone, ethyl acetate and methanol of leaves of *Cymodoceaserrulata*.

The hexane fraction of *Syringodiumisoetifolim* reveals quinone and terpenoids. Chloroform extract was showing a strong green colour against HCl-Meyer's reagent test, indicating the presence of alkaloid and terpenoides test was also positive for chloroform fraction. Acetone extract revealed alkaloid and quinone. The ethyl acetate extract exhibited dark red colour against sulphuric acid indicating the presence of quinone and brown colour against sulphuric acid – chloroform test indicating terpenoid presence. The methanol fraction produced dark green colour for alkaloid test. Methanol extract also produced mild yellow colour against sodium hydroxide indicating the presence of flavanoids. These results are displayed in Figure 1 and Table 1.

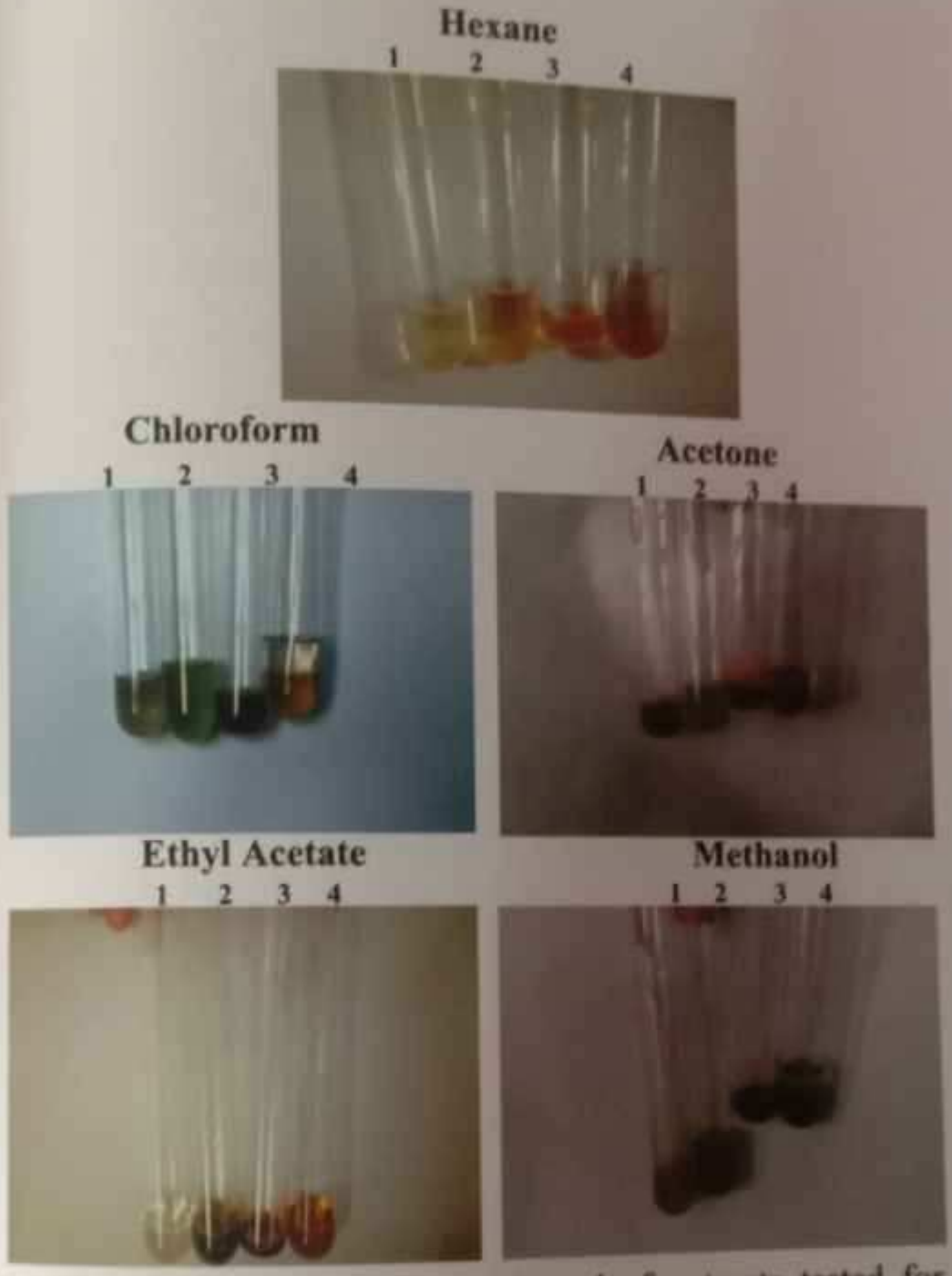

Figure 2: *Syringodium isoetifolium* leaf extracts tested for secondary metabolites. 1 - flavanoid, 2 - alkaloid, 3- quinones 4 - terpenoides.

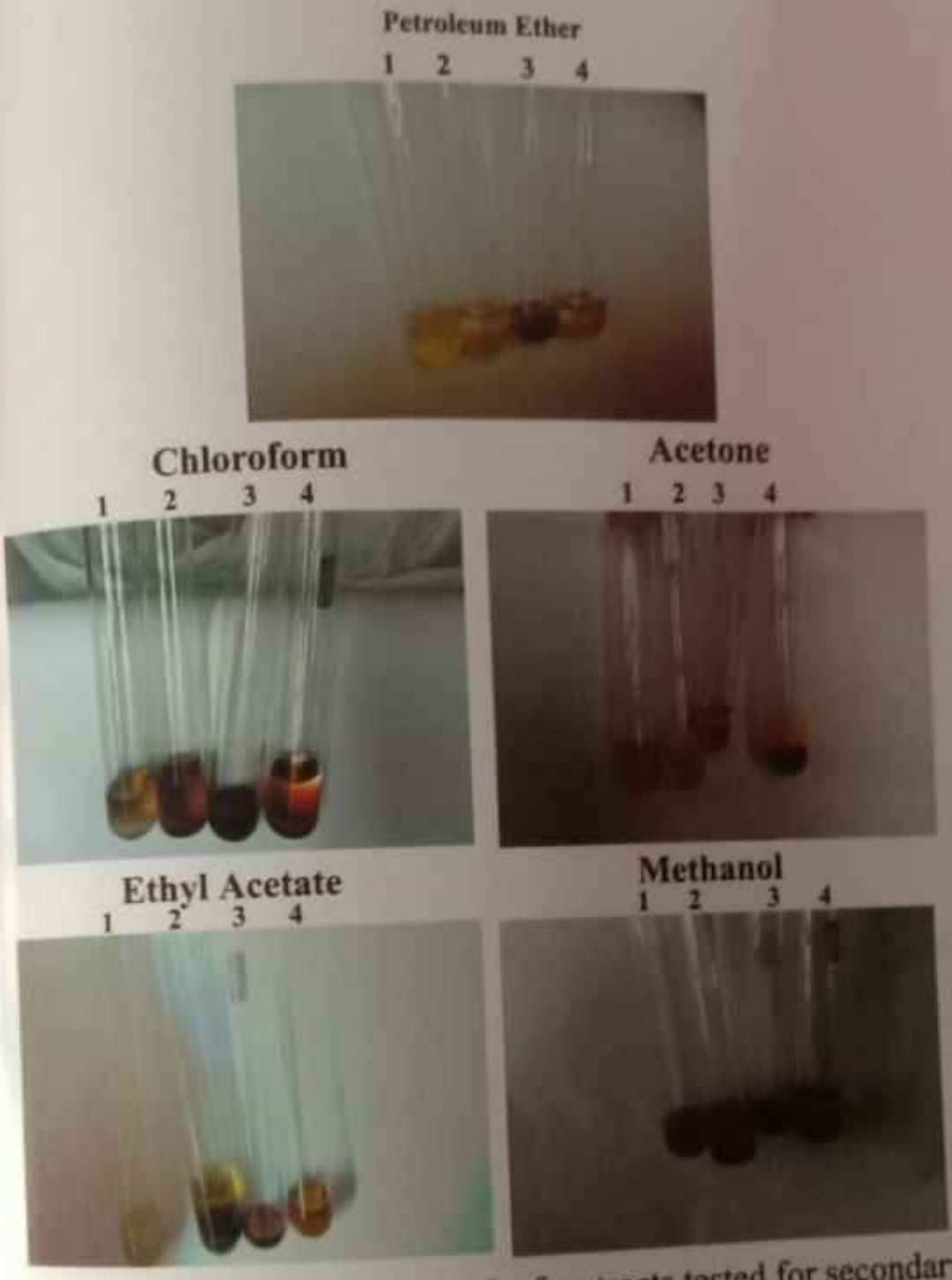

Figure 3: *Cymodoceae serrata* leaf extracts tested for secondary metabolites. 1- flavanoid, 2 - alkaloid, 3- quinones 4 - terpenoides.

Table 2: Preliminary screening for flavonoids, alkaloids, quinones and terpenoids in the leaf extracts of *Cymodoceaserrulata*

Active Component	Different extracts of *Cymodoceaserrulata* leaf				
	Petroleum Ether	Chloroform	Acetone	Ethyl Acetate	Methanol
Flavanoid	+	-	++	+	-
Alkaloid	-	-	+	+	-
Quinone	+	+	-	++	++
Terpenoid	+	++	++	-	-

(+) Positive sign indicates present, (-) Negative sign indicates absent, (++) relatively very high concentration

Both the species of sea grasses under study belongs to the same family. But when compared the distribution of secondary metabolites under consideration, it was observed that the quinones and terpenoides were widely distributed in all the polarity ranges from hexane to methanol. But terpenoides were not indicated in methanol fraction. In none of the fractions, flavanoids were strongly indicated. The difference in phytochemical distribution indicates the change in genetic constitution. These differences additionally support the differences in their genus though the morphology itself distinct (Dawes CJ, 1981).

The phytochemicals presented above are the integral part of the defense system, and may be responsible for the survival of *C. serrulata* and *S. isoetifolium* in the sea. They play a major role in the plant defense system by acting against pathogens and also as an antioxidant thereby preventing cells from damage (Kinghorn AD *et al.*, 2009).

Flavonoids are naturally occurring biological compounds that can act as potent antioxidants and can prevent cardiovascular disease by preventing the oxidation of LDL. They also reduce the risk of cancer by acting as a natural scavenger of

free radicals (Lekshmi S *et al.*, 2018).The presence of flavonoids estimated in petroleum ether, acetone and ethyl acetate extracts *of C. serrulata* implies the application of this extract not only as an anticancer and anti-inflammatory agent but also as an agent to treat various cardiac ailments (Doughari JH *et al.*, 2012).Raghupati RK *et al* (2013) observed that the flavonoids contributed significantly to the antioxidant property of the seagrass (Ragupathi*et al.*, 2013).Nanthakumar R*et al.*,(2013) reported that *C. serrulata* methanolic extract exhibits antioxidant property and cytotoxicity activity against the HeLa cells with growth inhibition of 40.47% at 100 µg/ml. Methanol extract of *S. isoetifolium* leaf also shows flavanoid presence. The presence of flavonoid chemical compounds in the extract of seagrass leaf shows their potential to be a natural chemical antifouling, antibacterial, antifungal, and other pharmaceutical raw materials (Dewi CSU *et al.*, 2018).

Alkaloids test was positive in the acetone fraction of *C. serrulata* and *S. isoetifolium*, and chloroform fraction of *S. isoetifolium* and ethyl acetate fraction of *C.* serrulata. Alkaloid is derived from plant sources, they are basic, they contain one or more nitrogen atoms in a heterocyclic ring and they usually have a marked physiological action on man or other animals (Evans WC, 2009).Alkaloid is often used in the field of pharmacology (Tanaka*et al.*, 2006). High alkaloid content and antibacterial activity is reported in the chloroform fraction of *S. filiforme*(Garcia GK *et al.*, 2020). The alkaloid content may be responsible for such activity. They also report 68 compounds in GC-MS analysis using the chloroform fraction including phenols and tannins. Alkaloids has anticancer effects (Lu J *et al* .,2012 ; Isah T *et al*, 2016 ; Arijit M *et al.*, 2019). Mani AE *et al.*, (2012), report that secondary metabolites was found in methanol extract of seagrass*S. isoetifolium*, such as saponins, phenols and alkaloids. Mani AE *et al.* (2012) also report remarkable antimicrobial activity and insecticidal activity in *S. isoetifolium.*

Quinones were one of the prominent secondary metabolite we found in the present study using *C. serrulata* and *S. isoetifolium.* The health benefits of quinines are reported by various researchers. Quinones, by their antioxidant activity, improve general health conditions. Many of the drugs clinically

approved or still in clinical trials against cancer are quinone related compounds. Quinones have also toxicological effects through their presence as photoproducts from air pollutants (Nahed El-N *et al*, 2011; Asche C, 2005).

Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer, and also to have antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, antiinflammatory, and immunomodulatory properties (Roselin J, 2011; Rahi T and Bishayee A, 2009 ; Wagner KH and Elmadfa I, 2003 ; Sultana N and Ata A, 2008 ; Shah BA *et al.*, 2009).

The quinone and terpenoides obtained from in chloroform may have anticancer activity since our result is similar with experiment of Perumal P *et al* (2002). The potential anticancer activity of the extracts of seagrass *C. serrulata* fractions was performed using hexane, chloroform, ethyl acetate and ethanol by Perumal P *et al* (2002). Their experimental outcome demonstrates secondary metabolites from chloroform fraction of seagrass *C. serrulata* which act as a potential anticancer drug against human colon cancer (HT-29 cell lines).

In the light of the above background information we proceed to our experiments for the photochemical analysis.

SUMMARY AND CONCLUSION

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Traditional folk treatment fromwild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals. In addition, some medicinal plants are still obscured within the plant which needs to be scientifically evaluated.In folk medicine, seagrasses have been used for a variety of remedial purposes.

This report demonstrating the active secondary metabolites such as flavanoids, alkaloids, quinones and terpenoidsis. An encouraging trend unraveling the potential of the Indian coastline as a source of marine organisms worthy of further investigation. These organisms are currently being investigated in detail with the objective of isolating biologically active unique molecules which would prove to be lead chemicals for drug discovery.

The present study explored the presence of flavonoids, alkaloids, quinine and terpenoides in two seagrass members such as *Syringodiumisoetifolim*and*Cymodoceaserrulata*belong to the family Cymodoceae.

This study demonstrates the phytochemicals present in *S.isoetifolim*and *C. serrulata* by extracting successively, using solvents based on polaritys.

The hexane fraction of *Syringodiumisoetifolim* reveals quinone and terpenoids. Chloroform extract was showing a strong green colour against HCl-Meyer's reagent test, indicating the presence of alkaloid and terpenoides test was also positive for chloroform fraction. Acetone extract revealed alkaloid and quinone. The ethyl acetate extract exhibited dark red colour against sulphuric acid indicating the presence of quinone and brown colour against sulphuric acid – chloroform test indicating terpenoid presence. The methanol fraction produced dark green colour for alkaloid test. Methanol extract also produced mild yellow colour against sodium hydroxide indicating the presence of flavonoids.

The petroleum ether fraction of *C.serrulata* indicated yellow against the test for flavonoids revealed their presence. The terpenoid test produced mild brown colour in colourless background for petroleum ether fraction. It is also produced

a positive red colour against sulphuric acid for the quinine test. Chloroform extract produced dark red for quinone and brown for terpenoids indicating the presence of quinone and terpenoids. Acetone fraction exhibited a mild green tinch against sulphuric acid-Meyer's reagent test for alkaloids, slight red for quinine and strong brown for terpenoide tests indicating all their presence. The ethyl acetate extract exhibited dark red colour against sulphuric acid indicating the presence of quinone and brown colour against sulphuric acid and chloroform indicating terpenoid presence.

The presence of the biologically active compounds of the said category reveals the possibility of S.*isoetifolim* and *C. Serrulata* in using as potent source of free radical scavengers,anticancer agents, and antimicrobial agents. The study brings out the medicinal value of S.*isoetifolim* and *C. Serrulata* those can be used as a nutraceutical compound in various food and pharmaceutical industries.

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