A Novel Approach To Investigate The Phytochemical, Anti-Bacterial Analysis And Wound Healing Effects Of *Musa acuminata* and *Allium sativum*

A DISSERTATION SUBMITTED TO

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI.

Affiliated To Manonmaniam Sundaranar University,

In partial fulfillment of the requirements for the award of the degree of

BACHELOR OF SCIENCE IN MICROBIOLOGY

SUBMITTED BY

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APRIL - 2021.

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "A Novel Approach To Investigate The Phytochemical Analysis, Anti-Bacterial And Wound Healing Effects Of *Musa acuminata* and *Allium sativum* " is a bonafide record of the original work completed by M. Abish Fathima Joharal (Reg. No: 18SUMB01), T. Ananshiya (Reg. No: 18SUMB02), C. Anantha Jothi (Reg. No: 18SUMB03), M. Asha (Reg. No: 18SUMB04), W. Bivina (Reg. No: 18SUMB05) during the academic year 2020 - 2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfillment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by Manonmaniam Sundaranar University.

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Signature of the Director **Signature of the Principal**

Signature of the External Examiner

DECLARATION

We hereby declare that the dissertation work entitled **"A Novel Approach To Investigate The Phytochemical , Anti-Bacterial Analysis And Wound Healing Effects Of *Musa acuminata* and *Allium sativum* "** is a bonafide record of the original work completed by us during the academic year 2020-2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfillment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by Manonmaniam Sundaranar University. We also affirm that this is an original work done by us under the supervision of **Dr. Joys Selva Mary Albert M.Sc., M. Phil., Ph.D.** Coordinator & Head of the department of Microbiology, St. Mary's College (Autonomous), Thoothukudi.

Signature of the Students **Signature of the Guide**

Place: Thoothukudi

Date:

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(M. ABISH FATHIMA JOHARAL, T. ANANSHIYA , C. ANANTHA JOTHI , M.ASHA, W.BIVINA).

CONTENT

ABBREVIATION

1. Mm - Millimeter
2. dm - diameter
3. gm - gram
4. ml - milliliter
5. °C - degree Celsius
6. µl - microliter
7. hrs - hour
8. min - minute
9. % - percentage
10. Kg - Kilogram
11. MHA - Muller Hinton Agar
12. PDA - Potato Dextrose Agar
13. DMSO - Dimethyl Sulfoxide

INTRODUCTION

1. INTRODUCTION

1.1 Prebiotics:

Prebiotics are a group of nutrients that are degraded by gut microbiota. Their relationship with human overall health has been an area of increasing interest in recent years. They can feed the intestinal microbiota, and their degradation products are short-chain fatty acids that are released into blood circulation, consequently, affecting not only the gastrointestinal tracts but also other distant organs. Fructo-oligosaccharides and galacto-oligosaccharides are the two important groups of prebiotics with beneficial effects on human health. Since low quantities of fructo-oligosaccharides and galacto-oligosaccharides naturally exist in foods, scientists are attempting to produce prebiotics on an industrial scale. Considering the health benefits of prebiotics and their safety, as well as their production and storage advantages compared to probiotics, they seem to be fascinating candidates for promoting human health condition as a replacement or in association with probiotics. This review discusses different aspects of prebiotics, including their crucial role in human well-being. Various types of microorganisms, known as gut microbiota, are inhabitants of the human gastrointestinal tract. It has been reported that there are 1010–1012 live microorganisms per gram in the human colon (Collins S., Reid G. 2016). The resident microbial groups in the stomach, small, and large intestine are crucial for human health. The majority of these microorganisms, which are mostly anaerobes, live in the large intestine (Louis P., Flint H.J., Michel C. 2016) . Although some endogenous factors, such as mucin secretions, can affect the microbial balance, human diet is the chief source of energy for their growth. Particularly, non-digestible carbohydrates can highly modify the composition and function of gut microbiota (Walker A.W., Ince J., Duncan S.H., 2010, 2011). Beneficial intestinal microbes ferment these non-digestible dietary substances called prebiotics and obtain their survival energy from degrading indigestible binds of prebiotics (Glenn G., Roberfroid M, 1995) . As a result of this, prebiotics can selectively influence gut microbiota. On the other hand, the gut microbiota (Bouhnik Y., Raskne L., Vicaut E., 2004). On the other hand, the gut microbiota affects intestinal functions, such as metabolism and integrity of the intestine. Moreover, they can suppress pathogens in healthy individuals through induction of some immunomodulatory molecules with antagonistic effects against pathogens by lactic acid

that is produced by Bifidobacterium and Lactobacillus genera (Roberfroid M., Morowvat M.H., Ghasemi Y., 2015). Various compounds have been tested to determine their function as prebiotics. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and trans-galacto-oligosaccharides (TOS) are the most common prebiotics. Fermentation of prebiotics by gut microbiota produces short-chain fatty acids (SCFAs), including lactic acid, butyric acid, and propionic acid. These products can have multiple effects on the body. The structure of prebiotics and the bacterial composition of gut determine the fermentation products (Zhou Z., Zhang Y., Zheng P., Chen X., Yang Y. 2013).

1.2 Phytochemical analysis:

Phytochemicals are chemicals of plant origin (Breslin and Andrew, 2017). Phytochemicals (from Greek phyto, meaning "plant") are chemicals produced by plants through primary or secondary metabolism (Molyneux *et al.,* 2007; Harborne, 1999).They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators (Molyneux *et al.,* 2007).

Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been established yet. Many antioxidant compounds can be found in fruits and vegetables including phenolics, carotenoids, anthocyanins, and tocopherols (Jakubowski W., Bartosz G. 1997). Phytochemicals under research can be classified into major categories, such as carotenoids and polyphenols, which include phenolic acids, flavonoids, and stilbenes/lignans. Flavonoids can be further divided into groups based on their similar chemical structure, such as anthocyanins, flavones, flavanones, and isoflavones, and flavanols. Flavanols further are classified as catechins, epicatechins, and proanthocyanidins. In total, there has been over 25,000 phytochemicals discovered and in most cases, these phytochemicals are concentrated in colourful parts of the plants like fruits, vegetables, nuts, legumes, and whole grains, etc. Approximately 20% of known plants have been used in pharmaceutical studies, impacting the healthcare system in positive ways such as treating cancer and harmful diseases (Naczk M., Shahidi F. 2006). Phytochemists study phytochemicals by first extracting and isolating compounds from the origin plant, followed by defining their structure or

testing in laboratory model systems, such as cell cultures, in vitro experiments, or in vivo studies using laboratory animals. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Suffredini I.B., Sader H.S., Goncalves A.G. 2004). Challenges in that field include isolating specific compounds and determining their structures, which are often complex, and identifying what specific phytochemical is primarily responsible for any given biological activity. Phytochemicals are bio-active chemicals of pant origin. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants (Boots A.W., Haenen G.R., Bast A.2008).They are regarded as secondary metabolities because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body, bark, leaves, stem, root, flower etc. i.e.. any part of the plant body may contain active components. Phytochemicals are non-essential nutritive plant material that consists of protective and disease preventive properties. They are found in fruits, vegetables, grains and other parts of the plant. The consumption of fruits and vegetables has been linked with several health benefits, a result of medicinal properties and high nutritional value (Valko M., Rhodes C.J. 2006). There are number of Phytochemicals each work differently. Few of the functions of phytochemicals are antioxidant, hormonal action, anti-bacterial effect, etc. most of the food contains Phytochemical except foods such as sugar and alcohol. Phytochemical journals covers the topics related to nutritive plant material. Antioxidants control and reduce the oxidative damage in foods by delaying or inhibiting oxidation caused by reactive oxygen species (ROS), ultimately increasing the shelf-life and quality of these foods (Ames B.N., Shigenaga M.K. 1993). Phytochemical analysis refers to the extraction, screening and identification of the medicinally active substances found in plants. Phytochemical are naturally present in the plants and shows biologically significance by playing an essential re in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. The secretion of these compounds is varying from plant to plant some produce more and some produce in minimal activity. Sometimes they can be harmful and sometimes they can be very helpful. Phytochemicals are chemicals of plant origin. Phytochemicals are chemicals produced

by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens or predators. The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These non-nutritive components are called phytochemicals. The qualitative analysis as well as quantification of phytochemicals of a medicinal plant is regarded as vital step in any kind of medicinal plant research. Traditionally used medicinal plants have recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations.Plants have limitless ability to synthesize aromatic secondary metabolites, most of and solubilize compounds of similar polarity (Joona K., Sowmia *et al., 2013)*. plant is Mangifera indica Linn., it is a large evergreen tree, belongs to the family Anacardiaccae. It is commonly known as 'Maram' in Tamil, 'Mango' in English, 'Aam' in Hindi and 'Aamra' in Sanskrit. Different varieties of mango have been cultivated throughout the world and recently cultivated in Iraq (Rajan *et al., 2011)*. It consists of about sixty genera and six hundred species, which are mainly tropical trees and shrubs. Its parts are commonly used in folk medicine for a wide variety of remedies. Many phenolic compounds have been detected in Mango peels, bark, Pulps and seed kernels. Several pharmacological activities of mango extracts have been reported including anti-inflammatory, antioxidant, anti-allergic, anthelmintic and antiamoebic. Herbal drinks are very popular, as they contain natural constituents especially phenolic compounds (Abdelghany *et al., 2010).*

The mango is a rich source of various polyphenolic compounds. The major polyphenols in the mangoare: mangiferin, catechins, rutin, quercetin, kaempferol, rhamnetin, anthocyanins, gallic and ellagic acids, propyl and methyl gallate, benzoic acid, and protocatechuic acid. The amounts of the different polyphenolic compounds in the mango vary from part to part (pulp, peel, seed, bark, leaf, and flower) (Talba *et al., 2014)*. Polyphenols are secondary metabolites of plants and are widely distributed in beverages and plant-derived foods. Human consumption studies

indicate 1 g of total polyphenols is frequently consumed per day and it is not anticipated that any acute or lethal toxicity would be observed through the oral intake route (Augustin Scalbert *et al., 2000)*. Phenolic compounds have the capacities to quench lipid peroxidation, prevent DNA oxidative damage, scavenge with 200ml of ethanol by maceration over night at room temperature. The extract was filtered then concentrated and divided into small portions, one for identification and the other part was partioning with n-butanol and ethyl acetate respectively, then each fraction was concentrated to small volume for further study. Moraceae the mulberry family of the Rose order. Artocarpus heterophyllus is one of the most important and widespread trees in tropical region useful tree in significant genus Artocarpus . The tree is reported native to the rainforest of Malaysia, Western ghats of India and also found in Eastern &Southern Africa, Brazil, Florida, Australia. All parts of tree exude sticky, white milky latex when injured. The young fruits are acrid, astringent, and carminative. The ripe fruits are sweet, cooling, laxative, aphrodiasic and also used brain tonic. The seeds are diuretic and conspitating. The wood is nervine, antidiabetic, and sedative useful in convulusions. They are organic substances and could be obtained in both primary and secondary metabolic process. They also provide a source of medicine since the earliest time. The medicinal importance of plant is due to the . These compounds are synthesized by primary or rather secondary metabolism of living organisms. These are many phytochemicals herbs and each works differently. These phytochemicals have various health benefits such as anti-oxidant, anti-inflammatory, anti-cancerous, antidiabetic and anti-hypertensive effectpresence of chemical constituents like alkaloids, glycosides, resins, volitale oils, gums and tannins. . These compounds are synthesized by primary or rather secondary metabolism of living organisms. These are many phytochemicals herbs and each works differently. These phytochemicals have various health benefits such as anti-oxidant, anti-inflammatory, anti-cancerous, antidiabetic and anti-hypertensive effect.

1.3 Anti-bacterial activity:

Antibacterial as well as antiviral activity of a molecule is completely

associated with the compounds that provincially kill bacteria and virus or slow down their rate of growth, without being extensively toxic to nearby tissues. Most recently discovered antimicrobial agents are modified natural compounds and this modification is done through chemical mode, for example, b-lactams (penicillins), carbapenems, or cephalosporin. Pure natural products, such as aminoglycosides, and other entirely synthetic antibiotics, for example sulfonamides, are also frequently used. The antimicrobial agents could be classified as the agents that can either be bactericidal, which kill bacteria, or bacteriostatic, which slow down the growth of bacteria. Antibacterial agents are the most important in fighting infectious diseases. But, with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry. Resistance is most commonly based on developmental processes taking place, for example, antibiotic therapy, that leads to inheritable resistance.

This increasing resistance of the microorganisms toward antibacterial agents has been responsible in recent years for serious health issues. Most infectious bacteria are resistant to a minimum of one of the antibiotics that are generally used to eliminate the infection. This problem motivates the study of new agents that can efficiently inhibit the growth of microorganisms. Nanoparticles have been considered for use in optical devices, fuel cells, catalysts, biosensors, drugs, superconductors, and gene delivery. Nanomaterials as a novel drug delivery vehicle also have been applied to enhance the physicochemical and medicinal effectiveness of drugs. Likewise, nanotechnology in pharmaceuticals and in microbiological study have shown favorable applications to resolve the issue of antibiotic resistance. In the past few years, different nanosized antibacterial agents such as carbon-based nanoparticles, metallic and metal oxide nanoparticles, and polymeric chitosan nanoparticles have been studied by researchers. Different types of metallic and metal oxide nanoparticles such as silver (Ag), silver oxide (AgO), titanium dioxide (TiO), zinc oxide (ZnO), gold (Au), calcium oxide (CaO), silica (Si), copper oxide (CuO), and magnesium oxide (MgO) have been found to show both antibacterial and antiviral activity. Antimicrobial agents including antiseptics and antibiotics are extensively used for infection control in community and nosocomial settings. Antiseptics such as

chlorhexidine digluconate, triclosan and povidone-iodine are used as surface disinfectants in hand hygiene and disinfection of surgical and catheter insertion sites. In addition to human use, antibiotics such as ampicillin, amoxicillin, imipenem, and many others, are widely used to treat a variety of bacterial infections in animals, thus favoring their use in animal and poultry farming. Unfortunately, enhanced use of antimicrobial agents leads to the development of resistant microbes, such as chlorhexidine and colistin resistant *Klebsiella pneumonia*, methicillin and linezolid resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci. Importantly, some of the antiseptics and antibiotics have also been reported to precipitate adverse systemic effects in patients (Cunha B.A. 2001). Povidone-iodine and triclosan has been shown to disrupt thyroid hormone homeostasis (Pietsch J., Meakins J.L. 1976), while colistin and vancomycin have been associated with renal toxicity (Pogue J.M., Lee J., Marchaim D., Chopra T *et al. 2011)*. Thus, it is imperative that new and safe antimicrobial agents are explored from alternative sources. Plant extracts have been used for centuries to combat infectious human diseases in different parts of the world. Such medicinal extracts are a mixture of several compounds, with many extracts reported to have potent antimicrobial activities against wide range of drug resistant microbes (Rios J.L, Recio M.C. 2005). Phenolics, terpenoids, alkaloids and lectins are some of the classes of compounds present in plant extracts that exhibit strong antimicrobial activity. Antimicrobial activity has been reported from various plant extracts such as *Brillantaisia lamium, Crinum purpurascens, Mangifera indica* and *Psidium guajava*, against variety of pathogens including *S. aureus, Enterococcus faecalis, Candida tropicalis, Cryptococcus neoformans*, and *Salmonella* Paratyphi. Thus, plant-based materials form an abundant source for antimicrobials, that could be economical, easy to process, and efficient against drug-resistant microbes. The Jackfruit is one such plant product that fits this description.

The fruit from the jack tree, botanically termed *Artocarpus heterophyllus*, belongs to the mulberry family, *Moraceae*. The fruit by itself is quite large, weighing approximately 4-10 kg, commercially inexpensive, and widely consumed in south-east Asia and Africa (Gupta D. Mann S. 2011).· In addition to jackfruit's nutritional value, various components of the fruit possess a

plethora of medicinal properties (Jagtap U.B. 2010). The fruity arils are known to contain a cocktail of phytonutrients such as carotenoids, isoflavones, saponins and phenols that are responsible for antioxidative and immunomodulatory properties. In addition, jackfruit leaf extract has been reported to possess antibacterial activities against *Escherichia coli, Listeria monocytogenes, Salmonella typhimurium, Salmonella enterica, Bacillus cereus, Enterococcus faecalis*, and *S. aureus* (Loizzo M.R, Tundis R. 2010). Heartwood has antibacterial activities against *Bacillus subtilis, Streptococcus mutans, Streptococcus pyogenes, S. aureus* and *Staphylococcus epidermidis*. In addition, extract derived from jackfruit seeds and shell powder have also been reported to demonstrate antibacterial activities against *L. monocytogenes*.
While parts of the ripe jackfruit including the pulpy aril and seeds are a culinary delicacy, the rough, fibrous appendage called 'rag' that make up 10-20% of the fruit are either discarded as non-edible fruit waste, or in some cultures, cooked and consumed. There are no known reports of rag's being used for medicinal purposes, although we have recently reported the use of the Jackfruit rag extract (JFRE) as a photo-sensitizer in solar cells (Ashok A, Mathew S.E. 2018). Since rags make up large parts of the fruit and considering the existing lack of clarity towards their use for human benefit, we set out to explore possible medicinal value of the rag, with specific focus on its potential as an alternative to antibiotics against human pathogenic bacteria.
Jackfruit (Artocarpus heterophyllus Lam.) is a dicotyl plant with woody stems that are native to India, Malaysia and Bangladesh, but can also grow well in other tropical-subtropical regions including Indonesia (Manuel NV et al, 2012; Orwa et al, 2009) . In addition to fruit, seeds, or young flowers are useful as a source of food, other jackfruit plant parts are also empirically often used as drugs. Jackfruit root is used to treat skin diseases, asthma, fever, and diarrhea. Jackfruit leaf is believed to cure ulcers. Jackfruit sap is believed to cure abscesses, snake bites, and swollen glands. While the seeds, often used to cure gall disease (National Tropical Botanical Garden, 2015). Several studies have been conducted to prove the usefulness of jackfruit plants in the medical field. Jha and Srivastava in his study found that jackfruit seed oil could inhibit the growth of Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus bacteria with minimum inhibitory concentrations (MIC) between 1.55-5.20 mg / ml (Jha S et al, 2013). Other studies have also shown the presence of antibacterial activity from jackfruit extracts to MSSA

and MRSA played by the substance artocarpesin (Manuel NV et al., 2012).

1.4 Wound healing activity:

Skin is the largest organ in the body and covers the body's entire external surface. Made up of three layers, the epidermis, dermis, and the hypodermis. Skin's structure is made up of an intricate network which serves as the body's initial barrier against pathogens, UV light, and chemicals, and mechanical injury, and regulates temperature and the amount of water released into the environment. A skin wound results from the breakdown of the epidermal layer integrity (Yousef H, Alhajj M, Sharma S.2017). Wound healing mostly means healing of the skin.Begins immediately after an injury to the epidermal layer and might take years. Dynamic process including highly organized cellular, humoral, and molecular mechanisms. Has 3 overlapping phases which are inflammation, proliferation, and remodeling. Any disruption leads to abnormal wound healing (Kangal MK, Regan JP 2020). Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling.

The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. Angiogenesis involves new blood vessel growth from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts excrete collagen and fibronectin to form a new, provisional extracellular matrix. Subsequently, epithelial cells crawl across the wound bed to cover it and the wound is contracted by myofibroblasts, which grip the wound edges and undergo contraction using a mechanism similar to that in smooth muscle cells (Nayak BS, Anderson M, Pereira LMP. 2007).

Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds. In this direction, a number of herbal products are being investigated

at present. Various herbal products have been used in management and treatment of wounds over the years (Raina R, Prawez S, Verma PK, Pankaj NK 2008).

Ficus benghalensis Linn. (Family: Moraceae) is a reputed plant in ayurvedic medicine and commonly known as "banayan tree" in ayurvedic literature. Milky juice from stem, seeds, or fruits of the plant is applied externally in rheumatism and to the soles of feet when inflamed, internally used in dysentery and diarrhea. All the parts of the plant have astringent, anti-inflammatory, antiarthritic, and antidiarrheal activities. The latex is useful in hemorrhage, diarrhea, and dysentery, as well as in hemorrhoid and inflammation (Patel MA, Patel PK, Patel MB.2010).

Traditionally, it is used for wounds, fever, swollen joints, inflammations, and ulcers (West Lafayette 2003).

Various scientific studies have been carried out on *F. benghalensis* and various pharmacological activities have been reported. It has been reported to possess immunomodulatory (Gabhe SY, Tatke PA, Khan TA 2006), hypoglycemic, antioxidant, antistress and antiallergic, and anthelmintic activities. A glucoside, bengalenoside was isolated from *F. benghalensis* and evaluated for hypoglycemic activity (Augusti KT 1975). Efforts are being made all over the world to discover agents that can promote healing and thereby reduce the cost of hospitalization and save the patient from amputation or other severe complications. The need for safer and effective wound-healing agents and the lack of enough scientific data to support the claims made in ancient literature prompted the present study. Artocarpus heterophuyllus lam (Moracceae) is a large tropical tree and is indigenous to india and popularly known as jack tree. The leaves are simple, alternste, coriaceous, entire dark, shiny green above oblong, oval or eliptic in form, 4-6 inches in length, glabrous, hairless with smooth skinned surface (Prajapati, Narayan, Das Purohit 2003). The leaves show the presence of sapogenin, cycloaratenone, cycloartenol, β-soitosterols and tannis (Nodig shbha S, Gurumadhava Rao 1993). The leaves are reported to be used in skin diseases and ash of leaves is useful in fever, boils, wound, skin diseases and vitiated condition of pitta and vata (Fernando M.R 1989). The seeds of the ripe fruits are used as vegetable and

believed to have rich nutritional values (Chadha Y.B 1985). In the present investigation, the wound healing effects of the methanolic extract of leaves was investgated on albino mice using excision wound models. Thai traditional medicine (TTM) is based on Thai ancient knowledge that has been compiled into various scriptures through experiences and observations by traditional doctors and passed onto the next generations. The list of medicinal plants most commonly used to treat wounds was selected from the Mukkharoka scripture, the National List of Essential Medicines of Thailand, and traditional usages. From the above criteria, the seed of black cumin (*Nigella sativa* L.) appeared in most of the recipes in Mukkharoka scripture for the treatment of wound and inflammation in oral cavity. Previous studies found that *N. sativa* and its major compound thymoquinone have been shown for their therapeutic benefits such as antibacterial, antioxidant, and anti-inflammatory activities.

The root of licorice (*Glycyrrhiza glabra* L.) was found as the main ingredients of Am-ma-ruek-ka-va-tee recipe while dry fruits of Indian nightshade (*Solanum indicum* L.) and dry fruits of Thai nightshade (*Solanum trilobatum* L.) were found as the main ingredients of Phra-sa-ma-vaeng recipe in the National List of Essential Medicines of Thailand. These medicines were used to treat sore throat, cough with sputum, and ailments in the oral cavity. The major compounds of these extracts had been proved to be glycyrrhizin (*Glycyrrhiza glabra*) and steroidal glycoside (solanidine and solasodine in *Solanum* spp.) respectively, with reported activities such as antibacterial, anti-inflammatory, antioxidant, and anticancer activities

Indian gooseberry (*Phyllanthus emblica* L.) fruits were found as the main ingredient in Tri-pha-la recipe; TTM used this recipe for symptoms in the upper respiratory tract and to balance the internal body. Gallic acid, protocatechuic acid, and tannins were found in its extract with bioactivities such as anti-inflammatory, antioxidant, and analgesic activities

The pericarp of mangosteen (*Garcinia mangostana* L.) had traditional use such as wound cleansing liquid on the skin and feet. The major compound of *G. mangostana*, α-mangostin, had

various proven bioactivities such as antioxidant, antibacterial, antifungal, anticancer, and anti-inflammatory activities.

The bark of *Mimusops elengi* L. was used for toothache; it consisted of many compounds such as steroids, flavonoid, terpenoids, glycoside, saponin, and tannin with bioactivities such as antioxidant and anti-inflammatory activities

Clove bud (*Syzygium aromaticum* L.) has been used in throat lozenge for pain relief and its major active compound is eugenol; therefore *S. aromaticum* extract many bioactivities such as antioxidant and antihyperglycemic activities.

Thus, the discovery of medicine is an effort of mankind over millions of years of search foe eternal health, longevity and remedies to relieve pain and discomfort, which prompted early man to explore his natural surroundings and try many plants, animal products, minerals and develope a variety of therapeutic agents pharmaceutical industries are giving importance to the compounds derived from traditional sources and less traditional sources like marine organisms.

Reason for the selection of the problem:

When peep into the previous literature several authors have done excellent work using various parts of the plants and its pharmaceutical effects using various organisms and animals. Even though there are several thousands of work available on the importance of medicinal plants, the medicinal property of some plants are lacking. Hence the present study is planned and in this study *Musa acuminate* and *Allium sativum* was obtained from Teacher's colony, Thoothukudi were assessed for its bio – potentials.

1.5 Musa acuminata:

Musa acuminata is one of the most widely distributed and consumed fruit in the world. Considering the nutritional aspects, it is one of the world's leading food crops with a great source of minerals, vitamins, carbohydrates, flavonoids, and phenolic compounds. The peels of

variety of fruits have gained attention as a natural source of antioxidants and phytochemical content which are rich in compounds with free radical scavenging activity. The research on Musa acuminata peel extract indicated that banana peel is potential source of bioactive compounds like flavonoids and polyphenols with wide range of medicinal properties.

Scientific classfication:

Musa acuminata

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Zingiberales

Family: Musaceae

Genus: Musa

Section: Musa sect. Musa

Species : M.acuminata

Fig 1.5 ***Musa acuminata***

1.6 Allium sativum:

Allium sativum, commonly known as garlic, is a species in the onion genus, *Allium*. Garlic cloves are used as a remedy for infections (especially chest problems). Digestive disorders, and fungal infections such as thrush. Garlic can be used as a disinfectant because of its bacteriostatic and bactericidal properties. The plant under study, namely Allium sativam Linn contains alkaloids, flavonoids, lignins, triterpenoids, fixed oils, fats, proteins and amino acids. A survey of literature revealed that not much work has been made to study wound healing activity of this plant; hence it was thought worthwhile to investigate the wound healing activity of Allium sativam Linn extract in efficient experimental models of wound in rats. Garlic has an unusually high concentration of sulfur-containing compounds (1-3%) and its therapeutic properties are largely due to one particular class of sulfur-containing compounds, the thiosulfinates. These bioactive components have been isolated from aqueous, ethanolic and fermented extracts of crushed garlic and have the potential to interact with a number of cellular

targets, particularly those exhibiting reactive sulfhydryl moieties, whose functions range from control of cell cycle to expression of crucial antioxidant and detoxification enzymes. Interactions with these processes may underlie garlic`s putative therapeutic potential. With attention to widespread usage of garlic in curing of various illnesses particularly infectious diseases, there is an absence of scientific investigation of application of this medicinal plant in wound healing.

Scientific classification :

Allium sativum

Kingdom:Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Amaryllidaceae

Subfamily: Allioideae

Genus: Allium

Species: A. sativum

Fig 1.6 ***Allium sativum***

AIM AND OBJECTIVES

2. Aim And Objectives

- To investigate the phytochemical constituent of acetonic extract of *Musa acuminata* and *Allium sativum*.
- To evaluate the Antibacterial activity of acetonic extract of *Musa acuminata* and *Allium sativum* against gram positive and gram negative organisms.
- To evaluate the ability of acetonic extract of *Musa acuminata* and *Allium sativum* against the microorganisms present in the patient's wound by agar well diffusion method.
- To determine the wound healing activity from the acetonic extract of *Musa acuminata* and *Allium sativum*.

REVIEW OF LITERATURE

3. Review Of Literature

(Robert A Rastall *et al*., 2002) Reviewed the Recent research in the area of prebiotic oligosaccharides and synbiotic combinations with probiotics is leading towards a more targeted development of functional food ingredients. Improved molecular techniques for analysis of the gut microflora, new manufacturing biotechnologies, and increased understanding of the metabolism of oligosaccharides by probiotics are facilitating development.

(Elkayam *et al*., 2003) Reviewed the Commercially available garlic preparations in the form of garlic oil, garlic powder, and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile.

(Gibson *et al*., 2004) Reviewed the Elaborated the prebiotics concept by certain criteria viz. resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; fermentation by intestinal microflora and selective stimulation of the growth, and/or activity of intestinal bacteria ssociated with health and wellbeing.

(Bornet F.R.*et al*., 2004) Reviewed the capacity of nondigestible carbohydrates to stimulate fecal bifido bacteria in healthy humans: A double-blind, randomized, placebo-controlled, parallel-group, doseresponse relation study.

(Roberfroid M.B. *et al*.,2004) Reviewed the Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics.

(Panitantum *et al*., 2004) Reviewed the benefits of prebiotics are that they modulate bowel habits, increase calcium absorption, and interact with lipid metabolism to reduce low density lipo protein (LDL) cholesterol.

(Suffredini I.B.*et al*.,2004) Reviewed the Screening of antibacterial extracts from plants native to the brazilian amazon rain forest and atlantic forest.

(Gibson *et al*.,2004). Reviewed the Prebiotics must be able to withstand acid hydrolysis in the stomach, able to move to large intestine without changes or being absorbed in small intestine so that they can be utilized by the indigenous microflora in the large intestine to enhance their growth.

(Ríos JL.*et al*., 2005) Reviewed the plants and antimicrobial activity. Several plant extracts inhibited the growth of Candida albicans while only one plant extract showed inhibitory activity against Saccharomyces cerevisiae. All the plant extracts which demonstrated good anti-inflammatory activities also showed better inhibitory activity against Candida albicans.

(Ford *et al*., 2005) Reviewed the Antimicrobial properties intrinsic to the suture and

the addition of an extrinsic coating have been theorized to reduce surgical site infections by decreasing bacterial adherence to the suture. Currently available products (Coated Vicryl Plus Antibacterial, Monocryl Plus Antibacterial, PDS Plus Antibacterial) use triclosan, an antimicrobial biocide, shown to reduce in vitro suture colonization with methicillin resistant and methicillin sensitive Staphylococcus aureus and Staphylococcus epiderm.

(Gabhe SY.*et al*., 2006) Reviewed the Evaluation of the immunomodulatory activity of the methanol extract of Ficus benghalensis roots in rats.Various extracts of the aerial roots of Ficus benghalensis were evaluated for potential immunomodulatory activity, using the in vitro polymorphonuclear leucocyte (human neutrophils) function test. The methanol extract was evaluated for immunomodulatory activity in in vivo studies, using rats as the animal model. The extracts were tested for hypersensitivity and hemagglutination reactions, using sheep red blood.

(Nayak BS.*et al*., 2007) Reviewed the Evaluation of wound-healing potential of Catharanthus roseus leaf extract in rats.Wound contraction together with increased tensile strength and hydroxyproline content support the use of C. roseus in the management of wound healing.

(Vernazza *et al*., 2006) Reviewed the Prebiotics are more useful as functional food than probiotics, because of their ability to survive the digestive process in the upper gastrointestinal tract.

(Naczk M.*et al*., 2006) Reviewed the Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Consumption of plant foods, particularly fruits, vegetables and cereal grains is encouraged because they render beneficial health effects. Phenolics and polyphenolics are among the most desirable food bioactives because of their antioxidant activity, brought about by a number of pathways, or due to other mechanisms.

(Valko M.et al.,2006) Reviewed the Free radicals, metals and antioxidants in oxidative stress-induced cancer.Oxygen-free radicals, more generally known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS) are well recognised for playing a dual role as both deleterious and beneficial species.

(GT Macfarlane *et al*., 2006) Reviewed the Prebiotics are short-chain carbohydrates that alter the composition, or metabolism, of the gut microbiota in a beneficial manner. It is therefore expected that prebiotics will improve health in a way similar to probiotics, whilst at the same time being cheaper, and carrying less risk and being easier to incorporate into the diet than probiotics.

(Stowell *et al*., 2007) reviewed the existing prebiotics and classified them based on a

set of common criteria. Inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), lactulose and poly dextose are recognized as the established prebiotics.

(Pinerio *et al*., 2008) reviewed in the technical meeting convened by Food and Agriculture Organization of the United Nations (FAO) the beneficial effect of prebiotics on food.

(Bergerat A *et al*., 2010) Reviewed the Dominant and diet-responsive groups of bacteria within the human colonic microbiota.The populations of dominant species within the human colonic microbiota can potentially be modified by dietary intake with consequences for health.

(Jagtap UB *et al*., 2010) Reviewed the Evaluation of antioxidant capacity and phenol content in jackfruit (Artocarpus heterophyllus Lam)

(Loizzo MR.*et al*., 2010) Reviewed the antibacterial activities on foodborne pathogens of Artocarpus heterophyllus Lam. (Moraceae) leaves extracts.

(Pogue JM.*et al*., 2011) Reviewed the Incidence of and risk factors for colistin-associated nephrotoxicity in a large academic health system.

(Spapen H *et al*., 2011) Reviewed the Retrospective evaluation of possible renal toxicity associated with continuous infusion of vancomycin in critically ill patients.

(Rajan, SS *et al*., 2011) Reviewed the Anti-enteric bacterial activity and phytochemical analysis of the seed Kernel extract of Mangifera indica Linnaeus against Shigella dysenteriae(Shiga, corrig.) Castellani and Chalmers.

(Joona, K.*et al*., 2013) Reviewed the Preliminary Antimicrobial Activity and Phytochemical Profile of Mangifera Indica L.The growth of lactic acid bacteria was not inhibited, the proliferation of Grampositive food spoilage bacteria was prevented and the growth of Gram-negative Escherichia coli was reduced.

(Zhou Z.*et al.,* 2013) Reviewed the Starch structure modulates metabolic activity and gut microbiota profile.The changes in starch structure during fermentation were investigated using scanning electron microscopy (SEM), high-performance liquid chromatography (HPLC) and Fourier transform infra-red spectroscopy (FTIR).

(Lee *et al*., 2013)eacid phenethyl ester on is suggested to disrupt the bacterial outer membrane and to provoke superoxide radical stress in bacteria, leading to protein and DNA damage .

(Van Sinderen D.*et al*., 2014) Reviewed the Molecular dialogue between the human gut microbiota and the host: A lactobacillus and bifidobacterium perspec tive.

(J. Oniye. *et al*., 2014) Reviewed the Phytochemical screening and In-vitro antibacterial activity of mangifera indica (Mango) Kernel on. Aeromonascaviae

(Joys Selva Mary Albert.*et al*., 2015) Antimicrobial activity of selected Sea grasses against human pathogenic bacteria and fungi. Reviewed the Present investigation is carried out on the antimicrobial activity of selected Sea grasses Cymodocea rotundata and syringodium isoetifolium.methanolic extract of these two sea grasses were tested against positive bacteria,gram negative bacteria and fungi.The two sea grasses exhibated maximum antibacterial activity against Staphylococcus aureus where as no activity was observed against pseudomonads aeruginosa.

(T. M. Abdel Ghany.*et al*.,2018) Reviewed the Antioxidant, Antitumor, Antimicrobial Activities Evaluation of Musa paradisiaca L. Pseudostem Exudate Cultivated in Saudi Arabia, Bio Nano Science.

(Ashok A.*et al*., 2018) Reviewed the Cost effective natural photo-sensitizer from upcycled jackfruit rags for dye sensitized solar cells.

(Agarwal *et al*., 2020)Wound represents the major health problem, both in terms of morbidity and mortality. Wound healing is a fundamental response to tissue injury that results in resortation of tissue integrityWound Healing Activity Of Plantain Banana Extracts).

(W. Kundarto.*et al*., 2020) healing activity of skin incision and skin burn from spray gel formula contains combination of banana peel.

MATERIALS AND METHODS

4. Materials And Methods

4.1 Collection of raw materials:

Peel of *Musa acuminata* and *Allium sativum* was collected from the Teacher's colony, Thoothukudi.

Fig 4.1 ***Musa acuminata***

Fig4.2 *Allium sativum*

4.1.1 Sample preparation:

Peel of *Musa acuminata* and *Allivum sativum* was separated and washed twice with running tap water and then with distilled water respectively. Then the materialwas dried for 3 - 4 days. The dried material were grinded to fine powder.

4.1.2 Preparation of extracts:

Peel of Musa acuminata and *Allivum sativum* is cut into a small pieces and soaked in 70% of acetonic for about 3 days. Then the solvent is blended, filtered and stored.

4.2 Phytochemical Analysis:

The peel of *Musa acuminata* and *Allium sativum* were analysed to identify the presence of phyto consitituents in the materials. Tests were carried out for glycosides, phenols, flavonoids and saponins by the following standard methods.

4.2.1 Test for glycosides:

Small amount of the extracts was put in 1 ml of water in a test tube followed by the addition of 1 ml of NaOH. A yellow precipitate indicates the presence of glycosides.

4.2.2 Test for phenols:

The extract (5mg) was dissolved in distilled water and 3ml of 10% lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

4.2.3 Test for flavonoids:

A few drops of concentrated hydro chloric acid were added to a small amount of the extract. Immediated development of red colour indicates the presence of flavonoids.

4.2.4 Test for saponins:

An amount 1ml of each extracts was diluted with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. The formation of foam of about 1cm indicates the presence of saponins.

4.3 Anti-bacterial Activity:

The extracts of *Musa acuminata* and *Allium sativum* (acetonic) were tested for the antibacterial activity against the bacteria *Staphylococcus aureus*, S*treptococcus sp* and *Escherichia coli.* Miller – Hinton agar plates was used to demonstrate the antibacterial properties of the crude extracts by well diffusion method. The prepared inoculums for each bacterial strain were inoculated in the plates. Aseptically by dipping a sterile swab in the inoculums removing the excess of inoculums by pressing and by rotating the swab firmly against the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plates through an angle of 60°C. The inoculums were left to dry at room temperature with the lid closed. Wells of 6mm were bored in the culture media by using well cutter. The stock acetonic extract of *Musa acuminata* and *Allium sativum* were prepared in DMSO and the extracts in aliquots of volume of 10µl was added into corresponding wells with the help of micro pipette. The plates were incubated at 37°C for 24-48 hrs for bacterial culture. Zone of inhibitions (ZOI) as indicated by the clear zone i.e. without growth of organism around the well was measured.

4.3.1 Bacterial strains:

A total of three bacterial strains including two gram-positive *Staphylococcus aureus, Streptococcus sp and* one gram negative *Escherichia coli* were selected. The bacterial cultures were maintained in nutrient agar slants at 37°C and 40°C respectively. Each of the microorganisms was freshly cultured prior to susceptibility testing by transferring the into a separate tube containing nutrient broth over night.

S.NO	Bacterial Strains	Gram (+/-)
1.	*Staphylococcus aureus*	+
2.	*Streptococcus sp*	+
3.	*Escherichia coli*	–

4.4 Wound healing activity:

1. Ointment formulation (Britishpharmacopoeia,1993)
2. Animal model
3. Wound healing activity
4. Incision wound model (Potawale*etal*.,2007)
5. Constant waterflow technique

4.4.1 Ointment formulation (British Pharmacopoeia,1993)

A control ointment base was formulated without any drug content. Cream was formulated by using 10% extract (10 gm of Sample A *(Musa acuminata)* and sample B *(Allium sativum*) were incorporated in 100 gm of cream base).The standard drug for screening wound healing activity is povidone iodine ointment (5%w/w) which was bought commercially.

4.4.2 Animal model:

Albino rats (150-250gm) of either sex were procured from animal house were used for the present study. The Albino rats were divided into four groups of six rats. Group I rats were treated

with simple ointment base (control). Group II rats were treated with reference standard povidone iodine ointment. Group III and IV rats were treated with 10% ointment only.

Fig 4.3 Albino rat

4.4.3 Wound healing activity:

The excision wound healing activity was studied by the method described by Luisa *et al.,* (2003) and Farahpour and Habibi. (2012).The skin area on the dorsal thoracic region of the mice was removed by using a suitable depilatory (Anne French hair removing cream) one day prior to the experiment. Alcohol(70%) was used as antiseptic for the shaved region before making the wound. The surgical procedures were carried out under sterile conditions. The experimental animals were anesthetized with anesthetic ether. After successful anesthesia mice were fixed in a dorsal posture on a surgery table. Circular full thickness surgical wounds with diameters of 5mm,1cm away from the back bone were made using 5mm biopsy punch. Using this excision wound method, the epidermal, dermal, hypo-dermal and panniculuscarnosus layers were removed completely. After making surgical wounds, all mice were randomly marked using an non-toxic color. The animals were divided into the following four groups of six animals each (both male and female) and were treated as given below:

Group I Normal control group received petroleum jelly

Group II Standard group received povidone iodine ointment

Group III Drug treated group received 10% w/w of *Musa acuminate*

Group IV Drug treated group received 10%w/w of *Allivum sativum*

The drugs were topically applied daily until the formation of complete epithelial layer, starting from the first day of wound excision. All the animals were monitored daily and observed for any

Wound fluid, evidence of infection and any other abnormalities. The diameters of the wound were measured immediately by using Vernier caliper.

The wound area of each animal was measured from the first day of wounding to the days (8th and 16th) until the healing was complete. The wound closure was measured at regular intervals of time to see the percentage of wound closure and epithelialization time that indicates the formation of new epithelial tissue to cover the wound.

The percentage of wound contraction was determined using the following formula:

$$\text{Percentage of wound contraction} = \frac{\text{Initial day wound size} - \text{Specific day wound size}}{\text{Initial day wound size.}} \times 100$$

The number of days required for falling of the scar without any residual of the raw wound gave the period of epithelialization.

4.4.4 Incision wound model (potawale *et al*., 2007):

Animals were anaesthetized and para vertebral incisions (2.5-3.0 cm long) were made through the entire length of skin. After the incision was made, the parted skin was kept together and stitched with nylon thread at 0.5 cm apart with curved needle. The two test formulations, cream base and povidone iodine ointment were applied on wound once daily for 7 day. The sutures were removed on day 8 and wound tensile strength was measured on day 10 by using constant water flow technique.

4.4.5 Constant water flow technique:

On the 10th day the animals was secured to the operation table, under light

ether anaesthesia. A line was drawn on normal skin on either side of wound, 3 mm away from the wound line. Two Allis forceps were firmly applied on the lines facing each other. On one side the forceps was hooked firmly to metal rod fixed to the operation table. The other forceps was connected to a leak proof graduated polythene container through a string running over a pulley. The polythene container was connected to water reservoir placed at suitable height through a rubber tube kept occluded with a pinchcock. To measure wound tensile strength, the tube was released to allow a constant and continuous flow of water from the reservoir in to the polythene container. As the weight gradually increases, it acts as a pulling force to disrupt the wound. As soon as the gapping of the wound was observed, the rubber tube was clamped and the polythene container was weighed.

RESULT AND DISCUSSION

5. Result And Discussion

5.1 SOLVENT EXTRACTION:

Musa acuminata and *Allium sativum* were coarsely chopped and were soaked in 70% acetone solution. This homogenized mixture or slurry was left at room temperature for about 48 hrs. The slurry of *Musa acuminata* and *Allium sativum* was collected after 48 hours at room temperature the completion of this reaction was indicated by the conversion of yellow transparent liquid turned to amber and later to an opaque black liquid. The slurry was filtered after 48 hrs of incubation at room temperature and used for further analysis. Neem extract was prepared according to the method of Agbenin & Marley (2006). The mixture was allowed to stand for 4 hrs, and the homogenate was filtered (Whatman No.1). The completion of this extraction shows black greeny colour and this extraction shows maximum inhibitory zones against the gram + ve and gram - ve organisms. The extraction of *Musa acuminata* and *Allium sativum* were showed good results in phytochemical analysis and contain antibacterial activity.

Fig 5.1 Extraction of *Musa acuminata* in acetonic solvent

5.2 PHYTOCHEMICAL ANALYSIS :

The qualitative chemical test for the extracts was performed. The result reveals the presence of phytochemicals considered as active medicinal chemical constituents. The preliminarily phytochemical test indicates the presence of flavonoids, glycosides and phenols in the acetonic extract of *Musa acuminata* . The preliminarily phytochemical test indicates the presence of flavonoids and saponins in the acetonic extract of *Allium sativum* . The results were summarized in Table 5.2.1. The presence of bioactive compounds indicates the medicinal value of the extracts of *Musa acuminata* and *Allium sativum*, because their possible use as natural additives emerging from a growing tendency to replace synthetic antioxidants with natural ones.

Table 5.1 Phytochemical test for acetonic extract of *Musa acuminata* and *Allium sativum*

S. No.	Compound	*Musa acuminata*	*Allium sativum*
1.	Glycosides	+	-
2.	Phenols	+	-
3.	Flavonoids	+	+
4.	Saponins	-	+

Fig 5.2 Test for glycosides in the acetonic extract of *Musa acuminata*

Fig 5.3 Test for phenols in the acetonic extract of *Musa acuminata*

Fig 5.4 Test for flavonoids in the acetonic extract of *Musa acuminata*

Fig 5.5 Test for saponins in the acetonic extract of *Musa acuminata*

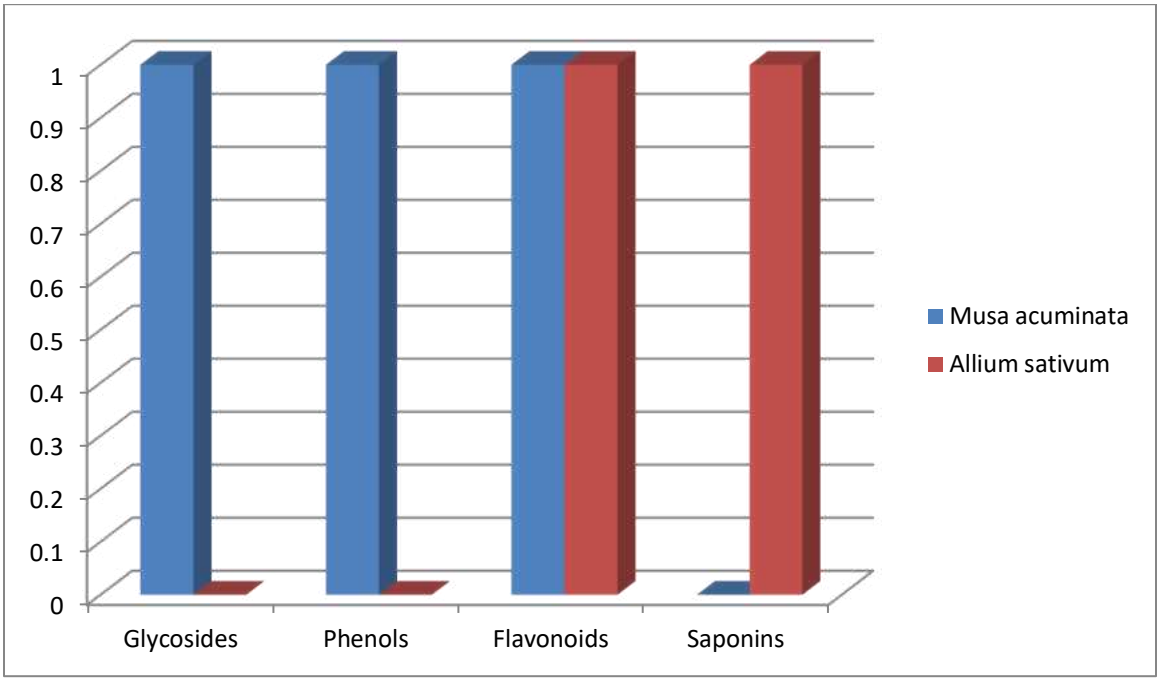
Fig 5.6 Test for phenols in the acetonic extract of *Allium sativum*

Fig 5.7 Test for saponins in the acetonic extract of *Allium sativum*

Fig 5.8Test for flavonoids in the acetonic extract of *Allium sativum*

Fig 5.9Test for glycosides in the acetonic extract of *Allium sativum*

Graph 5.1 Phytochemical test for acetonic extract of *Musa acuminata* and *Allium sativum*



5.2.1 DISCUSSION:

The phytochemical analysis were carried out and the results were tabulated. Fresh leaves of *F. religiosa* & *C. limonia*, seeds of *P. dactylifera*, stems of *S. chirata*, black seeds of *Sesamum indicum* and roots of *R. sativus* for screening were found to possess flavonoids (Watal *et al*., 2014). The phytochemical screening of *Musa acuminata* indicates the presence of glycosides(formation of yellow precipitate), phenols (formation of bulky white precipitate) and flavonoids (formation of red colour precipitate).Flavonoids are also present in the extracts as a potent water –soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have a strong anticancer activity. It also helps in managing diabetes induced oxidative stress. Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells. Interestingly, saponins are present in *S. chirata* which is supposed to be of maximum medicinal value out of *F. religiosa* & *C. limonia*, seeds of *P. dactylifera*, stems of *S. chirata*, black seeds of *Sesamum indicum* and

roots of *R. sativus* as it possesses majority of identified phytoconstituents (Gupta *et al*., 2014) . Similarly the present study carried out were also contain the saponins in *Allium sativum* . The phytochemical screening of *Allium sativum* indicates the presence of flavonoids (formation of red colour precipitate) and saponins (formation of foam). In addition, it has been found that saponins have anti-tumour, antioxidant and anti-mutagenic activities and can lower the risk of human cancer by inhibiting the growth of cancer cells. Saponins protect against hypercholesterolemia and antibiotic properties. The presence of wide range of phytochemical constitutes indicates that the extract taken could be used in a multitude of ways which may be beneficiary to a population. The finding of this study correlate with the finding of (Abaoab *et al*., 2013). Phytochemical may protect humans from a host of diseases.

Phytochemicals are non- nutritive chemicals that have protective or disease preventive properties. The flavonoids and phenolic compounds in the extract taken have been reported to exert multiple biological effects including antioxidants, free radical scavenging abilities, anti inflammatory, anticarcinogenic etc.,

5.3 Anti-bacterial activity:

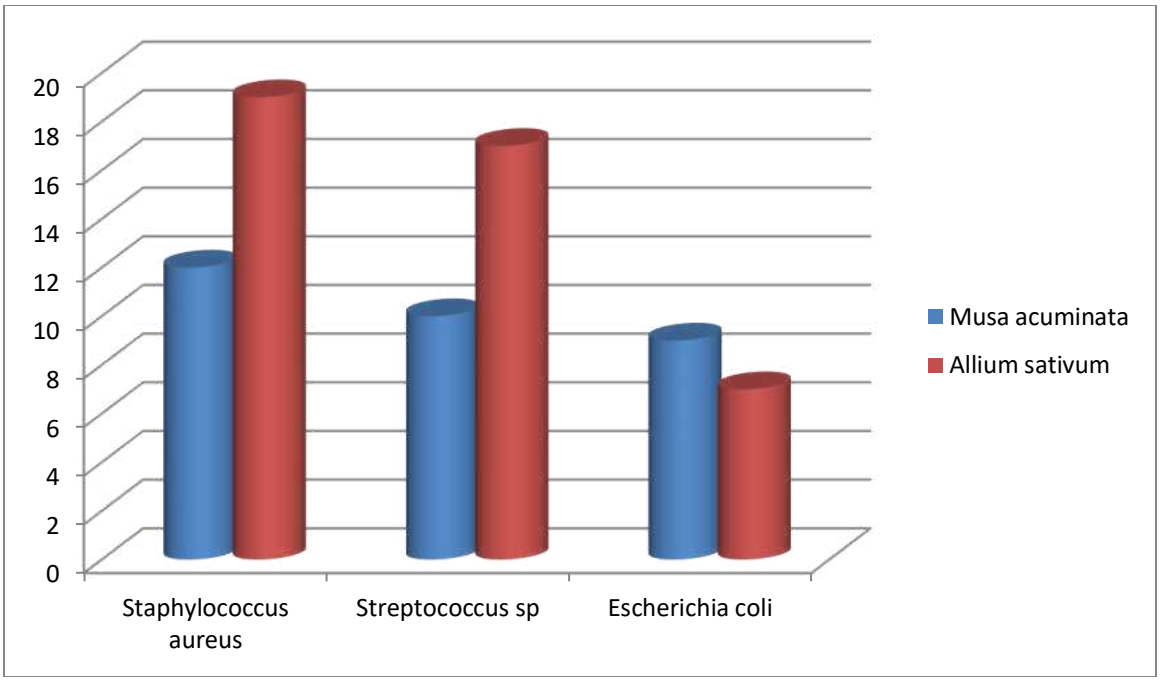
The results suggest that extract of *Musa acuminata* and *Allium sativum* has antibacterial activity assay against *Staphylococcus aureus, Streptococcus sp* and *Escherichia coli* was determined by measuring the diameter of inhibition expressed in mm. *Musa acuminata* and *Allium sativum* showed varied in the exploitation of antibacterial activity of zone of inhibition from 8-20mm against *Staphylococcus aureus, Streptococcus* and *Escherichia coli* . The degree of antibiotic property depends upon several factors such as age of the plant, duration of storage, temperature, preparation of the media, PH (Joys Selva Mary Albert, 2015). *Musa acuminata* showed minimum activity against the *Staphylococcus aureus* (12 mm) and *Streptococcus* (10 mm) and *Escherichia coli* (9 mm). The extract of *Allium sativum* showed maximum activity against *Staphylococus aureus* (19 mm) and *Streptococcus*(17 mm) and *Escherichia coli* (7 mm).The differences further observed in the antibacterial effect of *Musa acuminata* and *Allium sativum* extract studied against *Staphylococcus aureus, Streptococcus sp* and *Escherichia coli* in the present study may be due to permeability barriers.

In *Staphylococcus aureus, Streptococcus sp* and *Escherichia coli* the outer membrane is fairly affect barrier for the extract and also active compound persists. The susceptibility of *Staphylococcus aureus, Streptococcus sp* and *Escherichia coli* in acetonic extract of *Allium sativum* is more than in acetonic extract of *Musa acuminata* may be due to the presence of saponins as it was positive in phytochemical analysis of acetonic extract of *Allium sativum* . It can be concluded that *Allium sativum* is more effective and bioactive components can be purified for pharmaceutical products.

Table 5.2 Antibacterial activity of acetonic extract of *Musa acuminata* and *Allium sativum*

S.no	Method	Sample	Zone Of Inhibition (mm)		
			S. aureus	*Streptococcus sp*	*E.coli*
1.	Well cut method	*Musa acuminata*	12	10	9
2.	Well cut method	*Allium sativum*	19	17	7

Graph 5.2 Antibacterial activity of acetonic extract of *Musa acuminata* and *Allium sativum*



5.3.1 Disscusion:

Antibacterial activity of acetonic extract of *Musa acuminata* and *Allium sativum* had been assessed by measuring the diameters of zones of growth inhibition on some strains of *Staphylococcus aureus, Streptococcus sp and Escherichia coli* and the results were tabulated. The finding suggests that both these extracts are good antibacterial agents against gram+ve and gram-ve organisms. This antibacterial activity is due to the presence of flavonoids, phenols, saponins and glycosides. A study by (Karuppiah *et al*., 2013) showed that the methanolic extract of cloves had the moderate antibacterial activity against *E.coli, vibrio sp*. Our findings showed that the acetonic extract of *Musa acuminata* had the minimal antibacterial activity against the tested bacteria including *Staphylococcus aureus, Streptococcus sp and Escherichia coli*. Inhibition growth of the highest zone had been shown by acetonic extract of *Allium sativum* against *Staphylococcus aureus* (19 mm) and *Streptococcus* sp (17 mm). Inhibition growth of the moderate zone had been shown by acetonic extract *of Musa acuminata* against *Staphylococcus aureus* (12 mm) and *Streptococcus* sp (10 mm).The comparative study analyzed with our findings and the result predicted that the inhibition growth of zone of acetonic

extract of *Allium sativum* was higher than the inhibition growth of zone of acetonic extract of *Musa acuminata* against *Staphylococcus aureus, Streptococcus sp and Escherichia coli* . The growth inhibition was less active against *Escherichia coli* (9 mm) and (7 mm). The methanolic extract of *Zingiber officinale* which did not present antibacterial effect against *S. aureus* and *E. coli* (Samy *et al*., 2010). Our present study, verified an inhibitory action of acetonic extract of *Allium sativum* against *Staphylococcus aureus, Streptococcus sp and Escherichia coli*. Antibacterial resistance is the global problem. Therapeutic options are limited because of wide emergence of multidrug resistance.

5.4 Wound healing activity:

Plant products are potential wound healing agents, and largely preferred because of their wide spread availability, non-toxicity. There is an increasing interest to use plants in wound healing because of lower side effect and management of wounds over the years (Mohammad Reza Farahpour, 2019). In excision wound study table wound contraction progress identically with providing iodine ointment and in wound treatment with formulation of the acetonic extract of *Musa acuminata* and *Allium sativum ,* in this four group complete healing was observed in *Allium sativum* on 16^{th} day. On 16^{th} day the wound concentration of standard (Povidone iodine ointment) and the test were found to be significant when compared to control. However on 16^{th} day the test group exhibited 63.00% and 85.73% healing with 10% of acetonic extract of *Musa acuminata* and *Allium sativum* and the standard (Povidone iodine ointment) group showed 90.06% of healing in incision wound.

Tensile strength of group 1 control was found to be 121.69 ± 0.33 and standard was found to be 128.52 ± 0.30 while acetonic extract of *Musa acuminata* and *Allium sativum* was found to be 291.3 ± 3.09 and 362 ± 4.05 respectively table 5.3. The topical application of drug is an efficient therapy method of destroying microbial population because of the availability of drug at the infected wound site lead to enhance wound healing activity. The virulence capacity of microorganism, amount of inoculums and post immune response are important response that cause massive damage during infection . The tensile strength of 10% (w/w) methanolic extract of *Acanthus polystachyus* , showed significant increase in breaking

strength by 35.8, 32.7 and 31.2% respectively, when compared to normal negative control ($P < 0.001$) but failed to reach statistical significance (Asrade *et al.,* 2018) .The wound contaction of chloroform fraction 20 % (w/w) of the extract of *Ficus exasperata* showed significant contraction of 55.73 % from the 19th day (Victoria *et al*., 2014) . When compared to other the study of acetonic extract of *Musa acuminata* and *Allium sativum* showed complete recovery of wound in 18 days.

Table 5.3 Effect of topical application of *Musa acuminata* and *Allium sativum* on excision wound model (wound area mm).

TREATMENT		% WOUND CONTRATION IN EXCISION WOUND MODEL		
		0 DAY	*8TH DAY*	*16TH DAY*
Control (Simple ointment) (Group 1)		521.02 + 3.15	316.55 + 3.30 (39.24)	119.55 + 3.23 (77.05)
Standard (Povidone iodine ointment (Group 2)		523.47 + 2.42	218.09 + 2.00 (58.33)	49.17 + 0.75 (90.06)
Musa acuminata (Group 3)	10% w/w	510.12 + 1.21	331.20 + 9.09 (35.67)	186.72 + 2.03 (63.00)
Allium sativum (Group 4)	10% w/w	503.62 + 2.57	225.92 + 4.13 (55.14)	71.82 + 1.43 (85.73)

Graph 5.3 Effect of topical application of *Musa acuminata* and *Allium sativum* on excision wound model

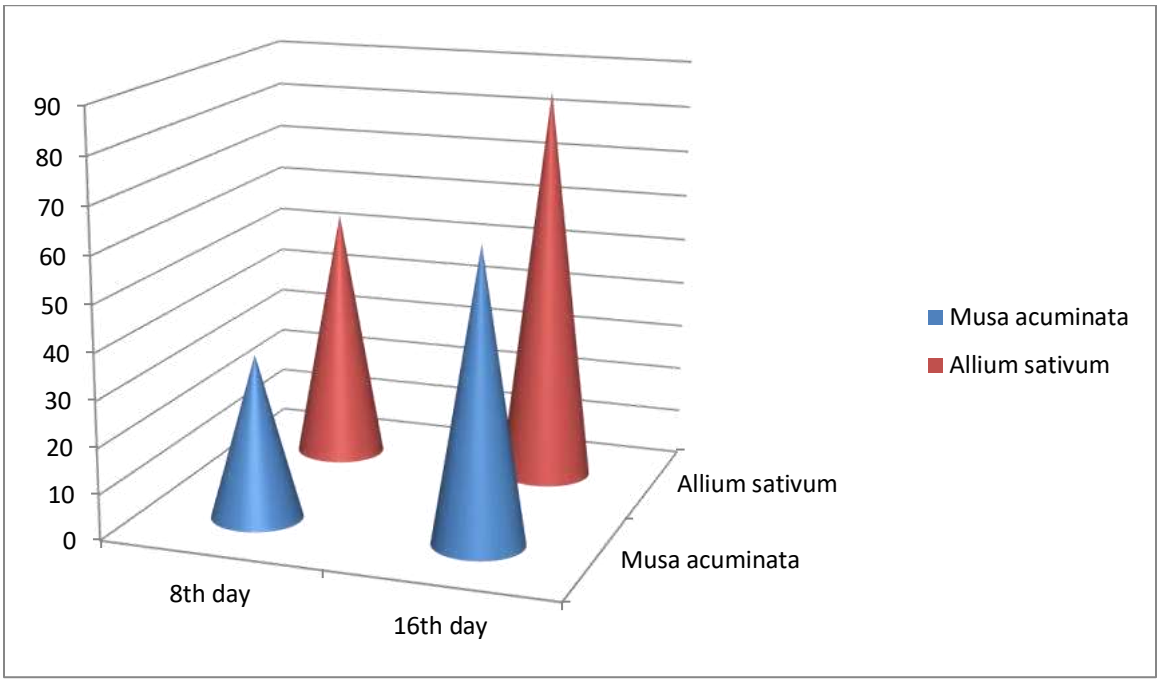
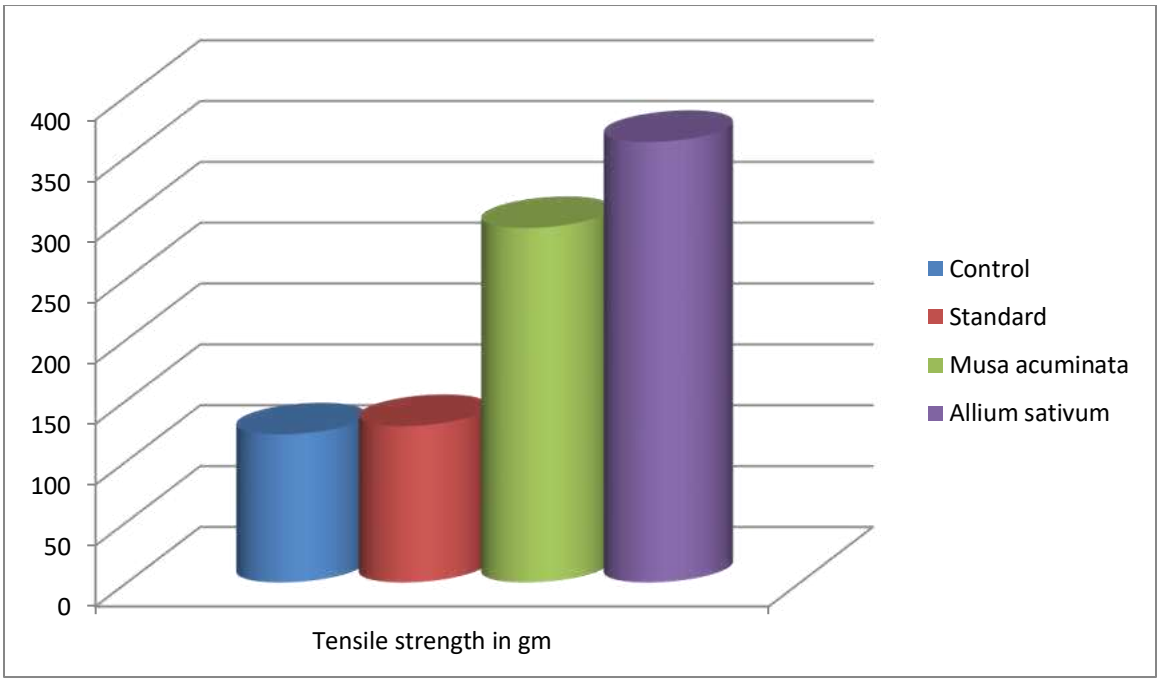


Table 5.4 Effect of topical application of *Musa acuminata* and *Allium sativum* on incision wound model.

Treatment		Tensile strength (gm)
Control (simple ointment) (Group 1)		121.69 $\pm$ 0.33
Standard (povidone iodine ointment) (Group 2)		128.52 $\pm$ 0.30
Musa acuminata (Group 3)	10% w/w	291.3 $\pm$ 3.09
Allium sativum (Group 4)	10% w/w	362 $\pm$ 4.05

Graph 5.4 Effect of topical application of *Musa acuminata* and *Allium sativum* on incision wound model



5.4.1 Discussion:

Plant products are potential wound healing agents, and largely preferred because of their wide spread availability, non-toxicity. There is an increasing interest to use plants in wound healing because of lower side effect and management of wounds over the years (Mohammad reza farahpour 2019). Our extract prepared from the acetonic solvent of *Musa acuminata and Allium sativum* has been reported to possess phytochemical properties and antibacterial activity and hence it is used in the treatment of wounds. The comparison analyzed with our present study and the result predicted that the acetonic concentration of *Allium sativum* showed 85.73 % wound contraction in excision wound model than the acetonic concentration of

Musa acuminata showed 85.73 % wound contraction in excision wound model. The values of the tensile strength of the incision wound treated with the EA fraction on day 10. Both the high dose (10% (w/v)) and the low dose (5% (w/v)) of EA fraction exhibited wound breaking strength values comparable to the one of the control group. The tensile strength of the mice treated with 5% (w/v) EA fraction ointment was higher than the one of the group treated with 10% (w/v) . The best healing was observed for the group treated with the EA fraction ointment on the 10th day of treatment (Fu Wang *et al*., 2016). Our findings proved that the tensile strength of acetonic extract of *Allium sativum* showed 362 $\pm$ 4. and the tensile strength of acetonic extract of *Musa acuminata* showed 05 291.3 $\pm$ 3.09. From our findings we predicted that the effect of wound healing of acetonic extract of *Allium sativum* is effective than the acetonic extract of *Musa acuminata.*

CONCLUSION

6. Conclusion

The *Musa acuminata* and *Allium sativum* which is taken up for many researches concerning the phytochemical and pharmaceutical applications. The Antibacterial screening carried out for the extract and compound of *Musa auminata* and *Allium sativum* showed that they exhibits significant activity against the organisms tested. The extract *Musa acuminata* and *Allium sativum*, exhibits significant activity against all the test organisms indicating that the extract posses such compound responsible for the activity. This study suggest that *Musa acuminata* and *Allium sativum*, can be reproductively used in the pharmaceutical area because of it's possible activity reported.

The antibacterial activity of *Musa acuminata* and *Allium sativum* was screened against the organisms isolated from the wound sample, carried out by agar well diffusion assay. The *Allium sativum* extract showed maximum zone of inhibition against the isolated pathogens than the other extract of *Musa acuminata*.This study paved way to identify the unexplored phytoconstituents that will prove promising their use in pharmacology .

Wound healing is the process by which damaged tissue is restored as closely as possible to it's normal state. Wound concentration is the process of shrinkage of the area of the wound. It is mainly dependent upon the type and the extent of damage, the general state of health and the ability of the tissue repair. Wound healing ability of *Musa acuminata* and *Allium sativum*, to treat the wound. Among the stuided extract, the *Musa acuminata* extract is more in healing power. However it needs further evaluation of clinical settings before consideration for the treatment of wounds. Hence, by this method we could produce antibiotics against some diseases and wound healing capacity from the *Musa acuminata* and *Allium sativum* extract.

SUMMARY

7. Summary

Prebiotics are compounds in food that induce the growth or activity of beneficial microorganisms such as bacteria and fungi. Dietary prebiotics are typically nondigestable fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them. They were first identified and named by Marcel Roberfroid in 1995.

In this study, Musa acuminata and *Allium sativum* which was collected from the Teachers colony, Thoothukudi. *Musa acuminata and Allium sativum* was cut into small pieces and soaked in 70% of acetone for about 3 days. Then the solvent was blended. After 3 days of soaking, the extracts were collected by filtering through Whatmann Filter Paper no 1. The solvents were evaporated and the crude extracts were stored for further use.

The acetonic extract of *Musa acuminata* and *Allium sativum*, were analyzed to identify the presence of phytoconstituents. Tests were carried out for glycosides, phenols, flavonoids and saponins by the following standard methods. The result indicates the presence of glycosides, phenols, flavonoids in the acetonic extract of *Musa acuminata* and in the acetonic extract *of Allium sativum* the result indicates the presence of flavonoids and saponins.

The diabetic foot wound swab was collected from the Government Hospital in Thoothukudi. The collected swab was processed to isolate the microflora present in the sample. The swab sample was inoculated on Nutrient broth. The plates were observed for growth and organisms were identified based on gram staining and biochemical charaterization. The pathogen isolated from the swab was identified as *Staphlococcus aureus and Streptococcus sp*. The acetonic extract of *Musa acuminata and Allium sativum* were tested against both gram +ve and gram –ve bacteria *Staphylococcus aureus, Streptococcus sp and Escherichia coli*. Muller-Hinton agar plates were used to demonstrate the antibacterial properties of the acetonic extract of *Musa acuminata* and *Allium sativum* by agar well diffusion method to determine the antibacterial activity. In our present study, the antibacterial activity of acetonic extract of *Musa acuminata* and *Allium sativum* showed significant activity against *Staphylococcus aureus*, *Streptococcus sp* and *Escherichia coli.* The acetonic extract of *Allium sativum* showed maximum zone of inhibition against *Staphylococcus aureus* and *Streptococcus sp* than the acetonic extract of Allium sativum against *Staphylococcus aureus* and *Streptococcus sp*. The acetonic extract of *Musa acuminata and Allium sativum* showed moderate zone of inhibition against Escherichia coli.

The excision wound healing activity was studied by the method described by Luisa A. DiPietro., (2003) and Farahpour and Habibi., (2012). The wound healing effect of acetonic extract of *Musa acuminata* and *Allium sativum* were investigated in this study. Povidone iodine ointment was used as standard. The acetonic extract of *Musa acuminata* and *Allium sativum* showed a significant contraction of wound which is equivalent to the standard which demonstrates

the rapid action of the extract in wound healing. The acetonic extract of *Musa acuminata* showed less contraction than the acetonic extract of *Allium sativum*

This study throws a light on the concept of utilization of *Musa acuminata* and *Allium sativum* and identifies the unexplored phytoconstituents that will prove promising in pharmacology and drug discovery. Hence, further research needs to be done to study the structural and functional properties of the identified phytoconstituents that are effective against *Staphlococcus aureus*, *Streptococcus sp* and *Escherichia coli*.

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WOUND HEALING BY *MUSA ACUMINATA* AND *ALLIUM SATIVUM*

ABSTRACT:

Allicin, the active component of garlic, has been shown to have antimicrobial and anti-inflammatory properties. Banana (*Musa acuminata*) peel is a rich source of many nutrients and considered high in carbohydrates. It has been traditionally used to treat diarrhea, anemia and ulcers. Some studies have shown that banana peels possess antioxidant and anti-inflammatory properties Fibroblasts play a key role in wound healing. Here we hypothesize that fibroblasts are being activated by allicin, leading to more organized and rapid wound repair. The data tells us thatallicin is acting on fibroblasts as there were more proliferating fibroblasts in the alloy *allium sativum* treated sites than in *Musa acuminata*. So it is concluded allium sativum extract increase the role of wound healing and decrease the rate of infection than *Musa acuminata*.

KEY WORDS: *Allium sativum*, *Musa Acuminata*, wound healing, fibroblasts.


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ANTI – MICROBIAL ACTIVITY BY *MUSA ACUMINATA* AND *ALLIUM SATIVUM*

Abstract:

Musa acuminata is used widely because of its nutritional values. In past, there are studies that show banana plant parts, and their fruits can Be used to treat the human diseases. There are no studies that relate the antibacterial activity of banana peel against periodontal pathogens. Garlic (*Allium sativum*) has been used for a long time as a spice or traditional medicine. Garlic contains allicin compound which has the role as anti-infective agents. This study aims to determine the potential of garlic as an antimicrobial agent in *Staphylococcus aureus* and *Corynebacterium diphtheriae* bacteria in vitro and determine the activity of allicin as an antibacterial in silico. Hence, the aim of this study is to determine the antimicrobial activity on *Musa acuminata* and *allium sativum.*

KEY WORDS: *Musa acuminata*, *Allium sativum*, anti – microbial activity, phytochemical activity.

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A NOVEL APPROACH TO INVESTIGATE ANTIDIABETIC EFFECT OF PROBIOTIC CURD CONTAINING *Lactobacillus sp.*, IN ALBINO RAT.

A DISSERTATION SUBMITTED TO

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

Affiliated To Manonmaniam Sundaranar University.

In partial fulfilment of the requirements for the award of the degree of

BACHELOR OF SCIENCE IN MICROBIOLOGY

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This is to certify that this dissertation work entitled **"A novel approach to investigate the antidiabetic effect of probiotic curd containing *Lactobacillus sp.* in albino rat."** is a bonafide record of the original work completed by **Ms R.. Deepa, R. Esakkipriya, A. Esakkiammal, S. Fathima Barkana, J. Jebina. (Reg. No. 18SUMB06 -18SUMB10)** during the academic year 2020 – 2021 in St. Mary's college (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirements for the award of the degree of bachelor of science in Microbiology prescribed by Manonmaniam Sundaranar University.

Signature of the Guide

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Signature of the Guide **Signature of the H.O.D**

Signature of the Director **Signature of the Principal**

Signature of the External Examiner

DECLARATION

We hereby declare that the dissertation work entitled **"A novel approach to investigate the antidiabetic effect of probiotic curd containing *Lactobacillus sp.*, in albino rat."** is a bonafide record of the work completed by us during the academic year 2020 – 2021 in St. Mary's college (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirements for the award of the degree of bachelor of science in Microbiology prescribed by the Manonmaniam Sundaranar University. We also affirm that this is a original work done by us under the supervision of **Dr. Joys Selva Mary Albert,** Coordinator & Head of department of Microbiology, St. Mary's college (Autonomous), Thoothukudi.

Signature of the Students **Signature of the Guide**

1.

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Place : Thoothukudi

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ABBREVIATION

1) Mm - Millimeter
2) dm - diameter
3) gm - gram
4) ml - millilitre
5) °C - degree Celsius
6) µl - microliter
7) hr - Hour
8) min - Minute
9) % - Percentage
10) kg - Kilogram
11) Mb - Megabase.
12) cfu. – Colony forming unit.
13) MHA - Mueller hinton agar
14) EMB - Eosin methylene blue
15) MRS - De man Rogasa and sharpe agar
16) NB - Nutrient Broth
17) NA - Nutrient Agar
18) E.Coli - Escherichia coli
19) S.aureus - Staphylococcus aureus
20) A.niger - Aspergillus niger

CONTENT

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INTRODUCTION

1.INTRODUCTION

Curd is regarded as a complete and natural food. It is a product of the milk which is white in colour and resulted due to bacterial action. Daily consumption of fresh and curd helps in preventing some diseases by virtal of the nutrients and probiotics bacteria, it contains. It is a functional food, which boosts natural as well as acquired immunity and improves stamina. Being the richest sources of probiotics, curd offers beneficial and healthy microflora to our alimentary canal. Curd is prepared either from cow or buffalo's milk of the family bovidae. The curd prepared from buffalo's milk is denser (16-19%) than cow's milk (12%) due to higher total solids. The microorganisms involved in the formation of curd are that such a *Streptococcus cremons , Streptococcus lactis , Streptococcus thermophillu , Lactobacillus ulgaris, Lactobacillus acidophilus, Lactobacillus helveticus* and *Lactobacillus cremoris* (Madhu *et al.,*2013).Curd is formed by the process of lactic acid fermentation so a small amount of fermentated curd is used as a starting materials. Addition of 0.2% citrate is recommended for rendering pleasant aroma to dahi. Firm and uniform consistency, sweet aroma, palatable taste and the manufacturing conditions determine the compostion of curd. Curd forms the richest source of probiotics known as so far. Curd is an natural and healthy food which prevents from numerous enteric diseases . It contains lactic acid bacteria and a perfect balance of proteins, carbohydrates, fats, vitamins, minerals and water. Curd boosts the immune system and systematic host immunity. Further it activates macrophages, enhances the level of immune globulins , NK cells activates and cytokinesin host cells. .
Probiotics contain Living microorganisms such as lactic acid bacteria which provide health benefits to the host. curd promotes the health of the host by boosting the immune system. It strengths natural immunity by stimulating oh mucosal and sytem host immunity which is manifestal through activated

macrophages , Increased levels of immune globulins, higher levels of Natural killer (NK) cell activities and cytokines in the host cell (Ashraf and shah *et al* , 2011) .Curd bacteria can trigger a cascade of immunological dense mechanism by binding the recognition of receptors , such as Toll like receptors Stimulate (TLRs) expressed on the surface of epithelial cells . These may also CD4 the production of immune cells such as CD4 + T – cells in HIV Patients . + T – cells mediate and control the balance of pro-inflammatory cytokines and chemokines . So curd can be used for the treatment of allergy , urinogentital infection , HIV , Cancer , Infections of helicobacter Pylori , liver diseases , inflammatory bowel diseases (IBD) , Irritable bowel syndrome (IBS) and Pancreatitis (Shadnoush and Shaker , 2013) . Depending on the immune system of host , different probiotics include adherence and colonization of the gut , suppression of growth or invasion by pathogenic bacteria , Improving of intestinal barrier function and production of antimicrobial substances. Researchers believe that food plays an increasingly important role in society with respect to diseases prevention and human well being . For this reason , the microbial aspects of food safety should seriously be taken into considersion (Havelaar AH *et al ,* 2009 ; Mor – Mur M . *et al* ,2010) . Due to their essential role in production and the favour and providing food preservation , *lactic acid bacteria* (*LAB* eg : *Lactobacilli*) are highly important in fermentation foods . It is found that the use of these bacteria as probiotics starters leads to the production of stable intestinal flora , competition with potentially harmful bacteria , and the prevention of certain diseases (Khan I *et al* , 2016) ; (RiazRajokaMs . *et al.,* 2017) . In addition , these bacteria are considered as effective means for enhancing food safety and stability due to consumers (Castellano . P . *et al* .,2008) . Further , *LAB* can act as an antagonist against Some pathogenic bacteria.To confer health benefits , probiotics needs to survive in the acidic conditions of the stomach, as well as the acidic conditions of some fermented foods and tolerate bile salt in the small intestine for colonization and metabolic activity . (Munoz – Quezada *et al* , 2013) . Future more , it is very

important that they can maintain their viability during the production , processing and storage of the fermented products . (Gandhi A *et al* , 2015) . On the other hands , the risks of the transmission of antibiotic resistance of genes to pathogenic and commensal bacteria in the gut should seriously to be known as a by using a probiotics in the foods (Adimpong DB *et al* , 2012 ; Khorrani . S *et al* ., 2018 ; Wang cy *et al.,* 2010).The term probiotics was first used by Lilly and still well (1965) do describe the substance secreted by one microorganisms that stimulates the growth of another Parker (1974) proposes that probiotics are defined as live microorganisms, which when administered in adequate amount confer health benefit do the host (Anonymous , 2002) Probiotics are also defined as live microbial feed supplements that improve the intestinal microbial balance of host .The most important clinical significance also probiotic therapy is prevention and treatment of the gastro intestinal infection and diseases (parvez *et al* , 2006). Elie Metchnikoff , A Russian Scientists working at the pasteur institute in Paris is credited with giving attention to health believe benefits of fermented milk . In 1994, the World Health Organization [WHO] regards that probiotics stimulate the immune defence system . When prescribed by antibiotics resistance is termed as a microbial interferons therapy (Zhou *et al ,* 2005 ; Botes*et al ,* 2008).Curd is obtained by coagulation milk in a sequential process called curdling . It can be a final dairy product or the first stage in cheese making. The coagulation can be caused by adding rennet or any edible acidic substance such as lemon juice or vinegar and then allowing it to coagulate . The increased acidity cause the milk proteins (casein) to tangle into solid masses or curds . Milk that has been left to sour (raw milk alone or pasteurized milk with a added lactic acid bacteria) will also naturally produce curds , and sore milk cheeses are produce this way. Producing cheese curds is one of the first steps in cheese making; the curds are pressed and drained to varing amounts for different styles of cheese and different secondary agents (molds for blue cheese etc.) are introduced before the desired aging finisbes the cheese .

The remaining liquid which contains only whey proteins is the whey . In cow's milk , 90 % of the proteins are caseins .In Indian English , used only in the Indian subcontinent , curd or curds instead refers to the traditional homemade yogurt (also known as dahi) while paneer and chhena are used to denote curdled milk . Though people often consider curd and yogurt to be the same , there's a thin line of difference between the two . The preparation of curd requires a *lactobacillus* bacteria , while yogurt is made using two specific strains of bacteria called lactobacillus *delbrueckiisubsp.bulgaricus* and *Streptococcus thermophilus* bacteria . Other strains of lactic acid bacteria may also be added. Curd is a traditional yoghurt or fermented milk product , originating from the Indian subcontinent usually prepared from cow's milk and sometimes buffalo milk or goat milk . It is popular throughout the Indian sub continent. The word curd is used in the English of Indian to refer to (naturally probiotics) homemade yogurt , while the term yogurt refers to the pasteurized commercial variety as heat treated fermented milk.

1.1 PROBIOTIC:-

Fermentative bacteria like *LAB* produce hydrogen peroxide to protect themselves from oxygen toxicity. The accumulation of hydrogen peroxide in growth media,and it's antagonistic effects on *Staphylococcus aureus* and *Pseudomonas*, have been demonstrated by researchers. *LAB* beginning Cultures have been used as starter cultures to create fermented foods since the of the 20th century. Elie Metchnikoff won a nobel price in 1908 for his work on *LAB. Lactobacilli* administered in combination With other probiotics benefits cases of irritable bowel syndrome (IBS), although the extent of efficacy is still uncertain. The probiotics help treat IBS by returning homeostasis when the gut microbiota. Experiences unusually high levels of opportunistic bacteria. In addition, *Lactobacilli* can be administered as probiotics, during cases of infection by the ulcer-causing *bacterium Helicobacter pylori. Helicobacter pylori* is linked to Cancer, and antibiotic resistance impedes the success of current antibiotic based

irradication treatments. When probiotic *Lactobacilli* are administered along with the treatment as an adjuvant, its efficacy is substantially increased and side effects may be lessened. Also, *lactobacilli* are used to help control urogenital and vaginal infections, such as bacterial vaginosis (BV). *Lactobacilli* produce bacteriocins to suppress pathogenic growth of certain bacteria as well as lactic acid and H2O2 (hydrogen peroxide). Lactic acid lowers the vaginal pH to around 4.5 or less, hampering the survival of other bacteria, and H2O2 restablishes the normal bacterial microbiota and normal vaginal pH In children, *lactobacilli* such as *Lacticaseibacillus rhamnosus* (previously *L. rhamnosus*) are associated with a reduction of atopic eczema, also known as dermatitis, due to anti-inflammatory cytokines secreted by this probiotic bacteria. In addition, lactobacilli with Other probiotic organisms in ripened milk and yogurt aid development of immunity in the mucous intestine in humans by raising the number of LgA (+).The term probiotics derived from the latin prefix pro which means for and the greek noun Bios (bios) which means "life". Hamiltin – Miller JM *et al.,*2003). According to the currently adopted definition by FAO/WHO, probiotics are : " live micro-organisms which when administered in adequate amounts confer a health benefit on the host."(Isolauri E *et al*.,2001, sanders ME *et al*.,2003). *Lactic acid bacteria (*LAB) from the genera *lactobacillus, streptococus, Enterococcus, the genus Bifido bactetium some yeasts and non-pathogenic strains Escherichia coli* are the most common types of microbes proved to be probiotics. (KootteRs *et al*., 2012).Gut flora consists of micro organisms that live in the digestive tracts of animals and is the largest reservoir of human flora with concentration of 10 14 cells / ml and approximate weight of 1.5 kg. In this context, gut is synonymous with intestinal tract and flora with microbiota and microflora. The human body, consisting of about 10 trillion cells, carries about ten times (100.109), as many micro organisms from 300 to 1000 different species in the intestines. (Turnbaugh PJ *et al*., 2007). In result our gut microflora (gut microbiom) carries more than 3

million genes, which provides a broad range of functions and abilities for dynamical changes according ti the factors of the environment. (Burcelin R *et al.,* 2011). The metabiotic activities performed by these of an organ, leading some to liken gut bacteria to a "forgotten" organ. (Quigley EM, 2010). The metagenome includes genomes of all microorganisms which humans are hosts for as they can live in various organs such as skin, lungs, vagina, mouth and intestinal tract. (Dethlefsen L *et al.,* 2007).It is shown that the gram negative *bacteroidetes* and the gram-positive firmicutes account for more than 90% of all phylotypes of gut bacteria. (Tsukumo DM *et a1*. 2009). Other phyla that are found as part of the gut microbes includes the gram – negative *proteobacteria* and *verrucomicrobia* ad well as the gram-positive *Actinobacteria*. (kootteRs et al., 2012. More than 90% of the bacterial population are obligate anaerobes (*Bacteroides , Eubacterium, Bifidonacteinrium , Fusobacterium , Peptostreptococcu)*Tsukumo DM *et al.,* 2009) .The gut microflora has its importance in controlling and regulating of different physiological processes . Once it is set , the gut microflora can be influenced by various internal or external factors , which can modify it's ecological structure. Such factors are antibiotics per biotics and probiotics and thus ready of processes in human body can be altered considering this the gut microbiota is a key organ in nutritional metabolism and possibly its imbalance contributes to the development of obesity (Ley RE *et al.,* 2006) and diabetes. (Larsen N *et al.,* 2010). It had been proved that administration of probiotics which contains *lactobacillus acidophilus and lactobacillus casei* significantly delayed the progression of high fructose-induced glucose intolerance and especially L. Casei decreases the plasma glucose levels. (Andersson *U et al*., 2010). At the same time increased number of *bifidobacterium* species correlates with inproved glucose tolerance and insulin, secretion. (Y in YN *et al.,* 2010). It is interesting to note that gaining body weight is associated with increase number of bacteria from phyla firmicutes and bacteroidetes (Larsen N *et al*., 2010) and antibiotics treatment of obese mice modify the gut microflora which leads to decrease

of body weight and improves glucose tolerance (vrieze A *et al.,* 2010). Obesity and diabetes are metabotic disorders with epidemiology distribution in USA and Europe. (Shameddeen H *etal*.,2011). Diabetes and impaired glucose tolerance are connected with obesity, which is widely discussed else where. (Schwiertz A *et al.,* 2009, Turnbaugh PJ *et al.,* 2009). Now a days about 190 millions people worldwide are with diabetes and until 2025 this number is expected to reach 300 millions. It is thought that in bulgaria about 2.5 – 3% of the population suffers from diabetes.The pathogenesis of diabetes mellitus is complex and one of the factors implemented is the oxidative stress. Probiotic – containing foods have been reported to suppress oxidative stress. *L. acidophillus* and *L. Casei* also attenuate oxidative stress and have antidiabetic effects. It was shown that *L.casei* decreased the oxidative stress (villarinin M *et al*., 2008) and suppressed the effector functions of CD4+ 7 cells, accompanied by reducing the proinflammatory molecules, (So JS *et al*., 2008) . Thus having antioxidant, immune- modulatory effects and antidiabetic effects. Basic risk factors for development of T2D are age above 40 years, overweight, family predisposition, etc. Chronic stress., Of muscle activity and inappropriate diet are considered to be the main triggering causes of T2D (stefanov TS etal.,2011) .Recent studies have shown that did it factor implemented in development of only 10-20 percentage of case of diabetes whereas the way of alimentation and social behavior prodominate (Burcelin.Retal.,2011).

1.2 IMPORTANCE OF PROBIOTICS:-

The benefits of curds in manufacturing health, treatment of diseases and management have been investigated in animal models and human subjects.Some studies have proved that curd in maintaining health, treatment of AIDS, Cancer, Diabetes, Insomia and Hepatic diseases.

1.3 MICROBIAL SPECIES WITH APPLICATIONS AS PROBIOTICS :

As far as nutrition is concerned only the strains classified as lactic acid bacteria are of significance and among them the one's with the most important properties is an applied context are those belonging to the the genera *lactococcus* and *bifidobacterium* (W.H Holzapfal *et al* .,2001) . Two other species playing an important role in food industry, particularly dairy products, although not strictly considered as probiotics for *streptococcus thermophilus* and *lactococcuslactis* , two of the most commercially important lactic acid bacteria (G.E Felis *et al* .,2007). It is important to mention that since probiotics activities are strain related strain identification is recommended in order to establish their suitability and performance for industrial application. This is achieved by a combination of phenotypic tests followed by genetic identification using molecular techniques like DNA / DNA hybridization 16 sRNA sequencing and so forth.

MICROORGANISMS CONSIDERED AS PROBIOTICS:-

Lactobacillus species, Bifidobacterium species , L . acidophilus, L . casei, L . crispatus, L .plantarum, L . reuteri, L . rhamnosus, B . adolescentis, B . longum.

1.3.1 DESIRABLE PROBIOTICS PROPERTIES :

In order for a potential probiotic strain to be able to exert its beneficial effects it is expected to to exhibit certain desirable properties. The ones currently determined by in vitro tests are;

a . Acid and bile tolerance which seems to be crucial for oral administration

b. Adhesion to mucosal and epithelial surface an important property for successful immune modulation competitive exclusion of of pathogens as well as prevention of pathogen adhesion and colonization.

c. Antimicrobial activity against pathogenic bacteria

d. Bile salt hydrolase activity

Nevertheless, the value of these parameters is still under debate as there are matters of relevance , vivo and vitro discrepancies, and lack of standardization of procedure to be considered. As there are no specific parameters essential to all probiotic application, best approach to establish a strains properties is target population and target physiologic function specific studies (A . Mercenier *et al* ., 2018 , M . Saarela *et al* ., 2000).Viability is by definition a prerequisite for probiotics functionality as it potentiates mechanisms such as adherence , reduction of gut permeability and immuno modulation and constitutes an industrial challenge (C . M. Galdeano *et al* ., 2004 , B . Kosin *et al* ., 2006) .thus for certain probiotics change of optimal growth during the in a initial production steps might be sufficient and they may not need to retain good viability during storage (S . J . Lahtinanetal ., 2012 .)

1.3.2 MECHANISMS OF PROBIOTICS ACTIVITY:-

Probiotics have various mechanism of action although the extract manner in which they exert their effects is still not fully elucidated . These range from bacteriocin and short chain fatty acid production , lowering of gut pH , nutrient competition to stimulation of mucosal barrier function and immuno modulation . The Latter in particular has been the subject of numerous studies and there is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by including phagocytosis and IgA secretion , modifying T – cells responses , enhancing Th1 responses and attenuating Th2 responses (F . Guarner *et al*., 2003, C. E MC Naught et al ., 2001 E . Isolauri *et al* ., 2001)

1.3.3 PROBIOTICS OF FOOD PRODUCTION:-

The range of food products containing probiotics strains is wide and still growing . The main products existing in the market are dairy – based ones including fermented milks , cheese , ice cream , butter milk , milk powder , and yogurts , the latter accounting for the largest share of sales (C. Stanton *et al .,* 2001).Non dairy food application include soy based products , nutrition bars , cereals , and a variety of juices as appropriate means of probiotics delivery to the consumer (J . A Ewe *et al* ., 2010 , V.M Sheehan *et al* ., 2007) . The product pH for instance is a significant factor determining the incorporated probiotics survival and growth and this is one of the reasons why soft cheeses seem to have a number of advantages over yogurt as delivery systems for viable probiotics to the gastrointestinal tract (C. Kehagiar *et al* ., 2006) micro encapsulation technologies have been developed to protect the bacteria from damage caused by external environment . By the introduction of a straw delivery system containing a dry form of the probiotic beverage manufacturers can now provide it to the consumer . In addition , viable spores of a spore forming probiotic are available in the market offering advantages during processing , In the same time , the potential of lantibiotics – substances with antimicrobial properties- production by bifidobacteria is being explored in order to be applied in the food area (D.E pszczola 2012 ,D.J. o' sulluivan 2012).

1.3.4 HEALTH BENEFITS OF PROBIOTICS:-

There is increasing evidence in favour of the claims of beneficial effects attributed to probiotics , including improvement of intestinal health , enhancement of the immune response , reduction of serum cholestorst and cancer prevention . These health properties are strain specific and are impacted by the various mechanism mentioned above . While some of the health benefits are well documented others require additional studies in order to be established . In fact ,

There is substantial evidence to support probiotic use in the treatment of acute diarohoeal disease , prevention of antibiotic – associated diarrhoea , and improvement of lactose metabolism, but there is insufficient evidence to recommend them for use in other clinical conditions.

1.4 LACTOBACILLUS:-

Lactobacillus is a genus of gram-positive, aerotolerant anaerobes or microaerophilic, rod shaped, non-spore-forming bacteria. Until march 2020, the genus *Lactobacillus* comprised over 260 phylogenetically, ecologically, and metabolically diverse species; a taxonomic revision of the genus in 2020 assigned. *Lactobacilli* to 25 genera including the Homo fermentative genera *Lactobacillus, Holzapfelia, Amylolactobacillus, Bombilactobacillus, Companilactobacillus, Lapidilactobacillus, Agrilactobacillus, Schleiferilactobacillus, Loigolacto bacillus, Lacticaseibacillus, Latilactobacillus, Dellaglioa, Liquorilactobacillus, Ligilactobacillus* and *Lactiplantibacillus* and the *heterofermentative Furfurilactobacillus, Paucilactobacillus, Limosilactobacillus, Fructilactobacillus, Acetilactobacillus, Apilactobacillus, Levilactobacillus, Secundilactobacillus* and *Lentilactobacillus.*properties of genera.

SCIENTIFIC CLASSIFICATION

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Lactobacillales

Family: Lactobacillaceae

Genus: *Lactobacillus*

Lactobacillus species constitute a significant component of the human and anima lmicrobiota at a number of body sites, such as the digestive system, and the female genital system. In women of European ancestry, *Lactobacillus* species are normally a major part of the vaginal microbiota. *Lactobacillus* forms biofilms in the vaginal and gut microbiota, allowing them to persist during harsh environmental conditions and maintain ample populations. *Lactobacillus*

exhibits a mutualistic relationship with the human body, as it protects the host against potential invasions by pathogens and in turn, the host provides a source of nutrients. *Lactobacilli* are among the most common probiotic found in food Such as yogurt, and it is diverse in its application to maintain human well-being, as it can help treat diarrhea, vaginal Infections, and skin disorders such as Eczema.

1.4.1 METABOLISM:-

Lactobacilli are homofermentative, i.e hexoses are metabolised by glycolysis to lactate as major end product, or heterofermentative, i.e. hexoses are metabolised by the Phosphoketolase pathway to lactate, CO2 and acetate or ethanol as major end products. Most *Lactobacilli* are aerotolerant and some species respire if heme and menaquinone are present in the growth medium.Aerotolerance of lactobacilli is manganese-dependent and has been explored (and explained) in Lactiplantibacillus plantarum (previously *Lactobacillus plantarum*). *Lactobacilli* generally do not require iron for growth.The *Lactobacillaceae* are the only family of the Lactic acid bacteria that includes homofermentative and heter of ermentative organisms; in the *Lactobacillaceae*, homofermentative or heterofermentative metabolism is shared by all strains of a genus. *Lactobacillus* species are all homofermentative, do not express pyruvate formatelyase, and most species do not ferment pentoses.In *L.crispatus*, pentose metabolism is strain specific and acquired by lateral gene transfer .

1.4.2 GENOME:-

The genomes of *lactobacilli* are highly variable, ranging in size from 1.2 to 4.9Mb(megabases).Accordingly, the number of protein-coding genes ranges from 1,267 to about 4,758 genes (in *Fructilactobacillus sanfranciscensis* and *Lentilactobacillus parakefiri*, respectively). Even within a single species there can be substantial variation. For instance, strains of *L. crispatushavegenome* sizes ranging from 1.83 to 2.7 Mb, or 1,839 to 2,688 open reading frames. *Lactobacillus* contains a wealth of compound microsatellites in the coding region of the genome, which are imperfect And have variant motifs. Many *Lactobacilli* also contain multiple plasmids.A recent study has revealed that plasmids encode the genes which are required for adaptation of *lactobacilli* to the given environment.

1.4.3 TAXONOMY:-

The genus *Lactobacillus* currently contain 44 species which are adapted to vertebrate hosts or to insects. In recent years, other members of the genus . *Lactobacillus* (formerly known as the Leuconostoc branch of *Lactobacillus*) have been reclassified into the genera *Atopobium, Carnobacterium, Weissella, Oenococcus,* and *Leuconostoc.* The pediococcus species *P. dextrinicus* has been reclassified as a *Lapidilactobacillus dextrinicus* and most *Lactobacilli* were assigned to *Paralactobacillus* or one of the 23 novel genera of the *Lactobacillaceae*. Two websites inform on the assignment of species to the novel genera or species.

1.4.4 HUMAN HEALTH:

1.4.4.1 VAGINAL TRACT:-

The female genital tract is one of the principal colonisation sites for human microbiota, and there is interest in the relationship between the composition of these bacteria and human health, with a domination by a single species being correlated with general welfare and good outcomes in pregnancy. In around 70% of women, a *Lactobacillus* species is dominant, although that has been found to vary between American women of European origin and those of African origin, the latter group tending to have more diverse vaginal microbiota. Similar differences have also been identified in comparisons between Belgian and Tanzanian women.

1.4.5 INTERACTION WITH OTHER PATHOGENS:

Lactobacilli produce hydrogen peroxide which inhibits the growth and virulence of the fungal pathogen Candida albicans in vitro and in vivo. In vitro studies have also shown that lactobacilli reduce the pathogenicity of C. albicans through the production of organic acids and certain metabolites. Both the presence of metabolites, such as sodium butyrate, and the decrease in environmental pH caused by the organic acids reduce the growth of hyphae in C. albicans, which reduces its pathogenicity. *Lactobacilli* also reduce the pathogenicity of C. albicans by reducing C. albicans biofilm formation. Biofilm formation is reduced by both the competition

From *lactobacilli*, and the formation of defective biofilms which is linked to the reduced hypha growth mentioned earlier. On the other hand, following antibiotic therapy, certain candida species can suppress the regrowth of lactobacilli at body sites.Where they cohabitate, such as in the gastrointestinal tract. In addition to its effects on C. albicans, *Lactobacillus sp.* Also interact with other Pathogens.For example, *Limosilactobacillus reuteri* (formerly *Lactobacillus reuteri*) can inhibit the growth of many different bacterial species by using glycerol to produce the antimicrobial substance called reuterin. Another example is *Ligilactobacillus salivarius* (formerly *Lactobacillus alivarius)*, which interacts with many pathogens through the production of Salivaricin , a bacteriocin.

AIMS AND OBJECTIVE

2 . AIMS AND OBJECTIVE

★ To prepare curd using cow's milk.

★ To isolate lactobacilli strain from the prepared curd.

★ To isolate *Staphylococcus aureus* from the diabetic wound sample.

★ To isolate *Escherichia coli* from urine sample.

★ To determine antimicrobial activity by well diffusion method.

★ To determine antidiabetic activity from probiotic curd.

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

Shulman GI (2000) reports that fermented milk products dahi, which contains probiotic bacteria.

Isolauri *E et al.,* (2001), sander ME *et al*., (2003) are said that the according to the currently adopted definition by FAO/WHO, probiotic are; “live microorganisms which when administered in adequate amounts confer a health benefit on the host

W.H. Holzapfel *et al.*, (2001) are stated that as far as nutrition is concerned only the strains classified as lactic acid bacteria are of significance and among them the ones with the most important properties in an applied context are those belonging to the general *Lactococcus* and *Bifidobacteriumi*.

C. Stanton *et al.,* (2001) stated that the range of food products containing probiotic strains is wide and still growing.

Sobngwi E, Mauvais-Jarvis F _*et al.*_ , (2001), informed that, economic aspects of diabetes and diabetes care currently attract considerable attention as the world Diabetes epidemic takes hold and the healthcare Activities of countries come under pressure to Accomplish more within constrained resources.

Shobhana R,. Rao PR, Lavanya A _*et al.,*_ (**2002**)estimated that,total annual cost Associated with diabetes in Latin America and the Caribbean to be US$65.216 billion. With a prevalence of 200,000 type 1 diabetics in India.

Patlak M. (2002), said that,The terms "Diabetes" and “Mellitus” are derived from Greek. “Diabetes” denotes "a passer through; a siphon" whereas the "Mellitus" denotes "sweet". It is thought that the Greeks named it so due to the excessive amounts of urine produced by diabetics attracted flies and bees.

Robert H.(2002),defines that , The insulin production is directly proportional to the amount of sugar (carbohydrate) consumed. The more sugar one consumes, the more insulin the body will have to produce, but, the tiny pancreatic beta cells were never designed to produce this level of insulin.

Hamilton – Miller JM *et al.,* (2003) are stated that the term probiotic derived from the Latin prefix pro which means for and the Greek noun bios(bios) which means 'life".

F. Guarner *et al*., (2003), are said that Probiotics have various mechanisms of although the exact manner in which they exert their effects is still not fully elucidated.

Barcelo A, Aedo C, Rajpathak S and Robles S. (2003),said that, The prevalence of diabetes in the WHO African Region Was estimated in 2000 to be at 7.02 million people. Of These, about 0.702 million had type 1 diabetes and 6.318 million had type 2 diabetes. In addition, close to 113,100 people died from diabetes related causes, 561,600 were permanently disabled, and 6,458,400 Experienced temporary disability.

C.M. Galdeano *et al.*, (2004) said that viability is by definition a prerequisite for probiotic functionality as it potentiates mechanisms such as adherence, reduction of gut permeability, and immunomodulation and constitutes an industrial challenge.

Votey SR. and Peters AL. (2004), included that catabolism and anabolism of carbohydrates, lipid and protein emanating from defective insulin secretion, insulin action,or both.

Belinda R. (2004) ,Insulin is a pancreatic hormone responsible for blood glucose level regulation. The hormone binds to its receptor sites on peripheral side of the cell membranes.

Anonymous. (2004),said that ,Insulin is a polypeptide hormone synthesized in humans and other mammals within the beta cells of the islets of Langerhans in the pancreasThe islets of Langerhans form the endocrine part of pancreas, accounting for 2% of the total mass of the pancreas,with beta cells constituting 60-80% of all the cells of islets of Langerhans .

Ley RE *et al*., (2006) ,larsen N *et al*., (2010) are said that the gut microflora has its important in controlling and regulating of different physiological process once it is set the gut microflora can be influenced by various internal or external factors which can be modify it's a ecological structure such factors or antibiotic prebiotic and probiotic and such variety of process in human body can be altered considering this the gut microbiota is a key organ in nutrition metabolism and possibly its imbalance to the development of obesity and diabetes.

Sicree R, *et al.,* (2006), said that, diabetics mellitus is a combination of heterogeneous Disorders commonly presenting with episode of hyperglycemia and glucose intolerance,as a result of Lack of insulin.

Piero MN (2006),it includes, various organ failure, progressive metabolic complications such as retinopathy, nephropathy, or neuropathy.

Kibiti CM. (2006),definesthat,A regular energy source is a prerequisite for every cell to function in the human body.

Njagi JM. (2006), informed that,Glucose is the body's primary energy source, which circulates in the blood as a mobilizable fuel source for cells .

Turnbaugh PJ *et al*., (2007) identified that the gut flora consists of microorganisum that live Gut Flora consist of microorganism that live in the digestive tract of animals and is the largest reservoir of human Flora with concentration of 10^{14}cell/ ml and approximate weight of 1.5 kg. In this context gut synonyms with intestinal track and flora with microbiota and micro flora human body, consisting of about 10 trillion cells carries about 10 times (100-10^{9}) as many microorganism from 300 to 1000 different species in the intestine

Dethlefsen L *et al*., (2007) are reported that the metagenome includes genome of all microorganism which human are host for us they can live in the various organs such as in skin, lungs, vagina, mouth and industrial track.

G.E. Felis *et al.*, (2007) are reported two other species playing an important role in food industry, particularly dairy products, although not strictly considered as probiotics are streptococcus *thermophilus* and *LactococcusLactis*, two of the most commercially important *Lactic acid bacteria.*

Castellano P, *et al*., (2008) reported that. In addition, these bacteria are considered as effective means for enhancing food safety and stability due to consumers' awareness and concern regarding chemical additives.

Botes, M., *et al.,* (2008) said that The use of probiotics in antibiotic resistance is termed as microbial interference therapy.

A. Mercenier *et al*., (2008) are observed in order for a potential probiotic strain to be able to exert its beneficial effects, it is expected to exhibit certain desirable properties.

Villarini M *et al*., (2008) are said that nowadays about 190 million people worldwide are with diabetes and until 2025 this number is expected to reach 300 million it is thought that in Bulgaria about 2.5 to 3 % of the population suffers from Diabetes. The pathogenesis of the diabetes mellitus is complex and one of the factors implement it oxidative stress probiotic containing food have been reported to suppress oxidation stress lactobacillus acidophilus and lactobacillus casei also attenuated oxidative stress and how antidiabetic effect it was shown that lactobacillus species decreases oxidative stress and suppress

So JS *et al.*, (2008) said that the effector function of CD4+, T cells, accompanied by reducing the prion inflammatory molecules

Tsukoma DM *et al.*, (2009) are reported that More than 90 % of the bacteria population or obligate anaerobes (*Bacteroides,Eubacterium,Bifidobacterium, fusobacterium, peptostreptococcusp*

Schwierz*et al*., (2009) are observed diabetes and impaired glucose tolerance are connected with obesity which is widely discussed elsewhere

Tsukumo *et al.,* (2009) are said that it is shows that the Gram Negative *bacteroidetes* and gram positive *firmicutes* account for more than 90 % of all phytotypes to gut bacterium

Kirigia JM, Sambo HB, Sambo LG and Barry SP. (2009), regards that, diabetes mellitus Poses a big economic burden with regards to health System costs, indirect costs arising from losses Occasioned by patient disability and premature Mortality, time spent by family members accompanying Patients when seeking care, and intangible costs in Terms of psychological pain to the family and loved Ones.

Quigley EM (2010) The metabolic activities performed by these microorganism resemble those of an organ leading some to like gut bacteria to a "forgotten" organ.

Havelaar AH *et al*., (2010) observed that, food plays an increasingly important role in society with respect to disease prevention and human well-being.

Mor-Mur M, Yuste J. *et al.,* (2010) said that, microbial aspects of food safety should seriously be taken into consideration.

Wang CY*, et al.,* (2010) isolated that, dilutions were spread on the MRS agar media, followed by incubating for 48 hours at 37°C under anaerobic condition in a jar with AnaerocultAstrip. After initial tests 16 gram-positive *bacilli* or *coccobacilli* and catalasenegative bacteria were chosen to study their most important probiotic criteria including antibiotic resistance, antagonistic activity, resistance to low pH, resistance to bile salt, biofilm formation, cell surface hydrophobicity, auto-aggregation and co-aggregation, and stability in storage conditions.

Shamseddeen H *et al.* ,(2011)described that obesity and diabetes and metabolic disorder with epidomological distribution in USA and Europe

Stefanov TS *et al*., (2011) this having antioxidant immune -modulatory effect and antibiotic effect basic risk factor for development of Td for age about 40 years overweight family practice portion except chronic stress of muscles activity and in operator diet it is considered to be the main triggering causes of T2D .

Burcelin R *et al.*, (2011) recent studies have shown that genetic factors are implement in development of only 10 to 20 % of case of diabetes whereas the way of alimentation and social behavior predominant

Burcelin R *et al.*, (2011) In result our gut microflora (gut microbium) carries more than 3 million genes which provides a broad range of function and ability for dynamic changes according to the factor of the environment

Adimpong DB, *et al.*, (2012) said that,normal flora of the mouth, gut and the vagina of the mammals

KootteRs *et al.*, (2012) are reported that the*latic acid bacteria (LAB)* from the genera *Lactobacillus, streptococcus, enterococcus the genus Bifidobacterium*some yeast and *nonpathogenic strain Escherichia coli* are the most common type of microbes proved to be probiotics.

Kootte RS *et al.*, (2012) Other phyla that are found as part of the gut microbes include the gram-negative *roteobacteria and verrucomicrobia a*s well as the Gram Positive Bacteria *Actinobacteria*

S.J. Lahtinen *et al.*, (2012) identified that thus for certain probiotic strain optimal growth during the intial production steps might be sufficient and they may not need to retain good viability during storage.

D.E. Pszczola *et al.*, (2012) are said that micro encapsulation technologies have been developed to protect the bacteria from damage caused by external environment. By the introduction of a straw delivery system containing a dry form of the probiotic beverage manufactures can now provide it to the consumer

Madhu, Prakash SM *et al.*, (2013) describe that,Curd is prepared either from cow or buffalo's milk of the family Bovidae. The microorganisms involved in the formation of curd are*Streptococcuscremoris, Streptococcus lactis, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus helveticus and Lactobacillus cremoris.*

Shadnoush M, *et al.*, (2013) identified that Curd bacteria can trigger a cascade of immunological defence mechanism by binding to recognition receptors, such as Toll- like receptors (TLRs) expressed on the surface of epithelial cells. These may also stimulate the production of immune cells such as CD4+ T- cells in HIV patients. CD4+ Tcells mediate and control the balance of pro inflammatory and anti- inflammatory cytokines and chemokines

Jena PK, *et al.,* (2013) Isolated that,the wide variety of natural media, including fermented food products (e.g., yogurt, cheese, sauerkraut, coffee, sausages, vegetables, sourdough, and kimchi), as well as the normal flora of the mouth, gut and the vagina of the mammals.

Muñoz-Quezada S, *et al.,* (2013) Isolated that,To confer health benefits, probiotics need to survive in the gut and to attach and colonize the intestine. Therefore, they must survive in the acidic conditions of the stomach, as well as the acidic environment of some fermented foods and tolerate bile salt in the small intestine for colonization and metabolic activity.

Aoudia N, Rieu A, Briandet R, Deschamps J et al., (2016) isolated for LAB can act as an antagonist against some pathogenic bacteria by producing various antimicrobial compounds such as bacteriocins organic acids, hydrogen peroxide, carbon dioxide, and diacetyl.

RiazRajoka MS, *et al.,* (2017) described that highly important in fermented foods. It is found that the use of these bacteria as probiotic starters leads to the production of stable intestinal flora, competition with potentially harmful bacteria, and the prevention of certain diseases.

SoltanDallal MM, Davoodabadi A, *et al.,* (2017) isolated that LAB can act as an antagonist against some pathogenic bacteria by organic acids, hydrogen peroxide, carbon dioxide, and diacetyl .

Son SH, Jeon HL *et al.,* (2017) identified that normal flora of the mouth, gut and the vagina of the mammals.

Khorrami S, Zarrabi A, ., (2018) reported that, the risk of the transmission of antibiotic resistance genes to pathogenic and commensal bacteria in the gut should seriously be taken into consideration by using probiotics in the foods.

MATERIALS AND METHODS

4 . MATERIALS AND METHODS

4.1 COLLECTION OF MILK SAMPLE:

The milk sample for the present study was collected from domestic farm and brought to the laboratory in sterile condition.

(Fig :4. 1)

4.2 PREPARATION OF CURD:

Fermentation in the milk consists essentially the gradual conversion of lactose lactic acid by lactose fermentation organisms . The lactose molecules is broken down by the enzymes lactose to glucose and galactose on its pathway (glycolytic pathway) towards lactic acid. Each of the simpler sugars is further acted upon by routes of acid end product. However , a portion of acid combines with the calcium of casein to form calcium lactate thus setting free the caseins and coagulation . It its iso- electric point (PH 4.6) is reached . The calcium combine .As CA HP04 in milk goes completely into solution more rapidly than calcium combined as caseinate during souring.

LACTIC ACID BACTERIA

$C_{12}H_{33}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$

Lactose Glucose Galactose

GLYCOTIC PATHWAY

$C_6H_{12}O_6 \longrightarrow 4C_3H_6O_3$

Glucose lactic Acid

Ca – caseinate + Lactic Acid → ca – lactate = casein(Curd form)

(Fig :4. 2)

4.3 ISOLATION OF LACTIC ACID BACTERIA :

- *Lactobacillus* strains were isolated from domestically prepared curd samples.
- Sample was serial diluted and diluted samples were plated onto De Man Rogosa Sharpe (MRS) medium for *Lactobacillus* isolation and incubated at 37°C for 48 hours under anaerobic condition.
- Isolated colonies with typical characteristics of *LAB* were picked from each plate and sub cultured into MRS broth and nutrient agar slant.

4.4 IDENTIFICATION OF LACTIC ACID BACTERIA (LAB)-*LACTOBACILLUS*:

- Further identification of the *Lactobacilli* was performed according to their morphological, cultural, physiological and biochemical characteristics as described by Bergey's Manual of systematic bacteriology.

4.4.1 SIMPLE STAINING:

The bacterial culture was smeared with a simple stain. A thin smear of pure isolatedColony was made on a clean glass slide dried in air and fixed by passing through flame of a burner. commonly used basic dyes were added such as methylene blue, and then examined under the compound microscope.

4.4.2 GRAM'S STAINING :

Isolated bacterial strains were identified by performing grams staining. A thin smear of pure isolate colony was made on a clean glass slide, dried in air and fixed by passing through flame of a burner. The smear was covered with crystal violet kept for one minute. The slide was washed with water, then covered with gram iodine and stand for one minute. The slide was again washed with water. Decolorized with alcohol, was achieved by rocking the slide gently for twenty seconds till the violet colour came off the slide and then washed with water immediately. This was counterstained with safranin for twenty seconds. Washed with water, blot dried and then examined under the compound microscope.

4.4.3 MOTILITY TEST:

The Isolated culture was tested for motility by hanging drop method and result was observed.

4.5 BIOCHEMICAL TEST:

4.5.1 Indole test :

Peptone water medium was prepared in the test tubes and the culture was inoculated into the medium, and the tubes were incubated at 37°C for 24 hours, after incubation kovac's reagent was added and the result was observed.

4.5.2 Methyl Red Test:

MR – VP broth medium was prepared in the test tubes and the culture was inoculated in to the medium and tubes were incubated at 37°C for 24 hours, after incubation few drops of methyl red indicator was added and the result was observed.

4.5.3 Voges Proskauer Test :

MR – VP broth medium was prepared in the test tubes and the culture was inoculated in to The medium and tubes were incubated at 37°C for 24 hours, after incubation 0.5 ml of alpha naphthol,0.2ml of KOH were added and the result was observed.

4.5.4 Citrate Test :

Simmon Citrate Agar slant was prepared and the culture was inoculated as a single streak on the agar slant surface, and the tubes were incubated at 37°C for 24 – 48 hours, after incubation The result was observed.

4.5.5 Catalase Test :

The catalase test was performed by slide method, on a slide, few drops of hydrogen peroxide were placed to which the culture was added and the result was observed.

4.6 IDENTIFICATION OF *ESCHERICHIA COLI* FROM URINE SAMPLE :

Peptone is the nitrogen source in EMB agar. Agar is a solidifying agent. Dipotassium phosphate is the buffer. Eosin Y and methylene Blue are the indicators. Methylene blue is also a selective agent. The accompanying micro flora which hinders the isolation of medically important organisms is inhibited by the dyes of the medium, especially gram- positives. Collect the urine sample from Dog; Take a sterile inoculation loop & dip it in to a culture as Urine sample. Inoculate the entire EMB agar surface of each plate, first in horizontal direction and then in vertical direction to ensure the even distribution of the organisms over the agar surface. Incubate the medium at 37° c for 24 hours and recorded the results.

4.7 ISOLATION OF *STAPHYLOCOCCUS AUREUS* FROM WOUND SAMPLE :

Staphylococcus is gram-positive cocci that are microscopically observed as individual organisms, in pairs, and in irregular, grapelike clusters**.** Growth of Staph.aureus is obtained by inoculation of sample on Blood agar media and it is confirmed by Gram's staining and different biochemical tests catalase Test.

.4.7.1 Blood Agar Media

Blood Agar media was prepared and sterilized by autoclave. Samples were inoculate on this media and were overnight incubated at 37 0C. Staph.aureus produces golden yellow colonies which are further identify by biochemical tests and Gram staining.

4.7.2 Biochemical Tests

The *Staphylococcus aureus* isolates were identified by the standard morphological and culture characteristics by performing Gram's stain, catalase test.

4.7.3 Gram's staining :

Thin smear of the isolates was prepared on clean glass slides using a sterile loop. The slides were air dried and then heat fixed by passing it through a flame and performed Gram's staining, smear was covered with crystal violet stain for 60 seconds then poured off and covered the smear with Lugol's iodine solution for 30 seconds. The iodine solution was poured off and the smear was decolorized with acetone iodine decolorizer until the ceased to come out of the smear. The slide was thoroughly washed with water. The slide was counter stained with diluted carbol fuchsin for 30 seconds. Washed with water, blotted with absorbent paper and air dried. Organisms that retained the crystal violet-iodine dye complexes, after Decolorizing with acetone-iodine, stain purple and were termed as Gram positive and those that lost that complex and become red due to counter stain (carbolfuchsin) were termed Gram negative .

4.7.4 Catalase Test:

This test is useful in distinguishing organisms that have the ability to produce catalase from those that lack this enzyme.This test was performed from a blood free non inhibitory medium for example nutrient agar. Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen.hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism.

$$2H202 \text{ ----------} \rightarrow 2H20 + 02$$

A small amount of culture to be tested was picked with glass rod and inserted into the 3% hydrogen peroxide solution in a clean tube.The production of gas bubbles indicated a positive test

4.8 IDENTIFICATION OF *ASPERGILLUS NIGER* FROM PEANUT:

Aspergillus niger isolated from the peanut. Take a sterile inoculation loop and dip in to the cultures *A.niger* inoculate the entire Rose Bengal agar surface of the each plate . First in horizontal and then the vertical direction. To ensure the even distribution of the organisms over the agar surface. Incubate the medium at 25^0 c for 48 hours and recorded the results. Morphology of *Aspergills niger* like others, Aspergillus niger are filamentous fungi, which means that they tend to form filaments (hyphae) and thus resemble the structure of a plant. When viewed under the microscope, *A. niger* consists of a smooth and colorless conidiophores and spores.In microscopy, the carbon black/dark brown color of the spores (as well as the conidia) is used to distinguish *A. niger* from other species in the same genus.

4.9 ANTIMICROBIAL ACTIVITY:

The Antimicrobial effect of isolates was evaluated by well diffusion test on MHA medium ,Rose bengal agar medium ,plated with two pathogens as;(*Staphylococcus aureus* is isolated from wound healing sample ;*Aspergillus niger* is isolated from)For this purposes.to determine the smallest amount of the antibiotic needed to inhibit the growth of the micro organisms. The resulting value is called the minimal inhibitory concentration (MIC) which is determined by measuring the diameter of growth inhibition (Clear) zone surrounding the disc.It used to demonstrate the Antimicrobial activityby ;Sample as curd ;18 hours nutrient broth culture of *Staphylococcus aureus, Aspergillus niger*, Antibiotic as streptomycin (for bacteria) , *ketacanozole* (for Fungi) as standard were used. By using ,the .sterile cotton swab and dip it in to a culture or cell suspension. Inoculate the entire agar surface of each plate, first in horizontal direction and then in vertical direction to ensure the even distribution of the organisms over the agar surface, using the swab then , the medium used with test organisms, that incubated plate was kept in side for few minutes .by using the well cutter ,wells were made in those plates at require distance. In each step of well cutting, the well cutter is thoroughly sterlize with alcohol. Using sterlize micropipette loaded the sample .The plates were incubated at 37C for 24 hrs for bacterial. For fungi the plate were incubated at 25C for 48 hrs. After that the incubation, inhibition of growth was analysed and results were recorded. Antimicrobial activity was evaluated by measuring the zone of inhibition in mms using graduated scale.

4.8 ANTIDIABETIC ACTIVITY

The antidiabetic effect of probiotic *Lactobacillus sp* in type2 diabetic rat.Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and a body imbalance in the metabolism of carbohydrates, fats and proteins. The treatment of diabetic mellitus can be done by giving synthetic drugs but the use of synthetic drugs can have a side effect on users. The type2 diabetics was induced to the albino wister rat .There are very limited studies on antidiabetic effect of lactic acid bacteria despite their potential in reducing the risk of Diabetes onset, reported that oral administration of *Lactobacillus casei* in albino rat ,significantly decreased plasma glucose levels and inhibited the Production of -cell specific CD4 T cells and

cytokines(interferon and interleukin-2) that are leading factors for induction of autoimmune diabetes.In contrast to these observations, in the present study dahi was used as a carrier of probiotics rather than direct administration of cultures. Feeding of a high fructose diet provides a dietary model of type 2 diabetes associated with insulin resistance, hyperinsulinemia , hypertriglyceridemia , and hypertension.

4.8.1 INDUCTION OF DIABETES :

- ★ Alloxan (2,4,5,6-tetraoxpyriminde; 2,4,5,6 pyrimidinetetrone) is an oxygenated pyrimidine derivative (Lenzen.,2008) and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wohler and Justus Von Liebig (Lenzen*et al.*,1998).

- ★ Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species
  .
- ★ This causes an insulin-depent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen,2008).

4.8.2 ANIMALS :

- Male wister rats weighing 190-210g were used in the experiments.
- They were maintained in standard environmental conditions.

  Temperature (25±20c).
  Relative humidity (55±10%).
  12 hrs dark/light cycle.

- They were fed with standard diet and water and libitum (Thirupathireddy and Ravikumar.,2006).

4.8.3 CHEMICALS AND REAGENT :

Alloxan monohydrate was obtained from S.D. Fine, Mumbai and all the other chemicals used were of analytical grade and were acquired from commercial sources.

Male wister albino Rat (Fig : 3)

4.8.4. ALLOXANE INDUCED DIABETIC RATS :

- Diabetes was induced by injecting Alloxane monohydrate of 150 mg/kg body weight intraperitonially.
- After one hour of alloxan administration the animal was given feed adlibitium and 5% dextrose solution for a day (24 hours) to overcome hypoglycemic phase.
- Animals were kept under administration for 48 hours and blood glucose was measured.
- Normally ,Fasting glucose level should be more than 250mg/dl.

- GROUP-I= Normal control (saline)
- GROUP-II= Diabetic control (Alloxane 150mg/kg) i.p
- GROUP-III= Diabetic rat + Glibenclamide 600µg/kg p.o
- GROUP-IV= Diabetic rat + 200mg/kg.p.o of sample
- Extracts were given orally for 7 days at maximum dose 200 and 400 mg/kg p.o…
- Fasting blood glucose levels were estimated after 7 days at time intervals of 0,1,3,5 and 7 hrs of drug administration (Kanner DM et al., 2006).

RESULT AND DISCUSSION

5. RESULT AND DISCUSSION

Curd is regarded as a complete and natural food. It is a product of the milk which is white in colour and resulted due to bacterial action. Daily consumption of fresh and curd helps in preventing some diseases by virtal of the nutrients and probiotics bacteria.Curd is prepared either from cow or buffalo's milk of the family bovidae. The curd prepared from cow's microorganisms involved in the formation of curd may be *Streptococcus cremons , Streptococus latis , Streptococcus thermophilus , Lactobacillus bulgaricus , Lactobacillus acidophilus , Lactobacillus helveticus* and *Lactobacillus cremoris* (Madhu *etal*.,2013). Curd is formed by the process of lactic acid fermentation so a small amount of fermented curd is used as a starting material. Addition of 0.2% citrate is recommended for rendering pleasant aroma to dahi.Curd forms the richest source of probiotics knowns as so far. Probiotics contain living microorganisms such as a lactic acid bacteria which provide health benefits to the host. Curd promotes the health benefits to the host curd promotes the health of the host by boosting the immune system.We isolate *Lactobacillusspecies* in MRS agar medium. The colonies were identified as creamy white , transparent , mucoid and smooth round in shape.

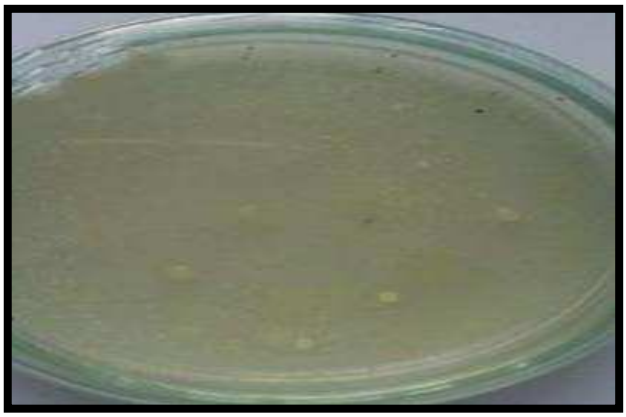


FIG :5.1 Growth of *lactobacillus* in MRS media

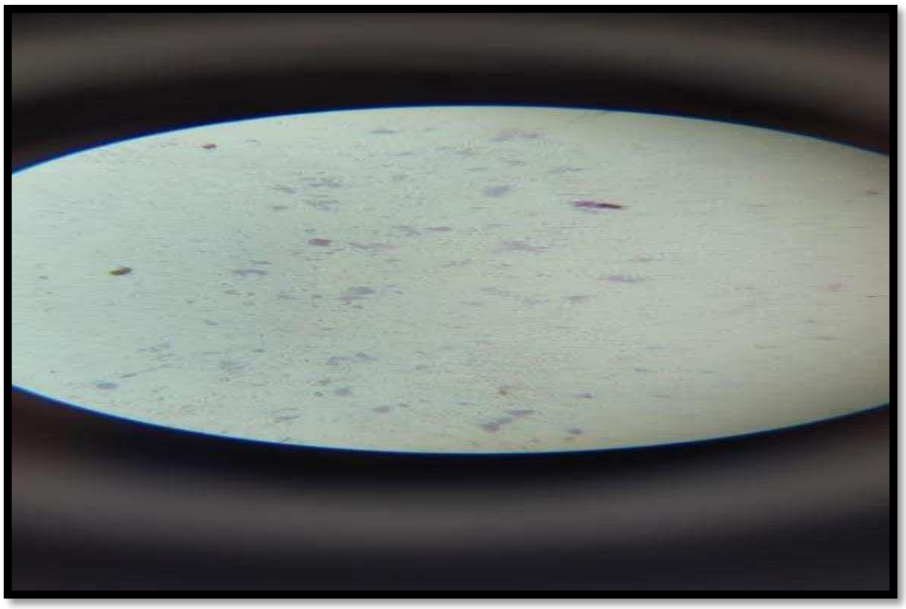


FIG : 5.2 Microscopical. Examination of *Lactobacillus.sp*

5.1 ISOLATION OF *LACTO BACILLUS*:

STAINING	LAB1	LAB2
Grams staining	Positive	Positive
Shape	Rod	Rod
Motility	Motile	Motile

5.1.1BIOCHEMICAL PROPERTIES OF *LACTOBACILLIUS*

TEST	RESULT
CATALASE TEST	NEGATIVE
METHYL RED	POSITIVE
STARCH HYDROLYSIS	POSITIVE
VOGES PRAUSKEUR	NEGATIVE
CITRATE	NEGATIVE

wound sample,urine sample and pea nut sample were use to isolate s.aureus , *E.coli* and *A.niger* respectively for that we have to determine the organisium morphology and biochemical characteristics .

Table 5.1.2 TO ISOLATE FROM THE WOUND AND URINE

STAINING	**LAB1**	**LAB2**
Gram's staining	Positive	Positive
Shape	Rod	Rod
MotilityL	Motile	Motile

BIOCHEMICAL PROPERTIES OF LACTOBACILLUS

TEST	RESULT
CATALASE TEST	NEGATIVE
METHYL RED	POSITIVE
STARCH HYDROLYSIS	POSITIVE
VOGES PRAUSKEUR	NEGATIVE
CITRATE	NEGATIVE

Wound sample , urine sample and peanut sample were used to isolates *S.aureus* , *E.coli* and *A.niger* respectively for that we have to determine organisms morphology & biochemical characteristics.

TABLE : 5.1 TO ISOLATES FROM WOUND AND URINE

STAINING	***Staphylococcusaureus***	***Escherichiacoli***
Gram's staining	Gram positive	Gram negative
Shape	Spherical	Rod
Motility	Non-motile	Motile

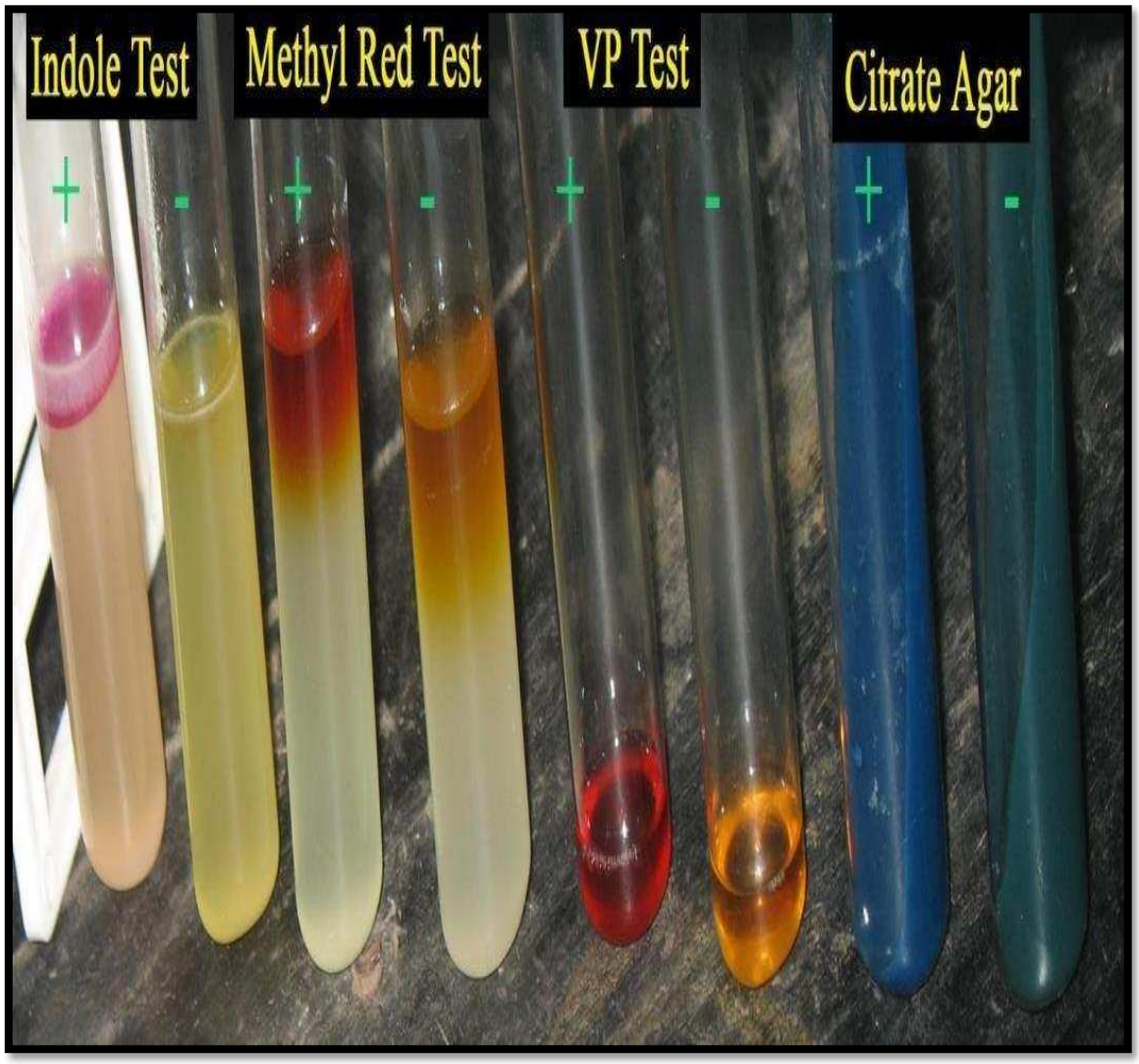


Fig: 5.3

TABLE : 5. 2 BIOCHEMICAL CHARACTERISTICS

S.NO	BIOCHEMICAL TESTS	IS1	IS2
1.	INDOLE	-	-
2.	METHYL RED	-	-
3.	VOGAS PROSKUER	+	+
4.	CITRATE TEST	+	+
5.	CATALASE TEST	+	+
6.	STARCH HYDROLYSIS	+	+
7.	UREASE TESTS	+	+

5.3 ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the curd either extract of against human pathogens was determined by measuring the diameter of zone of inhibition expressed in millimeter(mm) (Wilkinson,2007 and Joys Albert,2015). *Staphylococcus aureus* showed varied in the exploitation of antimicrobial activity of zone of inhibition from streptomycin(12mm) and curd(16mm). *Aspergillus niger* showed varied in the exploitation of antimicrobial activity of zone of inhibition from ketacanozole (14mm) and curd(10mm). The curd extract showed the maximum activity against the *staphylococcusaureus* and the minimum activity against the *Aspergillusniger*. When compare to the antimicrobial activity of *staphylococcus aureus* and *Aspergillus niger*, the extract shows

the significant activity against the test organisms than the streptomycin and ketoconazole. The observed in the antimicrobial effect of the staphylococcus aureus studied against gram positive bacteria in the present study may be due to difference in permeability barriers. From the result it was obvious extract of curd showed significant potency against the test organisms. The different in antifungal activity is due to the potential differences in the susceptibility of *Aspergillus niger.*

TABLE 5.3: THE EFFECT OF ANTIMICROBIAL ACTIVITY OF CURD AGAINST *STAPHYLOCOCCUS AUREUS*

TEST ORGANISIUM	SAMPLE	ZONE OF INHIBITION (dm)
Staphylococcus aureus	**Streptomycin**	**12**
	Curd	**16**

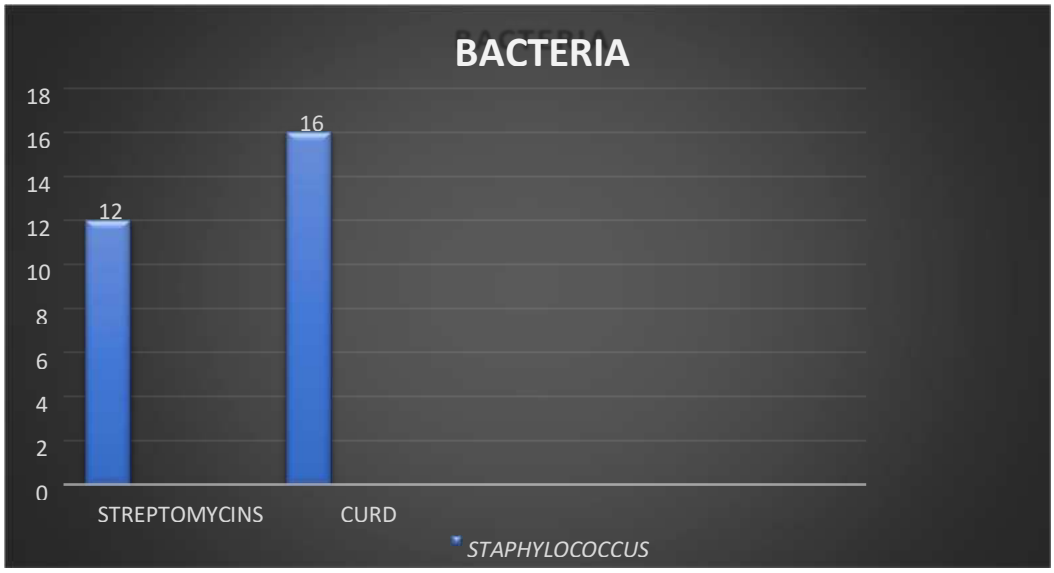
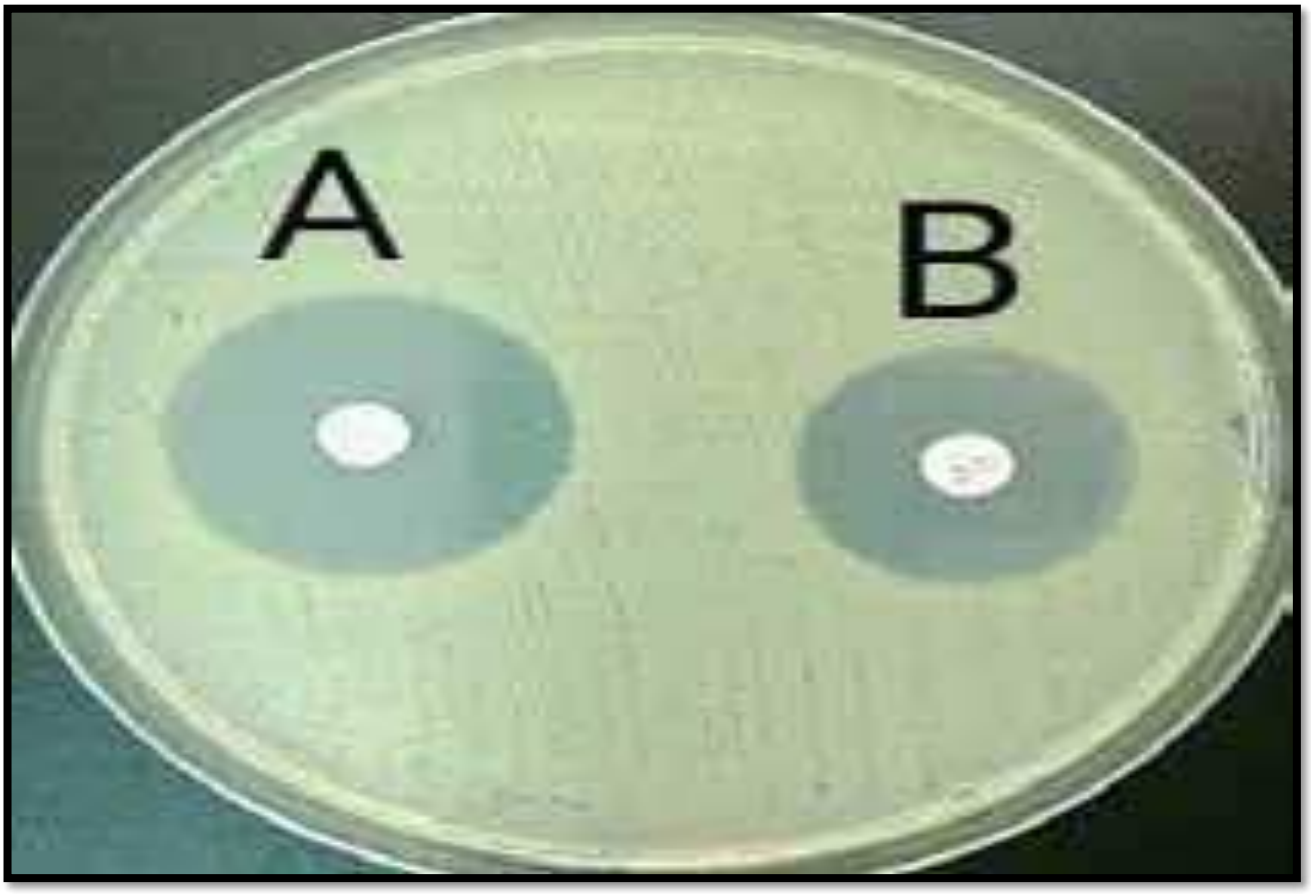


FIG : 5.4

Plate showing the inhibition towards *staphylococcus aureus*



A -Curd

B- antibiotic as streptomycin

FIG:5.5

TABLE 5.4: THE EFFECT OF ANTIMICROBIAL ACTIVITY OF CURD AGAINST *ASPERGILLUS NIGER*

TEST ORGANISMS	SAMPLE	ZONE OF INHIBITION (dm).
Aspergillus niger	**Water**	**-**
	Ketacanozole	**14**
	Curd	**10**

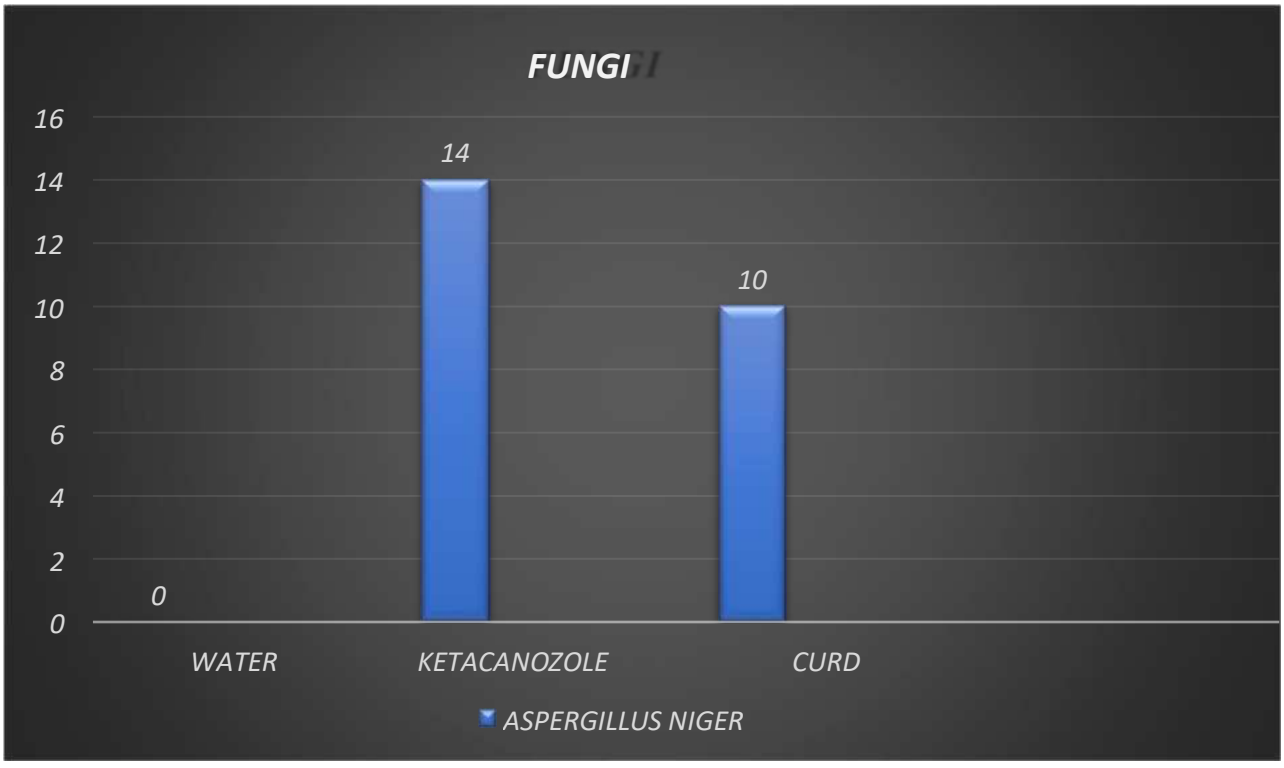
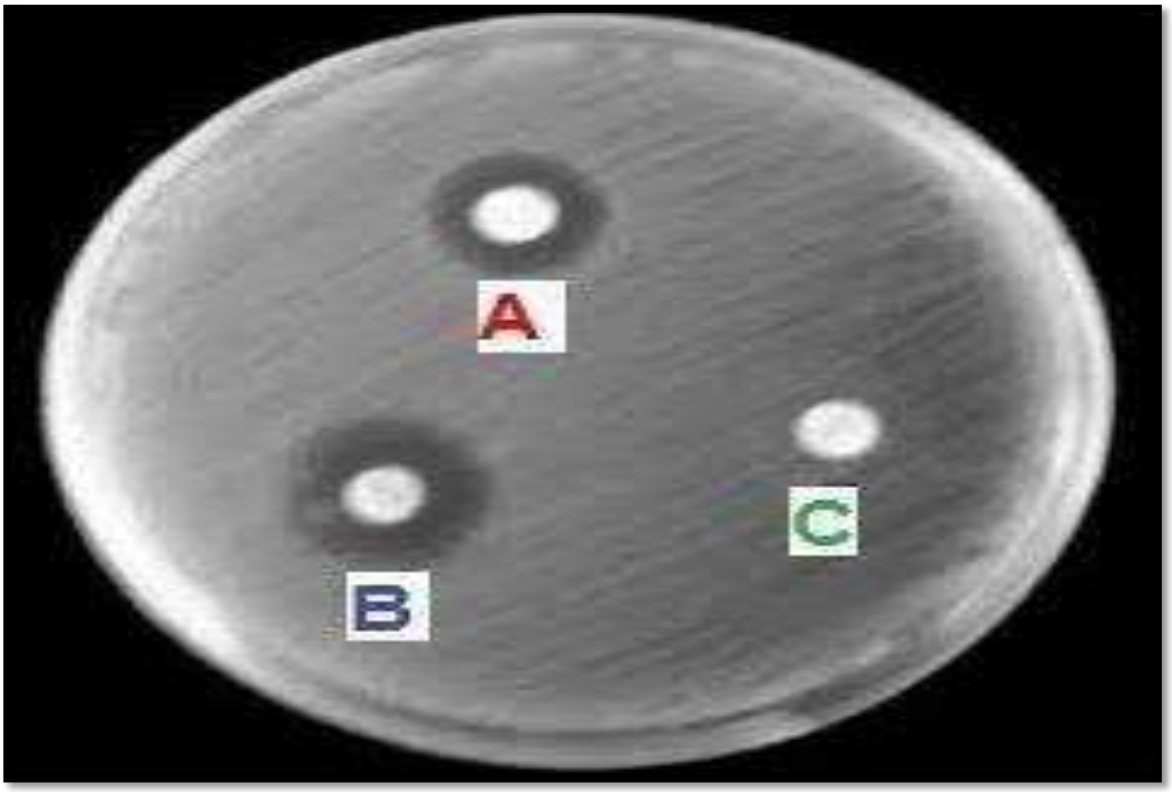


FIG : 5.6 .

Plate showing inhibition towards *Aspergillus niger*



A- Curd
B- Antibiotic as ketacanozole
C- Sample water

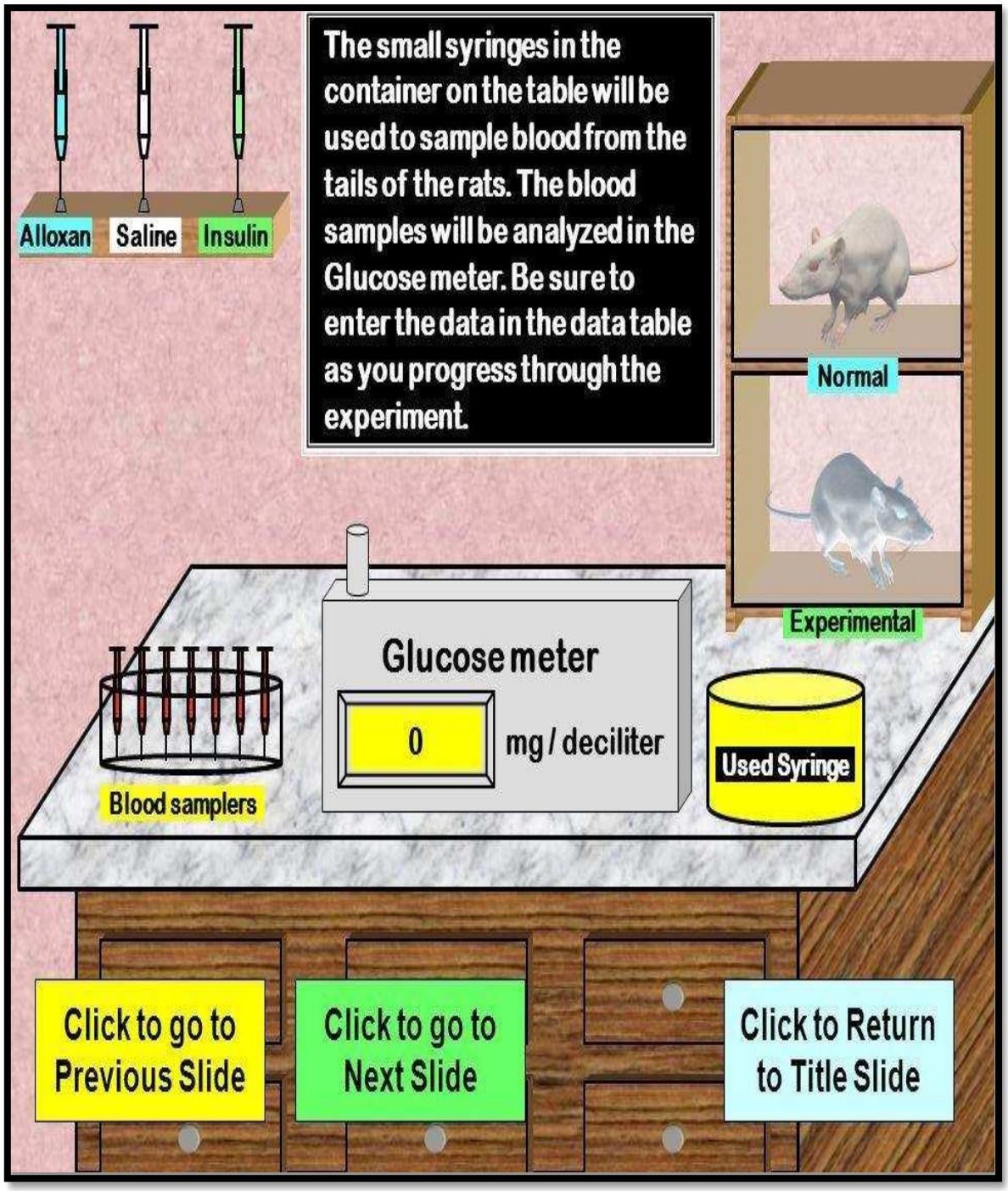
FIG: 5.7

5.4ANTIDIABETIC ACTIVITY

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and a body imbalance in the metabolism of carbohydrates, fats and proteins. Treatment of diabetes mellitus can be done by giving synthetic drugs, but the use of synthetic drugs can have side effects on users. So, the treatment of diabetes mellitus shifts to the use of herbal medicines from plants which are believed to be an α - glucosidase inhibitors.

Similar results obtained by Akpan and Radhika et al., who demonstrated that alloxan administration was associated with hyperglycemia.

The blood glucose level in normal rats is in the range of 85 to 132mg/dl (kohn D.F and Clifford C.B. 2002). Treatment with alloxan has increase the blood glucose level to a range of 250 to 270mg/dl after 5 days. Single dose administration of extracts at 400mg/Kg showed significantly reduced the blood glucose level on alloxan induced diabetic rat while control Glibenclamide 10mg/Kg significantly reduce the blood glucose level at 1st 2nd and 4th hour after single dose administration in alloxan induced diabetic rats were given in Table 5.4. Repeated dose administration with the rat. Reduced the glucose level in time dependent manner over the period of 3 weeks. However, animal treated with dose shows the significant decreases in blood glucose level on 21th days of treatment when compared to the standard found some incubation 7 to 21days were given in the table 5.5. The supplementation improved the glucose tolerance in the fasted normal rats.


Alloxan
Saline
Insulin
The small syringes in the container on the table will be used to sample blood from the tails of the rats. The blood samples will be analyzed in the Glucose meter. Be sure to enter the data in the data table as you progress through the experiment.
Normal
Experimental
Glucose meter
0
mg / deciliter
Used Syringe
Blood samplers
Click to go to Previous Slide
Click to go to Next Slide
Click to Return to Title Slide

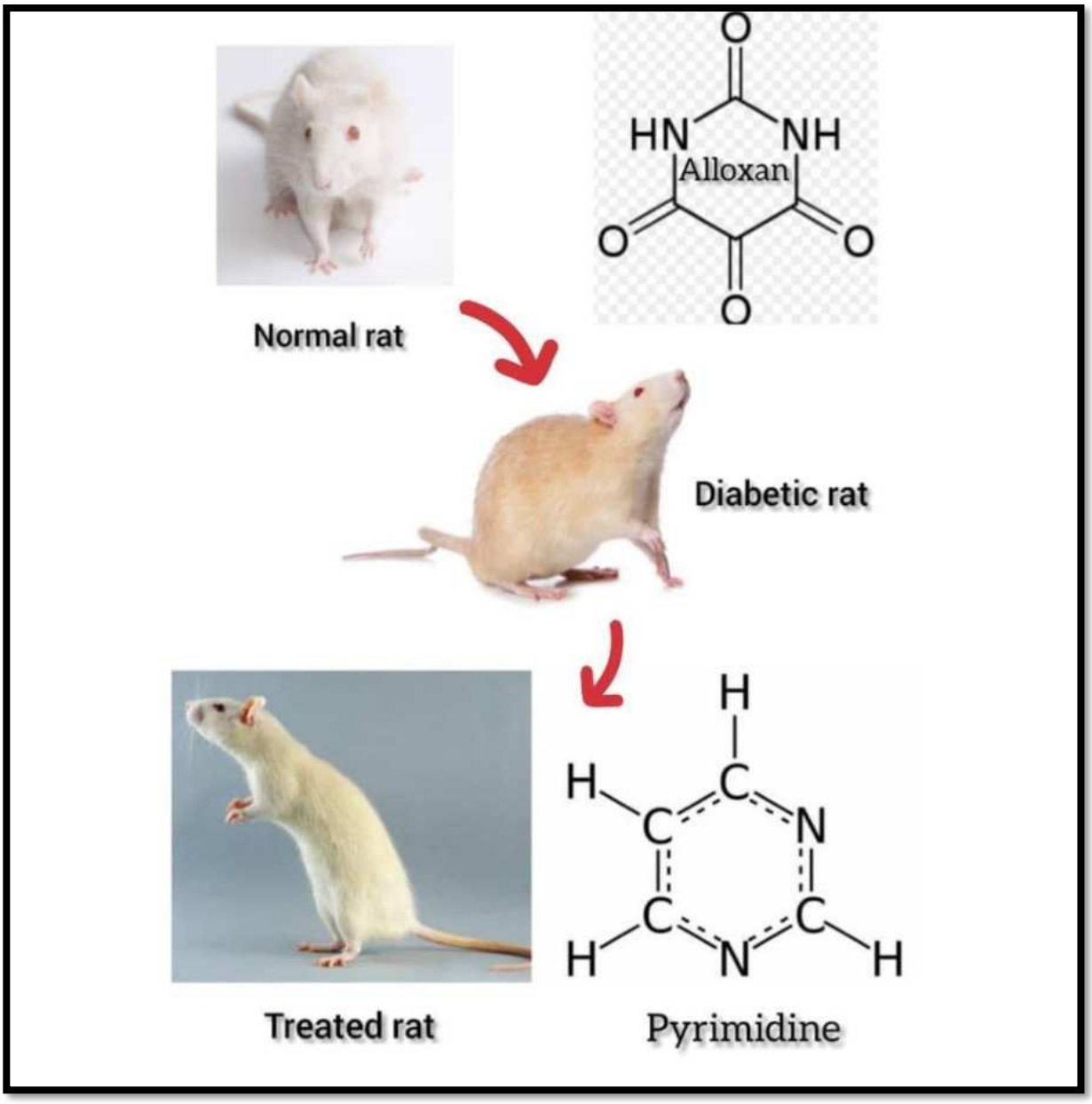


FIG:5.8

Table 5.5: Effect of single dose treatment of sample on blood glucose level on alloxan induced diabetic rats

GROUP	TREATMENT	DOSE	SERUM GLUCOSE (MG/DL)			
			Time after glucose administration in days			
			0^{th} day	7^{th} day	14^{th} day	21^{st} day
I	Normal control	10ml/kg	95± 0.57	95± 1.15	95± 1.15	96± 0.57
II	Control (3% v/v Tween 80)	10ml/kg	260± 0.57	264± 0.57	265± 0.57	255± 0.57
III	Standard Glibenclamide	10ml/kg	264± 0.57	102± 0.57	95± 0.57	85± 0.57
IV	Sample	200mg/Kg	261± 0.57	215± 0.57	185± 0.57	124± 0.57

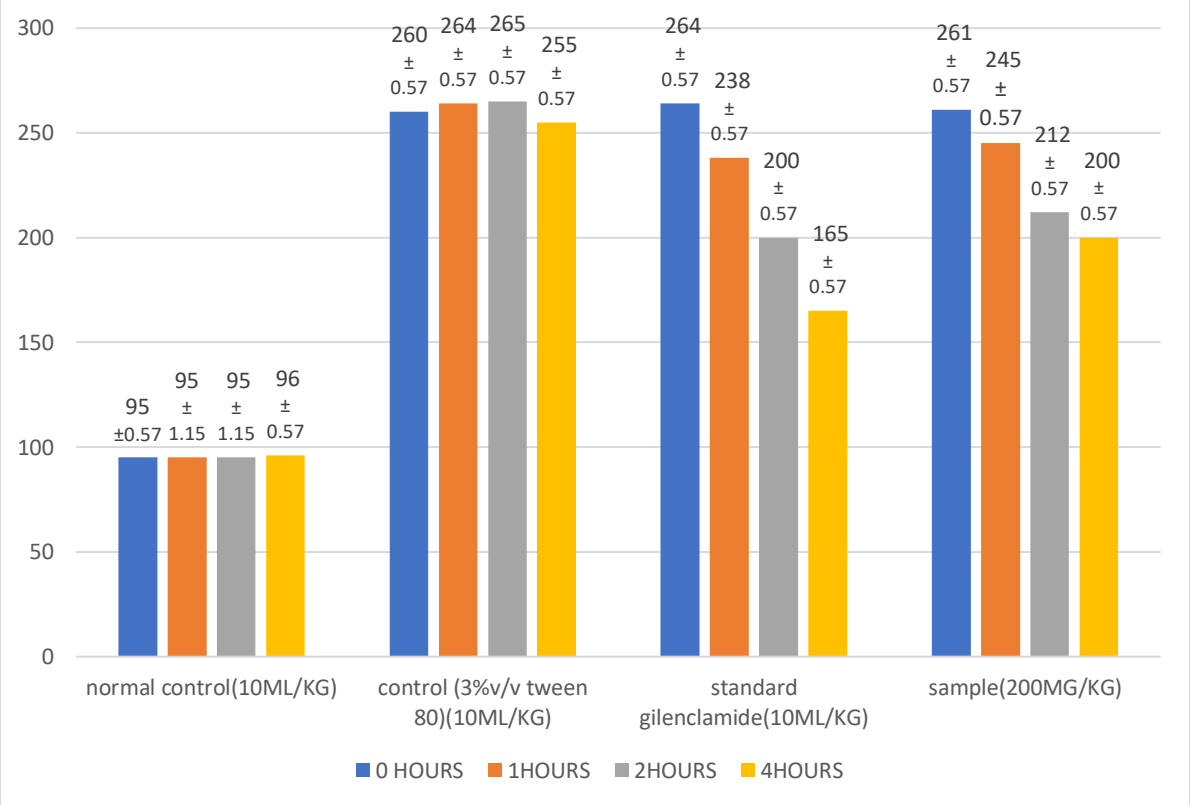


FIG 5.9:Effect of single dose treatment of sample on blood glucose level on alloxan induced diabetic rats

Table 5.6: Effect of repeated dose treatment of sample on blood glucose level on alloxan induced

GROUP	TREATMENT	DOSE	SERUM GLUCOSE (MG/DL)			
			TIME AFTER GLUCOSE ADMINISTRATION IN MINUTES			
			0 hour	1 hour	2 hour	4 hour
I	Normal control	10ml/kg	95± 0.57	95± 1.15	95± 1.15	96± 0.57
II	Control (3% v/v Tween 80)	10ml/kg	260± 0.57	264± 0.57	265± 0.57	255± 0.57
III	Standard Glibenclamide	10ml/kg	264± 0.57	238± 0.57	200± 0.57	165± 0.57
IV	Sample	200mg/Kg	261± 0.57	245± 0.57	212± 0.57	200± 0.57

diabetic rats

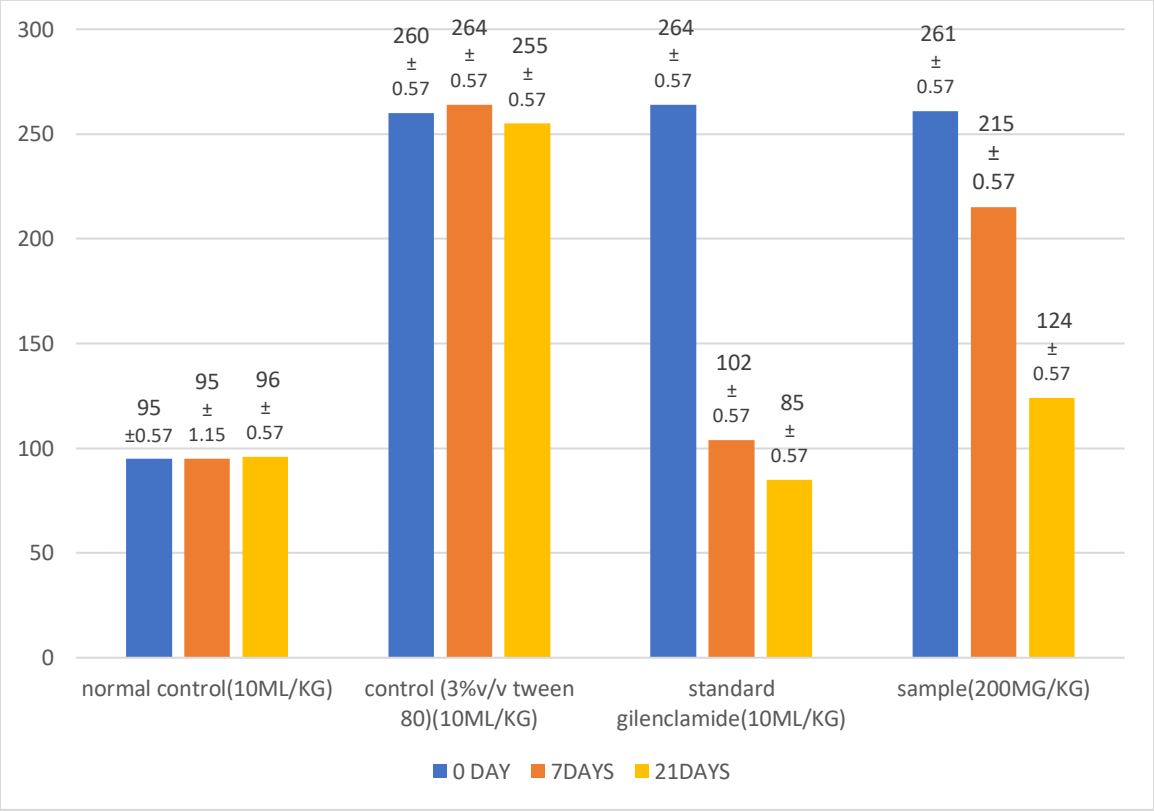


FIG:5.10 Repeated dose treatment of sample on blood glucose level on alloxan induced diabetic rats

Table 5.7 : Percentage inhibition of the glucose in alloxan-induced diabetic rats when sample orally administered in different days of study.

DAYS	STANDARD 10MG/KG	SAMPLE 200MG/KG
0	264± 0.57	261± 0.57
7	102± 0.57	215± 0.57
14	95± 0.57	185± 0.57
21	85± 0.57	124± 0.57

5.5 PROBIOTIC :

The probiotics are generally consumed as a part of the fermented foods with specially added active live cultures; such as in yogurt and soy yogurt, or as dietary supplements. Metchnikoff (1907) reported that there is a potentiality for the modification of the gut micro flora and the replacement of harmful microbes by beneficial bacteria i.e. probiotics Due to their potential of providing health benefits, Probiotics become an integral part of the complex world as food, nutritional supplements, biologics and pharmaceuticals. These acts as food supplements as well as preventive or curative drug which contains live nonpathogenic bacteria. It is mainly the bacteria and metabolites that are produced by them which impart these probiotics their health promoting properties.Probiotics are present as natural temporary constituent of the intestinal microflora with an insufficient concentration, but when administered in adequate amounts, it confer a health benefit on the host and enable to prevent or cure some diseases to certain extent .There has been a growing awareness among Indian consumers in recent years about the importance of nutrition, health, and quality of food they eat. Consumers are preferred to healthy diet instead of physical activities. They are switching towards health supplements which could have deleterious effect. An increase in sale of health products amongst the health conscious consumers globally showed the interest of consumers towards these products, creating new health food categories. At present, the probiotics is at nascent stage and awareness as food supplement is limited to urban areas. Regular use of probiotics could improve the quality of life and reduces the dependence on drugs and medical expenses. Probiotics presumably exert a dual effect by preventing/decreasing the intestinal colonization with pathogen microorganisms or interacting with the gut associated lymphoid tissue (GALT) to prevent inflammatory responses and promote a state of tolerance to themselves and possibly to foods.Benefits of probiotics include the strengthening of the immune system, the improvement of the skin's function, the protection of DNA, the protection of proteins and lipids from oxidative damage and the maintaining of individual intestinal microbiota in subjects receiving antibiotic treatment.The most promising probiotic strains include the members of the Genera- *Lactobacillus, Bifidobacterium and Enterococcus* . Probiotic cultures are available in fermented dairy products

and probiotic fortified foods and also in tablets, capsules, powders and sachets containing the bacteria in freeze dried form are also available.Different probiotic strains have shown their efficacy in various infections like urogenital infections, gut infections and oral infections. Experimental studies have showed probiotics' ability for gastric ulcer healing.

SUMMARY

6. SUMMARY

Curd is a natural and leading food which prevents from numerous enteric diseases. It contains *Lactic acid bacteria* and a perfect balance of proteins, carbohydrates, fats, vitamins, minerals and water. Curd boosts the immune system and enhances stamina .*Lactobacilli* are used as a probiotic, but scientific investigation of such strains is scanty. The current aims to isolates and identify *lactobacillus* strains from curd sample for probiotic characteristic and antibiotic resistance determination. Curd samples were screened for the presence of *Lactobacilli*. Curd samples were screened for the presence of *Lactobacillus*, and the isolates were identified by cultural methods, gram's staining and biochemical test, and antibiotic susceptibility tests.

Diabetes hyperglycaemia mellitus is a combination of heterogeneous disorders commonly presenting with episodes of and glucose intolerance, as a result of lack of insulin , defective insulin action, or both . Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both. The blood glucose level in normal rats is in the range of 85 to 132mg/dl. Classification of diabetes mellitus is based on its aetiology and clinical presentation. As such, there are four types or classes of diabetes mellitus viz; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types.

Diabetes mellitus has been known since antiquity, its treatments were known since the middle ages, and the elucidation of its pathogenesis occurred mainly in the 20th century. Non-progressing Type II diabetics almost went undiagnosed.That diabetics lacked a single chemical which was normally produced by the pancreas. Name of this chemical was later proposed to be insulin.This was a step forward in elucidation of the endocrine role of pancreas in metabolism and existence of insulin. These scientists proceeded on to isolate insulin from bovine pancreases, thereby leading to the availability of an effective treatment of diabetes mellitus, with the first clinical patient being treated.

Insulin is a polypeptide hormone synthesized in humans and other mammals within the beta cells of the islets of langerhans in the pancreas. The islets of langerhans form the endocrine part of pancreas, accounting for 2% of the total mass of the pancreas,with beta cells constituting 60-80% of all the cells of islets of langerhans. Insulin exhibits a multitude of effects in many

tissues,with liver, muscle, and adipose tissue being the most important target organs for insulin action. The basic physiological function of insulin is promoting the synthesis of carbohydrates, proteins, lipids, and nucleic acids. The effects of insulin on carbohydrate metabolism include stimulation of glucose transport across muscle and adipocyte cell membranes, regulation of hepatic glycogen synthesis, and inhibition of glycogenolysis and gluconeogenesis.

We investigated the effect of low-fat (2.5%) dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei*on progression of high fructose-induced type 2 diabetes in rats. Diabetes was induced in male albino wister rats by feeding alloxan in water. The body weight, food and water intakes, fasting blood glucose, glycosylated hemoglobin, oral glucose tolerance test.

Alloxan is a oxygenated pyrimidine derivative. Alloxan is an toxic glucose analogue ; which destroys insulin producing cells in pancrease when administered to rodents and many other animal speices. This causes an insulin dependent diabetes mellitus called “Alloxan diabetes”. Treatment with alloxan has increase the blood, glucose level to a range of 250 to 270mg/dl after 4 days. Single dose did not show the significant of blood glucose level on Alloxan induced diabetic rat. White control Gliclamide 10mg/ng significantly reduce the blood glucose level at 1^{st}, 2^{nd} and 4^{th} hour after single dose. Repeated dose administration with the rat, reduced the glucose level in time dependent manner over the period of 3 weeks. However animal treated with dose shows the significant decreases in blood glucose level on 21th days of treatment, when compared to the standard found some incubation 7 to 21 days. The supplementation improved the glucose tolerance in the tested normal rat At present, people in the modern world are getting health conscious and have therefore opted for changes in terms of diet and their lifestyle. The dietary changes include the supplementation of probiotics and probiotic based products. The importance of probiotics has been reported by several researchers and in reports that it promotes proper digestion, improves immune system, treats diarrhea which mainly originated due to antibiotic course or travelling and prevents ulceration caused due to helicobacter pylori ,and assists in nutrient assimilation particularly B vitamins and omega-3 fatty acids. These health benefits initiated the research to support the concept that there are clinical health benefits to ingest these micro-organisms . It is a majorfocus of attention of scientists across the world due to

their promising health benefits and their applications offers an innovative approach for development of novel probiotic formulations.The article reviews the reports available on probiotics, describes the functioning of probiotics in human ecosystems. The potential of probiotics in solving number of diseases such as gastrointestinal problems, lactose intolerance, treatment of acute diarrhea, cancer, diabetes, prevention and treatment of allergy related problems.

CONCLUSION

7. CONCLUSION

Dairy products, particularly yoghurt, continue to be the most important vehicles for delivery of probiotic bacteria to the consumer with the nondairy sector continuously evolving as well, as a result of food technology advances and the growing demand. A virtuous circle is therefore created: as the range of new products with improved sensory appeal widens, consumer acceptance increases and the food industry invests more on this growing market by development of new processes and products. Nevertheless, the development of probiotics for human consumption is still in its infancy. Further research, in the form of controlled human studies, is needed to determine which probiotics and which dosages are associated with the greatest efficacy and for which patients, as well as to demonstrate their safety and limitations.

Dairy products, particularly yoghurt, continue to be the most important vehicles for delivery of probiotic bacteria to the consumer with the nondairy sector continuously evolving as well, as a result of food technology advances and the growing demand. A virtuous circle is therefore created: as the range of new products with improved sensory appeal widens, consumer acceptance increases and the food industry invests more on this growing market by development of new processes and products. Nevertheless, the development of probiotics for human consumption is still in its infancy. Further research, in the form of controlled human studies, is needed to determine which probiotics and which dosages are associated with the greatest efficacy and for which patients, as well as to demonstrate their safety and limitations.Curd has been associated with health benefits for decades. Now, there is clear scientific evidence emerging as to which microbes are responsible for individual effects. Lactobacilli are particularly characterized in this regard, being associated with protection from pathogenic bacteria, modulation of the immune system to potentially reduced risk of allergies and cancer, reduction of radical oxidative species and cholesterol levels, and potentially benefiting in diabetes.In conclusion, probiotics have a large variety of beneficial actions in health and disease, reducing extrinsic nuisances, inhibiting of inflammation and having antioxidant capacity via reducing and preventing the formation of reactive free radicals with improvement of antioxidant defense in diabetic patients.

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ANNEXURE

10. ANNEXURE

EMB AGAR :

Composition		Amount g/l
Agar	-	13500
Peptone	-	10000
Lactose	-	5000
Sucrose	-	5000
Dipotassium phosphate	-	2000
Eosin Y	-	0.400
Methylene blue	-	0.065

NUTRIENT AGAR :

Peptone	-	5gm
Beef extract	-	3gm
Agar	-	15gm
Distilled water	-	1000ml
PH	-	6.8-7.0

NUTRIENT BROTH :

Peptone	-	5gm
Beef extract	-	3gm
Distilled water	-	1000ml
PH	-	6.8-7.8

MUELLER – HINTON AGAR **:**

Beef infusion	-	300gm
Casamino acids	-	17.5gm
Starch	-	1.5gm
Agar	-	17gm
PH	-	7.4
Distilled water	-	1000ml

LACTOBACILLUS MRS AGAR :

Composition	**Amount g/l**
Agar	- 12.00
Peptone	- 10.00
Yeast extract	- 5.00
Dextrose	- 20.00
Meat extract	- 8.00
Triammonium citrate	- 2.00
Sodium acetate	- 5.00
Magnesium sulphate Heptahydrate	- 0.20
Manganese sulphate Tetrahydrate	- 0.05
Dipotassium phosphate	- 2.00
Sorbitanmonooleate (Tween 80)	- 1.00

KAMARAJAR GOVERNMENT ARTS COLLEGE
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INTERNATIONAL WEBINAR ON
Emerging Microbial infections

Time: 10.40 a.m. to 11.20 a.m

Prof. Dr. P.K.Rajesh

Deputy Vice Chancellor,
Academic and International Affairs,
AIMST University, Malaysia

Topic: Viruses and vaccines-a viral perspective!

Time : 11.30 a.m to 12.15 p.m

Dr S Gokul Shankar

Senior Associate Professor,
Microbiology Unit,
Deputy Dean-Students & Alumni,
Faculty of Medicine,
AIMST University,Malaysia

Topic: Fungal infections- the tip of an iceberg

Time: 12.30 p.m to 1 .00 p.m

Dr. SRIDEVI VISVANATHAN

Senior Lecturer,
Biochemistry Unit,
Faculty of Medicine
AIMST University,Malaysia

Title : Molecular diagnostic approach in Covid -19

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VENUE : KANIVELAN AUDITORIUM **DATE: 17.03.2021** **TIME : 10.30 a.m to 1.00 p.m**

ANTIDIABETIC EFFECT OF PROBIOTIC CURD CONTAINING *LACTOBACILLUS .SP* IN ALBINO RAT.

Joys Selva Mary Albert , J. Jebina, S. FathimaBarkana, R. Deepa, A.Eskkiammal, R. Esakkipriya

Department of Microbiology,

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ABSTRACT

Curd is a natural and healing food which prevents from numerous enteric diseases. It contains lactic acid bacteria and a perfect balance of proteins, carbohydrates, fats, vitamins, minerals and water. Curd boosts the immune system and enhances stamina. Lactobacilli are used as a probiotic but scientific investigation of such strains is scanty. The current study aims to isolate and identify Lactobacillus strains from curd samples for probiotic characteristic and Antibiotic resistance determination. Curd samples were screened for the presence of Lactobacilli. The result of the study suggest that curd could be suitable for diabetic patient.

Key words : Curd , lactobacillus, probiotics, Antidiabetic , Antibiotic resistance.

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This is to certify that S. Fathima Barkana, Department of Microbiology, ST. Mary's College (Autonomous), Thoothukudi, has won "FIRST PLACE" in presenting a paper on "ANTIDIABETIC EFFECT OF PROBIOTIC CURD CONTAINING Lactobacillus Sp., IN ALBINO RAT" in the International Webinar on "Emerging Microbial Infections" organised by Department of Microbiology, Kamarajar Government Arts College, Surandai - 627 859 held on 17th March 2021.

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ANTI BACTERIAL , ANTI-INFLAMMATORY , ANTIULCER AND ANTICANCER ACTIVITY OF SQUID INK

A DISSERTATION SUBMITTED TO

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI.

Affiliated by Manonmaniam Sundaranar University,

In partial fulfilment of the requirements for the award of the degree of

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APRIL - 2021.

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "**Antibacterial, Anti-inflammatory, Anti ulcer and Anticancer activity of Squid ink**" is a bonafide record of the original work completed by A.JEFFI GETHZIAL , A.MAHALAKSHMI, M.MALATHI, K.HENCY KESIYA, P.JOTHI PRIYA PAIKARAY as a group project during the academic year 2020-2021 in ST. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirements for the award of the degree of BACHELOR OF SCIENCE IN MICROBIOLOGY prescribed by the University of Manonmaniam Sundaranar.

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LIST OF ABBREVIATIONS

µg - microgram

µl - microliter

DMSO - Dimethyl Sulphoxide

EPS - Extracellular polysaccharides

g - Gram

hrs - hours

IPS - Intracellular polysaccharides

Mg - Milligram

Min - minute

ml - Mililiter

mM - Millimolar

MR-Vp - Methyl red - Voges Proskauer

MSA - Mannitol salt agar

N - Normality

nm - Nanometer

Wt - Weight

WHO -World Health Organisation

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"AN EVALUATION OF ANTIMICROBIAL, ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY OF *Acacia arabica*"

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DECLARATION

We hereby declare that the dissertation work entitled **"AN EVALUATION OF ANTIMICROBIAL, ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITY OF *Acacia arabica*"** is a bonafide record of the original work completed by us during the academic year 2020-2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfillment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by the University of Manonmaniam Sundaranar. We also affirm that this is an original work done by us under the supervision of **Dr. C. Siluvai Kirubagari Aneeshia, M.Sc., Ph.D.**, Assistant Professor, Department of Microbiology, St. Mary's College (Autonomous), Thoothukudi.

Signature of the Students **Signature of the Guide**

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CONTENTS

LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	EXPANSION
1	**NDF**	Neutral Detergent fibre
2	**ADF**	Acid Detergent Fibre
3	**MJ**	Mega Joule
4	**PAM**	Protospacer Adjacent Motif
5	**NMR**	Nuclear Magnetic Resonance
6	**TLC**	Thin Layer Chromatography
7	**UV**	Ultra Violet
8	**MM**	Milli meter
9	**µG**	Micro gram
10	**KG**	Kilo gram
11	**MG**	Milli gram
12	**DPPH**	2,2-Diphenyl-1-Picryl Hydrazyl
13	**MR-VP**	Methyl red – Voges Proskauer
14	**P.O.**	Per Os

15	**G**	Gram
16	**DMSO**	Dimethyl Sulfoxide
17	**SD**	Standard Deviation
18	**SEM**	Standard error of mean
19	**ML**	Milli litre
20	**NM**	Nano meter
21	**µL**	Micro litre

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1. INTRODUCTION:

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals and minerals are the basis for treating human diseases (Firenzuoli F and Gori L, 2007). Medicinal plants are presently in demand and their acceptance is increasing progressively. Undoubtedly, plants play an important role by providing essential services in ecosystems. Without plants, humans and other living organisms cannot live in a way living should be. Anyway, herbals especially medicinal herbs have constantly acted as an overall indicator of ecosystem health (Singh JS, 2002). Medicinal plants have undoubtedly been considered by human beings since ancient times. It can be said that before the history and since the early humans recognized and exploited the plants around them for use as fuel, clothing, shelter and food; they became aware of their properties more or less. Medicinal plants have been transformed into one of the oldest sciences in countries such as China, Greece, Egypt and India. In ancient Persia, plants were commonly used as a drug and disinfectant and aromatic agent (Hamilton AC, 2004). In fact, the use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings have sought a tool in their environment to recover from a disease, the use of plants was their only choice of treatment (Halberstein RA, 2005). More than a tenth of the plant species (over 50 000 species) are used in pharmaceutical and cosmetic products. However, the distribution of medicinal plants across the world is not uniform (Huang H, 2011; Rafieian-Kopaei, 2012), and medicinal herbs are mainly collected from the wildlife population. Indeed, the demand for wildlife sources has increased by 8%-15% per year in Europe, North America and Asia in recent decades (Verma S and Singh S, 2008). The term medicinal plant refers to a variety of plants that have medicinal properties. These plants are a rich source of compounds that can be used to develop drug synthesis (Rasool Hassan B A, 2012).

The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, skin, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms (Phillipson J D, 2001). Human is mainly dependent on raw plant materials in order to meet

medical needs to maintain health and cure diseases (Jack D B, 1997). Medicinal plants are used for treatment because they have certain properties, including synergistic actions. The constituents of the plant may interact with each other, and this interaction can be beneficial for both or adverse to either of them or eliminate the harmful effects of both. Plant-derived compounds can dramatically improve hard-to-treat illnesses, such as cancer. Plant components are also characterized by their ability to prevent the development of certain diseases. The toxicity and adverse effects of conventional and allopathic medicines have also been important factors in the sudden increase in population demands and increase in the number of herbal drug manufactures as well as a reduction in the use of chemical drugs (Rasool Hassan B A, 2012). Knowing the history of any science is effective in understanding and using that science. Hence, the historical significance of the past and present and future to medicinal herbs will continue to be addressed. In this perspective review, we have highlighted and discussed the history, current challenges, development and future outlook of using medicinal plants and their active compounds.

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. (Hassan A and Rahman S *et al*., 2009)

Morphological description:

Rajvaidhya *et al*., (2012), reported about Acacia as perennial shrub or tree, 2.5–10 (–20) m tall, variable in many aspects. Branches spread forming a dense flat or rounded crown with dark to black colored stems; branchlets are purple- brown in color, shortly or densely pubescent, with lenticels. Bark thin, rough, fissured, deep red-brown. Spines (thorns) thin, straight, light-grey in color, axillary pairs, usually in 3–12 pairs, 5–7.5cm long in young trees, mature trees commonly without thorns. Leaves bipinnate 30–40 mm long, often with 1–2 petiolar glands and other glands between all or only the uppermost pinnate; pinnate 2–11 (–17) pairs, with 7–25 pairs of leaflets (1.5–7 mm long) per pinnae. Peduncles clustered at nodes of leafy and leafless branchlets. Flowers prolific, golden yellow, in globulus heads 1.2–1.5 cm in diameter. Pods are straight or slightly curved, 5–15cm long on a pedicel, 0.5–

1.2cm wide, with constrictions between the seeds giving the appearance of a string of pearls, fleshy when they are young, indehiscent, becoming black and hard at maturity. Seeds deep blackish-brown, smooth, sub-circular, compressed, areole 6–7mm long, 4.5–5mm wide. Seed weight ranges from 5,000–16,000 seed/kg. Subsp. *nilotica* is characterized by glabrous pods and twigs, or nearly so, while subsp. Kraussiana has strongly constricted white-grey hairy pods. Pods lightly, or not constricted in subsp. astringents.

Habitat and distribution:

Ashid Mohammad, Shamsi Shariq, Zaman Roohi and Itrat Malik (2014), reported the Babul tree is a strong light demander but is susceptible to frost. The greatest plus point is that it tolerates even insensitive droughts. It is a tree of miraculous adaptations. As its adaptation is very wide, this tree is distributed widely in India under different climatic conditions. It seldom extends above 500 m in altitude. It grows well in dry, hot arid climates with high mean maximum temperature regime (up to 500C) and very low minimum temperature (even below 0 0C) that is, even in deserts. The rainfall requirement ranges from 100 mm to 1000 mm. Babul is known for its endurance to very long hard summer seasons and in water logged areas. It can come up in saline or brackish water too. The long taproot is an adaptation to absorb water from depths and kinkily layers. It is called as a "Phreatophyte" meaning a plant, which is able to scavenge water from deeper soil layers. It tolerates salinity; pH is of range from 7.5 to 8.5. It can also come up in shallow soils and rocky areas. It is not a good coppice and root suckers are absent in the species. Babul is seen all over the Indian subcontinent to an altitude of up to 3000 meter. It is called Indian gum-Arabic tree. It is found very common in the forest fringes in Madhya Pradesh, Chhattisgarh, Jharkhand, and Uttar Pradesh, Rajasthan, Maharashtra and other dried parts of country.

Common name of *Acacia arabica*:

Unani Tibbi Name: Aqaqia, kikar, Mughilan. Arabic: Ummughilan. Persian: Kharemughilan, mughilan, madareghulan. Urdu: Babool, Kikar. Hindi: Kikar, Babool, Babula, Babura. English: Indian gum arabic, Black babool, Thorn acacia. Kannada: Jaali,Gobbli Tamil: Karuvelam. Telugu: Nallatuma. Thumma Sinhala: Babbulae. Latin: Acacia arabica. Assamese: Babal Acacia a. Gujrati: Baval, Kaloabaval. Kashmiri: Sak. Punjabi: Kikkar, Oriya: Babula, Babala. Marathi: Babhul, Babhula. Bengali: Babla. Malayalam: Veluthmadareghula.

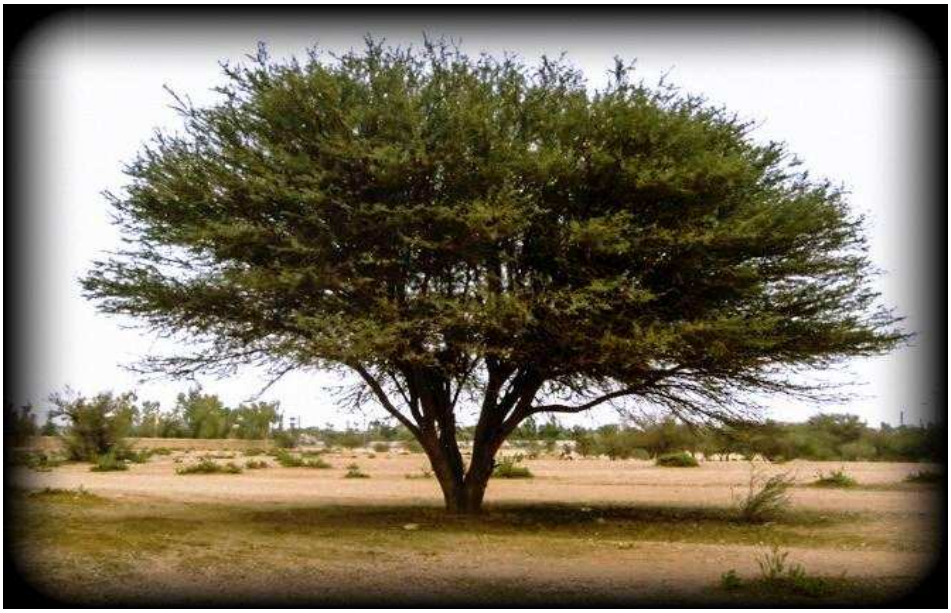

Plate 1.1 ***Acacia arabica***

Classification of *Acacia arabica*:

Kingdom: Plantae

Subkingdom: Tracheobionta

Super division: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabales

Family: Fabaceae

Genus : *Acacia*

Species: *arabica*

Synonyms: *Acacia nilotica* (Lam.), *Acacia scorpioides*, *Mimosa arabica*, *Mimosa nilotica*.

Nutritional value:

Raivaidhya*et al*., (2014), reports that the leaves contain 2.2–2.6% N, 16.9– 20.0% NDF, 13.3–14.1% ADF, 7.2–8.7 MJ/kg energy, 10–21% crude fibre and 6–9% condensed tannins. Pod and seed contain 1.6–2.2% N, 10 MJ/kg energy, 12– 18% crude fibre and 4–7% condensed tannins. Pods alone contain 2% N, 25% NDF, 17% ADF .In digestibility trials conducted in Zimbabwe, of several species browse species tested, intake of *A. nilotica*was the lowest. Nutritional value of the refined seed oils is done by rat bioassay and using peanut oil as control. The animals fed on 10 % seed oil diet showed poor growth performance and low feed efficiency ratio. The digestibility of the seed oil was 90 % as compared to 94 % for peanut oil. The seed oil in the diet of rats for 4 wk did not produce any abnormal serum lipids or histopathological findings. The seed oil was apparently non toxic. The de-oiled seed cake contains 21.9% protein and balanced amino acids but also contained anti-nutritional factors, tannins (4.2%) and saponins (2.4 %). The nutrient and amino acids composition of the detoxified seed meal (PAM) was almost similar to that of unprocessed seed meal except for anti-nutritional factors. PAM was nutritionally evaluated using rat bioassay produce in a comparative study with casein as standard. Nutritional indices, biochemical parameters and histopathology findings indicated the possibility of using PAM as supplementary feed for livestock animals.

Chemical constituents:

Phytochemical screening of the stem bark of *Acacia arabica* revealed that the plant contain amines and alkaloids (dimethyl tryptamine, 5-methoxy-dimethyltryptamine, and N-methyltryptamine), cyanogenic glycosides, cyclitols, saponins, fatty acids and seed oils, fluoroacetate, gums, non- protein amino acids, terpenes, hydrolyzable tannins, flavonoids and condensed tannins (Seigler, 2003). Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive anti-diabetic principles (Yasir*et al*., 2010).

The bark is also reported to contain (+) catechin, (-) epicatechin, (+) dicatechin, quercetin, gallic acid, (+) leucocyanidingallate, sucrose and (+) catechin-5-gallate (Sundaram and Mitra, 2007). Acacia gum contains chiefly arabin which is the mixture of calcium, magnesium and potassium salts of arabic acid. On hydrolysis arabic acid yields L-rhamnopyranose, galactopyranose, L- arabofuranose and taldobionic acid 6-d- glucuronosido-d-galactose. Further hydrolysis yields L-arabinose, D-galactose, d-glucuronic acid and

rhamnose. The gum also possesses enzymes like oxidases, peroxidases and pectinases (Rajvaidhya *et al*., 2014).

Previous studies on *Acacia arabica* (Bark) showed that the plant possess antibacterial activities against various organisms. Acetone, methanol and petroleum ether extracts showed antibacterial activity against *S. aureus, S. mutans, S. sanguis, S. salivarius, L. acidophilus and C. Albicans* (Ajaybhan *et al*., 2010).

Acacia Arabica seeds were reported to be active against *S. aureus, S. epidermidis, P. aeruginosa, K. pneumoniae, C. albicans and A. Niger* (Parmar*et al*., 2010). The fractions with antibacterial activity were subjected to the standard procedures of phytochemical screening for the identification of its various active constituents (Tannins, Anthraquinone, Saponin, Cardiac glycoside, Flavonoids, Reducing sugars, Catechol, Alkaloids, Terpenoids, Phenol, Carbohydrate test) (Trease and Evans, 1989 and Sofowora, 1993). A study shows that acetone and methanol extract of *A.arabica* bark was found to be most effective in exhibiting antibacterial activity. Literature survey has revealed few studies that are comparable with the present findings. Patel *et al*., (2009) reported maximum antibacterial activity of methanolic extract of *A.arabica* bark followed by chloroform and least with petroleum ether extract against *S. aureus, P. aeruginosa and E. coli.* Sharma *et al*., (2014) evaluated antibacterial activity of *A.arabica* bark extracts in different solvents against *S. aureus, P. aeruginosa and E.coli* and observed maximum activity with acetone followed by methanolic extracts. Other workers have also reported extracts made in methanol to be most potent against *S. aureus, P. aeruginosa, B. subtilis, E. coli* (Deen and Sadiq, 2002; Kavitha*et al*., 2013) and *S. typhi* (Mbatchou *et al*., 2011).

The methanolic extracts of *A.arabica* pods have been claimed against HIV-PR 9, 10. Currently, one group of researchers has tested the antiplasmodial activity of *A. nilotica* ethyl acetate extract against different chloroquine resistant and sensitive strains of Plasmodium falciparum. (Saurabh Rajvaidhya *et al*., 2012).

*Acacia arabica*bark has been used as demulcent, nutritive supplement, expectorant, styptic and tonic and have astringent, immunosuppressant, antibacterial, antitumour, antithrombotic, hypoglycemic and anti-helminthic activities (Rajvaidhya *et al*., 2012).

Gum:

Gum administered in the form of mucilage in diarrhoea, dysentery and diabetes mellitus (Nadkarni K M, 2005; Anonymous, 2003; Said H M, 1997; Kritikar K R and Basu B D, 2003).Fried in ghee, the gum is useful as a nutritive tonic and aphrodisiac in cases of sexual debility (Nadkarni KM, 2005 and Said H M, 1997). Powdered gum mixed with the white of an egg is applied on burns and scalds [Nadkarni K M, 2005)]. The gum is expectorant, antipyretic, cure lung troubles (Kritikar K R and Basu B D, 2003). The gum is said to be very useful in diabetes mellitus (Rushd I. KitabulKulliya, 19877; Chopra R N, Nayar S L and Chopra I C, 2002).

Bark:

The bark is a powerful astringent (Khare C P, 2007; Prasad G and Reshmi M V, 2007; Chopra R N, Nayar S L and Chopra I C, 2002; Waring E J, 2010) and bark is used in leucorrhoea, haemorrhages, wounds, ulcers and decoction in diarrhoea and vaginal secretions (Pullaiah T, 2006).The extract is an astringent and injected to allay irritation in acute gonorrhoea and leucorrhoea (Nadkarni K M, 2005). Decoction of bark is largely used as an astringent. Douche in gonorrhoea, cystitis, vaginitis, leucorrhoea, prolapse of the uterus and piles (Waring E J, 2010; Nadkarni K M, 2005; Dymock W, Warden C J H and Hooper D, 2005). It is used as demulcent (Prasad G and Reshmi M V, 2007; Chopra R N, Nayar S L and Chopra I C, 2010; Nadkarni KM, 2005) aphrodisiac (Gupta A K and Tandon N, 2004; Pullaiah T, 2006; Prasad G and Reshmi M V, 2007) and shows anti- viral properties, an extract of the bark completely inhibited the propagation of potato virus X. It is a powerful tonic (Lindley J, 2001). The ground bark mixed with seeds of *Sesamumindicum* Linn has been used for food (Anonymous, 2003). The decoction largely used as a gargle and mouth wash in cancerous and syphilitic affections (Nadkarni K M, 2005) sore-throat, toothache (Chatterjee A and Pakrashi S C, 2000; Prajapati N D, Purohit S S, Sharma A K and Kumar T, 2009; Anonymous, 2003; Narayan D P and Kumar U, 2005 and Anonymous, 2000) and dry powder applied externally in ulcers (Chatterjee A and Pakrashi S C, 2000; Prajapati N D, Purohit S S, Sharma A K and Kumar T, 2000; Narayan D P, Kumar U, 2005) Decoction of bark is a valuable application in prolapses ani. (Waring EJ,2010).Stem bark is used in diarrhoea, dysentery, diabetes, astringent, anthelmintic, in skin diseases, cough and bleeding piles; gonorrhoea and as an antiasthmatic, (Khare C P, 2007; Gupta A K and Tandon N,

2004), diuretic, leprosy, leucoderma, bronchitis, seminal weakness, utero-vesical disorders etc. (Pullaiah T, 2006; Prasad G, 2007). The infusion of bark is given in chronic diarrhoea and diabetes mellitus. The juice of bark mixed with milk is dropped into the eye for conjunctivitis. The burnt bark and burnt almond shell both pulverized and mixed with salt to make a good tooth-powder (Nadkarni K M , 2005).The powdered bark of the plant with little salt is used for treating acute diarrhoea. (Banso A, 2009).

Pods:

Seeds are eaten roasted or raw in times of acute Scarcity (Anonymous, 2003). Pods when green is used as fodder (Anonymous, 2000). Pods decoction is effective in urogenital diseases (Khare C P, 2007; Chatterjee A and Pakrashi S C, 2000; Prajapati N D *et al.,* 2009; Gupta A K and Tandon N, 2004; Anonymous, 2003; Narayan D P and Kumar U, 2005; Anonymous, 2000). Pods are Expectorant (Syed H M, 1997), used for impotency and dry cough (Gupta A K and Tandon N, 2004). Seeds are hypoglycaemic in normal rats, no such effect in diabetic rats. Seed oil is antifungal. Pods are used as an astringent in diarrhoea. (Dymock W *et al.,* 2005).

Antimicrobial activity:

An antimicrobial is an agent that kills microorganisms or stops their growth Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria, and antifungal are used against fungi. They can also be classified according to their function. Agents that kill microbes are microbicides, while those that merely inhibit their growth are called bacteriostatic agents. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis.

The main classes of antimicrobial agents are disinfectants (non-selective agents, such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body). The term "antibiotic" originally described only those formulations derived from living microorganisms

but is now also applied to synthetic agents, such as sulfonamides or fluoroquinolones. Though the term used to be restricted to antibacterial (and is often used as a synonym for them by medical professionals and in medical literature), its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth. In response, further advancements in antimicrobial technologies have resulted in solutions that can go beyond simply inhibiting microbial growth. Instead, certain types of porous media have been developed to kill microbes on contact.

Antimicrobial use has been common practice for at least 2000 years. Ancient Egyptians and ancient Greek used specific molds and plant extracts to treat infection. (Wainwright M, 1989).In the 19th century, microbiologists such as Louis Pasteur and Jules Francois Joubert observed antagonism between some bacteria and discussed the merits of controlling these interactions in medicine.(Kingston W, June, 2008). Louis Pasteur's work in fermentation and spontaneous generation led to the distinction between anaerobic and aerobic bacteria. The information garnered by Pasteur led Joseph Lister to incorporate antiseptic methods, such as sterilizing surgical tools and debriding wounds into surgical procedures. The implementation of these antiseptic techniques drastically reduced the number of infections and subsequent deaths associated with surgical procedures. Louis Pasteur's work in microbiology also led to the development of many vaccines for life-threatening diseases such as anthrax and rabies. (Ullmann A, 23 Dec, 2019). On September 3, 1928, Alexander Fleming returned from a vacation and discovered that a Petri dish filled with *Staphylococcus* was separated into colonies due to the antimicrobial fungus *Penicilliumrubens*. Fleming and his associates struggled to isolate the antimicrobial but referenced its therapeutic potential in 1929 in the British Journal of Experimental Pathology (Fleming A, 1929). In 1942, Howard Florey, Ernst chain and Edward Abraham utilized Fleming's work to purify and extract penicillin for medicinal uses earning them the 1945 Nobel Prize in Medicine.

Antioxidant Activity:

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative stress, plants and animals maintain complex

systems of overlapping antioxidants, such as glutathione and enzymes (eg., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C & E.

The term "antioxidant" is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. The former, industrial antioxidants have diverse uses: acting as preservatives in food and cosmetics, and being oxidation-inhibitors in rubber, synthetic plastics, and fuels. (Dabelstein W, Reglitzky A, Schütze A and Reders, 2007). Antioxidant dietary supplements have not been shown to improve health in humans, or to be effective at preventing disease.

Supplements of beta-carotene, vitamin A, and vitamin E have no positive effect on mortality rate (Bjelakovic G, Nikolova D and Gluud C, 2013; Abner E L *et al.,* 2011) or cancer risk.(Cortes-Jofre*et al.,* 2012 ; Jiang L *et al*., 2010). Additionally, supplementation with selenium or vitamin E does not reduce the risk of cardiovascular disease. (Rees K *et al.,* 2013; Shekelle P G *et al.,* 2004)

Inflammation:

Inflammation (from Latin: *inflammatio*) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants, (Ferrero-Miliani L, Nielsen O H, Andersen P S, Girardin S E; Nielsen, Andersen and Girardin, February, 2007) and is a protective response involving immune cells, blood vessels, and molecular mediators.

The five cardinal signs are heat, pain, redness, swelling, and loss of function (Latin *calor*, *dolor*, *rubor*, *tumor*, and *functiolaesa*). (Ferrero-Miliani L *et al.*,February, 2007) Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. (Abbas A B and Lichtman A, 2009).

Anti-inflammatory Activity:

Anti-inflammatory (or anti-inflammatory) is the property of a substance or treatment that reduces inflammation or swelling. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central

nervous system to block pain signalling to the brain. Plants have the ability to synthesize a wide verity of photochemical compounds as secondary metabolites. Many of the photochemical have been used to effectively treat the various ailments for mankind. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties (Mahesh B, Sathish S, 2008). Plants have a great potential for producing new drugs and used in traditional medicine to treat chronic and even infectious diseases. (Panda S K, Thatoi H N and Dutta S K, 2009). In the present review an attempt has been made to investigate the anti-inflammatory activity of *Acacia arabica.*

2. AIM & OBJECTIVES:

- To evaluate the Antimicrobial activity of Ethanol and Acetone extracts of seeds and bark of *Acacia arabica* against *Escherichia coli* and *Staphylococcus aureus*.
- To study the Anti-inflammatory activity of Ethanol and Acetone extracts of seeds and bark of *Acacia arabica*.
- To determine the Anti-oxidant activity of Ethanol and Acetone extracts of seeds and bark of *Acacia arabica*.

3. REVIEW OF LITERATURE:

Ayoub S M, (1982*). Acacia nilotica* have demonstrated the highest Molluscicidal Properties due to tannin activity (18-23%).

Hussein A *et al*., (1985) exhibited highest activity using acetone, alcohol and aqueous extracts of the fruits and stem bark of these species are reported against the two snail species which host schistosomes in the Sudan i.e. *B. truncatus* and *B. pfeifferi*.

Nath R *et al.,* (1992) studied aqueous or 90 % ethanol extracts of the plants of interest in rats orally dosed for 10 days after insemination with special reference to see the effect on foetal development. Leaf extracts of Moringaoleifern and Adhatodavasica were 100% abortive at doses equivalent to 175 mg/kg of starting dry material. Only the flowers of *Acacia arabica* and *Hibiscus rosa-sinensis* appeared to lack teratologicpotential at the doses tested.

Gilani A H *et al*., (1995), A methanol extract of *Acacia nilotica* pods (AN) caused a dose- dependent (3–30 mg/kg) fall in arterial blood pressure. Treatment of animals with atropine abolished the vasodilator response of acetylcholine (ACh), whereas the antihypertensive effect of the plant extract remained unaltered.Phentolamine (a α-adrenergic blocker) abolished the vasoconstrictor effect of norepinephrine (NE), whereas pretreatment of the animal with AN, did not modify the NE response. These results indicate that the antihypertensive effect of plant extract is independent of muscarinic receptor stimulation or adrenoceptor blockade

Rajvaidhya S *et al*., (2005), Inhibition of total proteolytic (caseinolytic), tryptic (by hydrolysis of benzoyl arginine p-nitroanilide) and chymotryptic (by hydrolysis of acetyltyrosine ethyl ester) activities by ten species of legume seeds on human and bovine pancreatic proteases were studied. Acacia seeds extract displayed morepronounced action on human trypsin and chymotrypsin, it was more effective in inhibiting the total proteolytic activity of the bovine system

Meena P *et al*., (2006) reported the chemopreventive activity of *Acacia nilotica* (Linn.) gum, flower and leaf aqueous extracts, on 7, 12–dimethylbenz (a)anthracene (DMBA) induced skin papillomagenesis in male Swiss albino mice. A significant reduction in the values of tumor burden, tumor incidence and cumulative number of papillomas was observed in mice treated by oral gavage with the *Acacia nilotica* gum, flower and leaf extracts as

compared with the control group.

Masahide Yamato *et al.,* (2006) studied about the Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia.

Meena PD *et al.,*(2006) studied about the anticancer and antimutagenic properties of *Acacia nilotica* on 7,12-dimethylbenz(a) anthracene-induced skin papillomagenesis in Swiss albino mice.

Mahesh G *et al* ., (2008) studied methanol leaf and bark extracts of *Acacia nilotica* showed significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens*.

Mohan S *et al*., (2008) examined comparative antimicrobial studies of Acacia species and *A. nilotica* exhibited highest activity against three bacterial strains *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi*.

Mohan S *et al*., (2008) has investigated comparative antimicrobial studies of Acacia species and *A. nilotica* exhibited highest activity against two fungal strains *Candida albicans* and *Aspergillusniger*.

Banso A *et al*., (2009) has studied the antimicrobial activity of ethanolic extracts of the stem bark against *Streptococcus viridans, Staphylococcus aureus,Escherichia coli, Bacillus subtilis* using the agar diffusion method and found the minimum inhibitory concentration of the stem bark extract of the plant ranged between 35 and 50 mg/ml while the minimum bactericidal concentration ranged between 35 and 60 mg/ml.

Hassan R *et al*., (2009) has tested antimicrobial activity of ethanolic extract of *Acacia arabica* in vitro against seven bacterial species and two fungal species by well-diffusion method and micro dilution methods. The result of this study revealed that ethanolic extracts of these plants were effective on bacterial strains.

Parmar B *et al.,* (2010) examined *Acacia arabica* for preliminary phytochemical analysis and characterization by various instrumental techniques. Methanolic extracts of *Acacia arabica* seeds was very good antibacterial activity and also minimum inhibitory

concentrating of different virus using HEL cell cultures HeLa cell cultures Vero cell cultures but Minimum inhibitory concentration (MIC) of Herpes simplex - 1 and 2, vaccinia virus, vesicular stomatitis and Herpes simplecx-1 (TK ACVI) were observed very good antiviral activity of *Acacia arabica* seeds DMSO extracts.

Rajendran A *et al.,* (2010), Fresh flowers of *Acacia Arabica* willd were extracted with 80% alcohol and the concentrated extract was fractionated in the usual way. The ethyl acetate fraction was found to contain isoquercetin. The structure was characterized by UV, NMR, Paper Chromatographic and Chemical studies. The yellow pigment was found to contain promising results with respect to acute and chronic anti-inflammatory studies. It also showed considerable percentage protection of bacteriostatic effect on *Bacillus subtilis*, a gram positive organism.

Sharma AK *et al*., (2010) to screen the hot aqueous extract of *A. nilotica* revealed both proliferative and inhibitory effects on the rat splenocytes and IL-10 release depending on the dose.

Patil RN *et al.,* (2010), investigated the antidiabetic effects of hydroalcohiolic extracts of *Acacia arabica* in diabetic rats. The Alloxan monohydrate was used to induce the diabetes in normal rats. The tolbutamide 80 mg/kg p.o. was used the standard antidiabetic throughout the study and the results indicated that 250 and 500mg/kg body weight of all hydroalcoholic test extracts reversed the altered glucose, cholesterol, triglycerides, LDL and HDL levels in diabetic rats significantly and in dose dependent manner.

AU Wawata*et al*., (2010) studied about the effect of aqueous methanolic stem bark extracts of *Acacia polyacantha* on sexual behaviour, serum testosterone levels in male wistar rats.

M. Dikko*et al.,* (2010) reported the evaluation of the Effect of Aqueous- methanolic Stem Bark Extract of *Acacia polyacantha* on Blood Glucose Levels of Alloxan Induced Diabetic Wistar Rats.

Mithun BH Pai*et al.,*(2010) studied about the Antifungal efficacy of *Punicagranatum, Acacia nilotica, Cuminum cyminum* and *Foeniculum vulgare* on *Candida albicans*: an *in vitro* study.

Sanjay Guleria*et al.,*(2011) studied about the Antioxidant Activity and Protective Effect Against Plasmid DNA Strand Scission of Leaf, Bark, and Heartwood Extracts from *Acacia catechu*.

Deboshree Biswas *et al.,* (2011) reported a paper on in vitro assessment of antisecretory activity of root extracts of *Acacia arabica* Lam. Willd against biodiversity of *E. coli* isolated from Bhilai-Durg region

Shazia B *et al*., (2011) has investigated the antimicrobial activity against medicinally important bacterial strains, such as *Pseudomonas aeuroginosa*. The anti-microbial activity was determined in extracts using agar well diffusion method. Result showed anti-bacterial activityagainst *Staphylococcus aureus, Pseudomonas vulgaris, Escherichia coli* and anti-fungal activity against *Streptococcus cereviceae*.

Malviya S *et al*., (2011) examined the plant extract and result revealed that potent antibiotic activity against four bacterial species: gram positive; *Bacillus subtilis, Staphylococcus albus, Streptococcus faecali*s; gram negative, *Escherichia coli* and two fungal species:*Candida albicans* and *Aspergillus flavus* examine by using paper disc diffusion method.

Pieter B Venter *et al.,* (2012) reported an analysis of commercial proanthocyanidins. Part 3: The chemical compositions of wattle (*Acacia mearnsii*) bark extract.

Naveeda Akhtar Qureshi *et al.,* (2010) studied about the Protozoidal activities of *Eucalyptus cammeldulensis, Dalbergiasissoo* and *Acacia arabica* woods and their different parts on the entozoic flagellates of *Heterotermes indicola* and *Coptoter mesheimi*.

Rahiman P *et al*., (2012) to screen the antimicrobial activity of *Acacia nilotica* and was found to give the most potent antimicrobial extract Noticeably no antimicrobial activity was found in methonolic bark extract of *Acacia nilotica* against the tested bacteria.

Dhabhai K *et al*., (2012) examined the antioxidant activity of ethyl acetate soluble fraction of *A. arabica* bark by in vitro lipid peroxidation model was carried out by tertiary butyl hydroperoxide induced lipid peroxidation and the most active fraction were identified by TLC and in vivo experiment in most active fraction were carried out with 50, 100 and 150

mg/kg oral dose in carbon tetra chloride induced hepatotoxicity in rats and it is hypothesized that flash chromatographic fraction of ethyl acetate extract exhibited maximum activity with in vitro lipid peroxidation and 150 mg/kg dose of carbon tetra chloride shows marked liver protection in in vitro model.10 The extracts, produced by 80% methanol, from leaf, bark and seed of three medicinal plants namely neem (Azadirachta indica A. Juss), kiker (*Acacia nilotica* L.) and jaman (Eugenia jambolana L.), were assessed fortheir antioxidant activity. The results showed that among the different parts of the investigated plants, neem leaf extract possessed highest activity to scavenge DPPH (71.54%) followed by kiker leaf and jaman leaf with contribution at 66.54% and 54.27%, respectively.44 Acacia species are rich source of polyphenolic compounds, known to have strong antioxidant properties that help in prevention and therapy of various oxidativestress related diseases including cardiovascular, neurodegenerative and cancer.

Bansal and Goel (2012), studied the different extracts [ethanolic, 50% hydroethanolic (50:50), 70%hydroethanolic (70:30) and aqueous] of young seedless pods were examined in pylorus ligation induced gastric ulcers in rats. Various parameters like, volume of gastricacid secretion, pH, free acidity, total acidity, ulcer index, mucin content and antioxidant studies were determined and were compared between extract treated, standard and vehicle control following ulcer induction. The mostactive extract was also evaluated in swimming stress induced and NSAID induced gastric ulceration. Results showed significant antiulcer activity in pyloric ligation induced ulceration. Even more the 70% hydroethanolic extract showed better protection as compared to 50% hydroethanolic extract. Further 70 % hydroethanolic extract also showed significant mucoprotection.

Sakthivel KM *et al*., (2012) studied the effect of *A. nilotica* extract against Dalton‘s ascitic lymphoma (DAL) induced solid and ascitic tumors in BALB/c mice. Experimental animals received *A. nilotica* extract (10 mg/kg.bw) intra peritoneally for 10 and 14 consecutive days before induction of solid and ascitic tumors, respectively. Treatment with *A. nilotica* extract significantly decreased the development of tumor.

Manoj Modi *et al*., (2013) reported that the extracts from *Acacia catechu* suppress HIV-1 replication by inhibiting the activities of the viral protease and Tat.

Mohammed Rahmatullah*et al*., (2013) studied about the Anti hyperglycemic and antinociceptive activity evaluation of 'Khoyer' prepared from boiling the wood of *Acacia catechu* in water

Khan *et al*., (2013), reported that traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases.

Amin B *et al*., (2013) has studied methanol, acetone and water extracts of different parts of *Acacia nilotica*, Calotropis procera, Adhatodavasica Nees, Fagonia arabica L. and Casuarinae quisetifolia L. to investigate the anti-bacterial activity against thirty four clinical isolates and two reference strains of H. pylori. Minimum inhibitory concentrations (MICs) of the extracts were determined using the agar dilution method and compared with some standard antibiotics like amoxicillin, clarithromycin, tetracycline and metronidazole, used in the triple therapy for H. pylori eradication. Methanol and acetone extracts from *Acacia nilotica* and Calotropisprocera exhibited stronger anti-H. pylori activity than metronidazole, almost comparable activity with tetracycline, but were found to be less potent than amoxicillin and clarithromycin.

Sunil K *et al*., (2013) investigated the petroleum ether, methanolic and water extracts for anti-diarrhoeal activity. Only methanolic extract showed significant anti-diarrhoeal activity against castor oil and magnesium sulphate induced diarrhoea and barium chloride induced peristalsis using swis albino rat.

Bhatnagar M *et al*., (2013) studied the potential of the polymeric component of aqueous extracts of gum Acacia (GA) and the seeds of *M. oleifera* (MSP) in wound management. The results revealed that both biopolymers were hemostatic and hasten blood coagulation. They showed shortening of activated partial thromboplastin time and prothrombin time and were non-cytotoxic in nature.

Hegazy GA *et al*., (2013) investigated the role of *Acacia arabica* extract as a hypoglycemic, anti hyperlipidemic, and antioxidant agent in streptozotocin-induced diabetic rats. The results found that *Acacia arabica* extract has good potential for hypoglycemic, hypolipidemic, and antioxidant properties, therefore, it can be further investigated for its efficacy in the treatment of diabetes in humans.

Farzana M *et al.,* (2014) stated that aqueous root extract of *A. nilotica* was analyzed for antiplasmodial activity in mice. Five groups, of five mice in each group were used. Group 1 or control, was administered with 10ml distilled water/kg body weight; groups 2, 3 and 4 were administered with 100, 200, and 400 mg extract/kg body weight, respectively, while group 5 was administered with 5 mg chloroquine/kg body weight. The results of this study showed that the aqueous root extract of *Acacia nilotica* is safe and has anti plasmodial activity.

Bukhtiar H *et al.,* (2014), investigated that the extract of *Acacia nilotica* (*A. nilotica*) have capacity to blocked platelet aggregation mediated by platelet agonists, arachidonic acid (0.75 µM), ADP (4.3 µM), platelet activating factor (800 nM) and collagen (638 nM) in a dose dependent manner. The findings revealed that the antiplatelet aggregatory activity of the extract of *A. nilotica* is mainly due to blockade of Ca2+ channels, although evidence also suggests that the involvement of protein kinase.

Daoud*et al.,* (2015), displayed that Agar well diffusion method was used to screen the antibacterial and antifungal activities of different solvent extracts.

Koudoro Yaya Alain *et al.,*(2015) reported Chemical characterization and biological activities of extracts from two plants (*Cissus quadrangularis* and *Acacia polyacantha*) used in veterinary medicine in Benin.

Muhammad Khalid*et al.,* (2017) reported the identification of oral cavity biofilm forming bacteria and determination of their growth inhibition by *Acacia arabica*, *Tamarixaphylla* L. and *Melia azedarach* L. medicinal plants.

Gupta A *et al.,* (2017) examined the extracts of herbs like Babool and Neem which have been used traditionally for oral care. The objective of the current clinical study was to investigate the efficacy of Babool Neem Toothpaste in oral hygiene and dental care. The study was conducted in patients of gingivitis & periodontitis and healthy subjects free from oral diseases. Babool Neem Toothpaste showed significant improvement in all the parameters assessed when compared to baseline. However, the similar results were also observed in Placebo Toothpaste Group. It could be concluded that brushing with Babool Neem Toothpaste produced significant improvement in various parameters viz. gingivitis, dental stains, plaque, Halitosis, microbial counts, clinical attachment loss and global efficacy

assessments. The results were statistically significant in comparison to the baseline within the same group and were assessed by investigator to be clinically significant.

Thangavelu L *et al*., (2018) investigated the hepatoprotective effects and possible mechanism of *Acacia catechu* in acetaminophen (APAP) induced hepatotoxicity using female Wistar rat model. Hepatotoxicity was induced by oral administration of acetaminophen (750 mg/kg body weight) for 24 h. The seed (400 mg/kg body weight) and bark (400 mg/kg body weight) extract's treated groups exhibited hepatoprotective effects and was compared with well-known clinical anti-dote N-acetylcysteine (NAC). When groups treated with acetaminophen, significant increase of liver weight/body weight ratio, liver function enzymes such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase(AST) and decrease of antioxidant enzymes such as glutathione (GSH) and superoxide dismutase (SOD) were observed. The histopathology of APAP treated groups also showed moderate degree of sinusoidal congestion, centrilobular necrosis with polymorph nuclear cells infiltration, marked vacuolations and congestion. However, pretreatment with seed or bark extract groups decreased LPO accumulation, reduced the liver function enzymes and increased antioxidant defense enzymes.

Karthikeyan R *et al.,* (2018) examined the antimicrobial efficacy of a mixed herbal powder extract (MHPE) against cariogenic microorganisms. The results revealed that the anti-adherence and anti-biofilm effect as well as the faster killing activity suggests that MHPE formula has effective antibacterial activity and could be a useful source of anticariogenic agents in near future.Sood R, et al (2018) investigated all the extracts of O. sanctum (crude extract, terpenoid and polyphenol) and *A.arabica* (crude extract, flavonoid and polyphenol) showed significant virucidal activity, however, crude extract ocimum and terpenoidocimum showed highly Significant to significant ($p < 0.001–0.01$) decrease in virus genome copy numbers with lowest dose tested. Similarly, therapeutic effect was observed in all three extracts of *O. sanctum* in comparison to the virus control, nevertheless, crude extract ocimum and terpenoidocimum maintained this effect for longer period of time (up to 72 h post-incubation). None of the leaves extracts of *A. arabica* had therapeutic effect at 24 and 48 h post-incubation, however, only the crude extract Acacia and polyphenols Acacia showed delayed therapeutic effect (72 h post inoculation). Prophylactic potential was observed in polyphenol acacia with highly significant antiviral activity compared to virus control ($p <$

0.001).

Aussara Panya *et al.,*(2019) reported a paper on Novel bioactive peptides demonstrating anti dengue virus activity isolated from the Asian medicinal plant *Acacia Catechu*.

4. METHODOLOGY:

Sample collection & preparation:

The seeds and bark of *A. arabica* was collected from Jagaveerapandiapuram, Thoothukudi, Tamilnadu. The pods were split to get the seeds. Collected seeds and bark were allowed to dry for 7 days and was powdered.

Plate 4.1 Bark of *Acacia Arabica*

Plate 4.2 Pods of *Acacia arabica*

Preparation of plant extract:

The powders were extracted using acetone and ethanol. 1.75 g of bark and seed powder was soaked in both acetone and bark (5% concentration)for 7 days. 2.5g of bark and seed powder was soaked in both acetone and bark (10% concentration) for 7 days. Then the powder and solvent mixture was kept to evaporate for a day. Now the remaining crude extract can be used to perform various tests.

Antimicrobial activity:

Isolation of bacterial culture:

To isolate *E. coli* and *S. aureus* from sewage and throat sample. Sewage sample was

collected to isolate *E.coli* and was diluted by serial dilution technique. 0.1 ml of sample was spread on EMB agar plates and the plates were incubated for 48 hours. Throat sample was collected using a swab and was inoculated in nutrient broth. After overnight incubation, 0.1ml sample was spread on Nutrient agar plate and was incubated for 24 hours. After incubation, a loopful of sample was streaked on a sterile Mannitol salt agar plate and incubated for 48 hours. The plates were observed for growth and organisms were identified based on Gram staining and biochemical characterization.

Biochemical test (IMVIC):

Indole production test:

Peptone water was prepared and sterilized and poured into test tubes. *Escherichia coli* and *Staphylococcus aureus* was inoculated into peptone water and were incubated for one day at 37°C. Few drops of Kovac's reagent were added to the test tube. Color change was observed.

Methyl red test [MR-Test]:

MR- VP broth was prepared and sterilized and poured into tubes. *Escherichia coli* and *Staphylococcus aureus* was aseptically inoculated into MR-VP medium. The culture tubes were incubated for one day at 37°C. Methyl-red indicator was added to the test tubes. Color change was observed.

Voges-Proskeuer test [VP-test]:

MR-VP broth was prepared and sterilized and poured into test tubes. *Escherichia coli* and *Staphylococcus aureus* was inoculated into MR-VP media tubes. The tubes were then incubated for one day. After incubation Barrit's reagent was added to the culture. Color change was observed.

Citrate utilization test:

Simmons citrate agar was prepared and sterilized and poured into tubes and kept in a slanting position and allowed to solidify. *Escherichia coli* and *Staphylococcus aureus* was carefully inoculated into Simmons citrate agar slant. The slant tubes were incubated for one day. Color change was observed.

Agar well diffusion method:

Antimicrobial activity was performed by agar well diffusion method (Bauer A W*et al*., 1966; Parekh J and Chanda S, 2007). Mueller-Hinton agar was prepared and sterilized, poured into plates and allowed to solidify. Mueller-Hinton agar plates were inoculated with *Escherichia coli* and *Staphylococcus aureus* using a swab. 0.25g of seed and bark extracts (at 5% concentration) were mixed with5ml of DMSO (Jack *et al.,* 1995). Then, a hole was punched aseptically with a sterile cork borer, and a volume of 0.5 µl of the extract solution was introduced into the well. Then the agar plates were incubated at 37°C for 24 hours. The plates were observed for zone of inhibition.

Anti-inflammatory activity:

Anti-inflammatory activity was assessed by the method suggested by (Winters *et al*., 1962) using carrageenan as phlogistic agent. The adult Wister albino rats of either sex weighing between 150 & 180 gm were housed in groups of four animals each. They were starved overnight during the experiment but had free access to water. The volume of paw of each animal was determined before giving any drug. Animals were divided into six groups each consisting of four animals. Extracts made at 10% concentration was taken. Group I served as control which received normal saline [1ml/Kg, (p.o)], Group II received standard drug Diclofenac sodium [10 mg/Kg (p.o)], Group III received 200mg/kg p.o. of ethanol bark extract. Group IV received 200mg/kg p.o.of ethanol seed extract. Group V received 200mg/kg p.o.of acetone bark extract. Group VI received 200mg/kg p.o. of acetone seed extract. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the albino rats. One hour after oral administration of the drugs, the paw edema was measured with the aid of a standardplethysmometerat 1, 2, 3 and 4 hours after the injection of carrageenan. Percentage inhibition of paw edema was calculated by comparing the control for each dose at different hours as given below.

Percentage inhibition= 1-Vt/Vc* 100

Where,

Vt= volume of paw edema in treated animals

Vc= volume of paw edema in control animals

Anti-oxidant activity:

Chemicals and reagents:

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid was purchased. DPPH is an organic chemical compound composed of stable free radicals. 5mM Sodium Nitroprusside, Griess reagent (1% sulphonil amide, 0.1% N 1-naphthylethylenediamine, 2% orthophosphoric acid), Phosphate buffer (pH- 7.4) and Methanol. All the chemicals and solvents were used of analytical grade.

DPPH free radicals scavenging activity:

Anti-oxidant activity was investigated by DPPH radical scavenging method (Blois, 1958). To a methanolic solution of DPPH (100 µM, 2.95 mL), 0.05 mL each of the extracts (10% concentration) dissolved in methanol was added at various concentrations (20-100 µg/mL). Equal amount of methanol was added to the control. Absorbance was recorded at 517 nm. Vitamin C (ascorbic acid) was used as standard antioxidant (positive control). Scavenging of DPPH radicals by the extract was calculated using the following formula and plot graph in compared to standard.

% DPPH free radical scavenging

(Absorbance of control – Absorbance of test) X 100Absorbance of control

5. RESULT AND DISCUSSION:

Antimicrobial activity:

Identification of bacteria:

The pathogens isolated from the sample were identified as *Escherichia coli* and *Staphylococcus aureus*, based on their growth in the selective plates such as EMB agar and Mannitol salt agar and by their biochemical characterization.

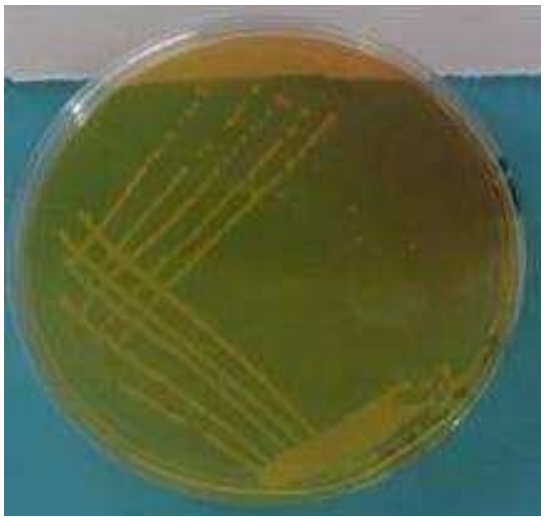

Plate 5.1 *S. aureus* on Mannitol Salt agar

Plate 5.2 *E. coli* on EMB agar

Biochemical Characterization:

Table 5.1: Biochemical characterization of the isolated pathogens

S.NO.	TESTS	*Escherichia coli*	*Staphylococcus aureus*
1.	Gram's Staining	-	+
2.	Indole	+	-
3.	Methyl Red	+	+
4.	VogesProskauer	-	+
5.	Citrate	-	+

Antimicrobial assay by agar well diffusion method:

The acetone and ethanol extracts of *Acacia arabica* seeds and bark were screened for their antibacterial activity against the isolated bacteria *Escherichia coli* and *Staphylococcus aureus* by agar well diffusion method. 0.5µl of extract was added to the well and was incubated. The growth of organism seeded in the medium after an incubation period of 24 hours was observed. The clear zone of growth inhibition was noted around the well due to diffusion of extracts and growth of bacteria. The diameter of the zone denotes the relative susceptibility of the test microorganism. The term susceptible implies that an infection caused by strain tested may be expected to respond favorably to the indicated antimicrobial agent for type of infection and pathogen.

Table 5.2: Antibacterial activity of extracts of *Acacia arabica*

S. No	Sample extract	Zone of inhibition (mm)	
		Escherichia coli	***Staphylococcus aureus***
	SEED		
1.	Acetone	22	24
2.	Ethanol	16	18
3.	DMSO	Nil	Nil
	BARK		
1.	Acetone	31	24.33
2.	Ethanol	17	14
3.	DMSO	Nil	Nil

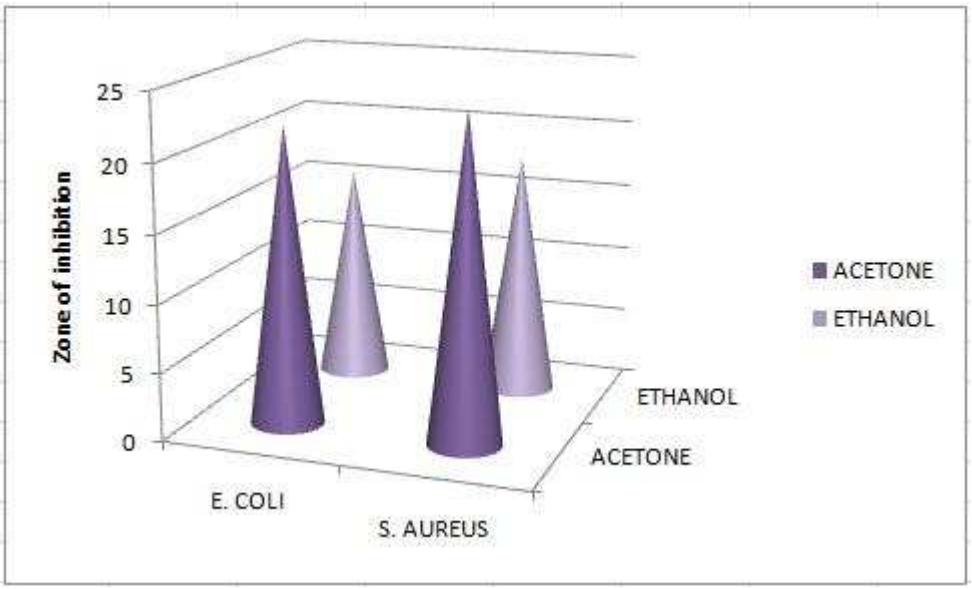

Figure 5.1 Zone of inhibition ethanol and acetone extracts of seed against *E. coli* and *S. aureus.*

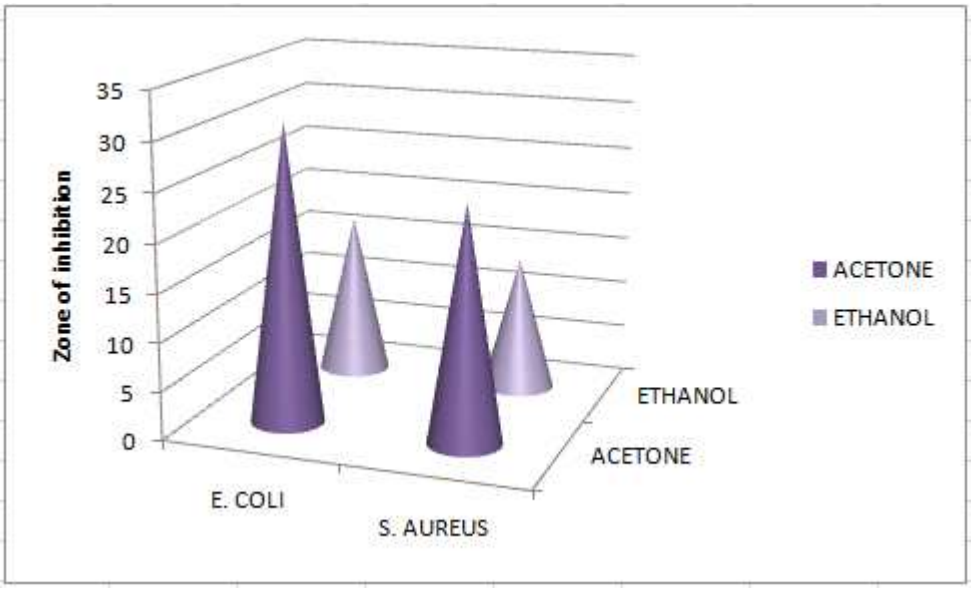

Figure 5.1 Zone of inhibition ethanol and acetone extracts of bark against *E. coli* and *S. aureus.*

The zone of inhibition was noted for both the bacteria namely *Escherichia coli* and *Staphylococcus aureus*. The acetone extract of bark showed maximum activity against

Escherichia coli and the acetone extract of seed showed significant activity against *Staphylococcus aureus*. Overall, acetone extracts showed significant activity than ethanol extracts. Among the extracts, bark showed significant activity than seeds of *Acacia arabica*.

Almost all antimicrobial components present in plants are aromatic compounds and can be easily extracted by initial ethanol/methanol extraction (Cowan, 1999). The antibacterial activity of extract used in this study could be chiefly due to the presence of phytoconstituents. Hassan *et al*., (2009) has tested antimicrobial activity of ethanolic extract of *Acacia arabicainvitro* against seven bacterial species and two fungal species by well diffusion method and micro dilution method. The result of the study showed that the ethanolic extract of the plant was effective on bacterial strains.

Mohan Lal Saini *et al.,* (2008), examined comparative antimicrobial studies of *Acacia* species and *Acacia nilotica* (*Acacia arabica*) exhibited highest activity against three bacterial strains *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*. Sharma *et al*., (2014) evaluated antibacterial activity of *Acacia arabica* bark extract in different solvents against *Staphylococcusaureus*, *Pseudomonas aeruginosa* and *Escherichia coli* and observed maximum activity with acetone extracts followed by methanolic extracts.

The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments. The results of the present study support the use of *Acacia arabica* for human and animal disease therapy and reinforce the importance of ethnobotanical approach as a potential source of bioactive substances.

Anti-inflammatory activity:

The anti-inflammatory effect of acetone and ethanol extracts of bark and seed of *Acacia arabica* were investigated in this study. The difference between the readings at time 0 minutes and the different time intervals was taken as the thickness of edema volumes are given in table 5.3.

Table 5.3: Anti-inflammatory effect of seed and bark of *Acacia arabica* on carrageen-induced paw edema in albino rats.

Drug treatment	Dose mg/kg	Mean changes in paw edema ± SEM			
		1 hour	2 hours	3 hours	4 hours
Control saline	1 mg/kg	0.30±0.01	0.80±0.00	1.24±0.00	1.27±0.01
Standard Diclofenac Sodium	10 mg/kg	0.20±0.00 (33.33%)	0.28±0.01 (65%)	0.25±0.02 (79.8%)	0.23±0.00 (81.8%)
Ethanol bark extract	200 mg/kg	0.14±0.00 (53.33%)	0.40±0.00 (50%)	0.38±0.00 (69.3%)	0.36±0.00 (71.6%)
Ethanol seed extract	200 mg/kg	0.17±0.01 (43.3%)	0.48±0.00 (40%)	0.42±0.01 (66.1%)	0.40±0.01 (68.5%)
Acetone bark extract	200 mg/kg	0.14±0.00 (53%)	0.32±0.00 (60%)	0.30±0.00 (75.8%)	0.28±0.00 (77.9%)
Ethanol seed extract	200 mg/kg	0.18±0.02 (40%)	0.37±0.01 (53.7%)	0.42±0.01 (66.1%)	0.32±0.01 (74.8%)

From the results it has showed, it is obvious that the acetone extract of bark showed maximum activity at 4 hour interval (77.9%) which is nearly to the standard value (81.8%), whereas ethanol extract of seed at 4 hour interval showed least activity (68.5%) comparatively. As the time increases the percentage of paw edema inhibition activity increases. It reveals that the acetone extract showed very significant anti-inflammatory activity than the ethanol extracts of *Acacia arabica* and also bark extracts has significant anti-inflammatory activity than the seed extracts.

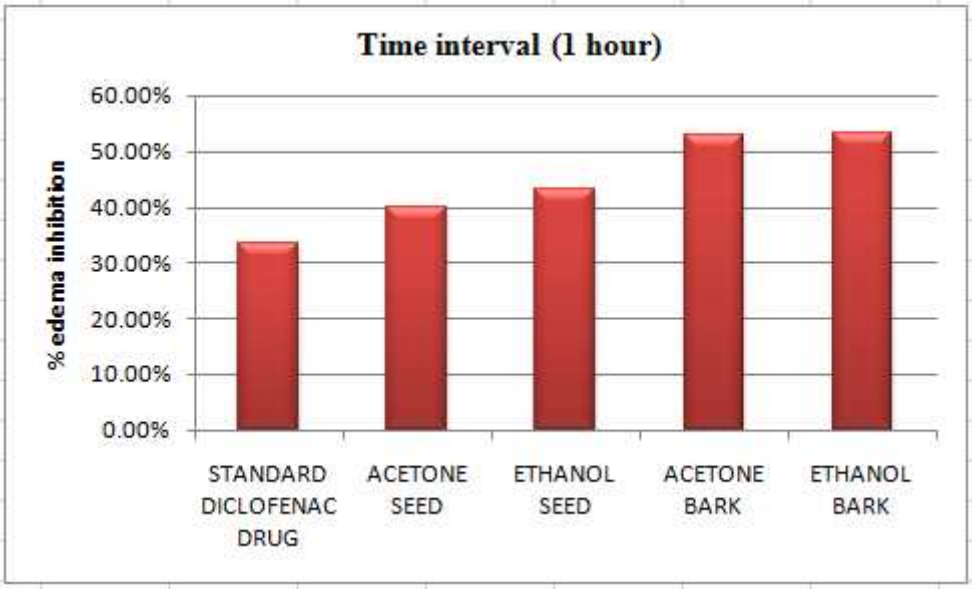

Figure 5.3 % edema inhibition at 1 hour interval

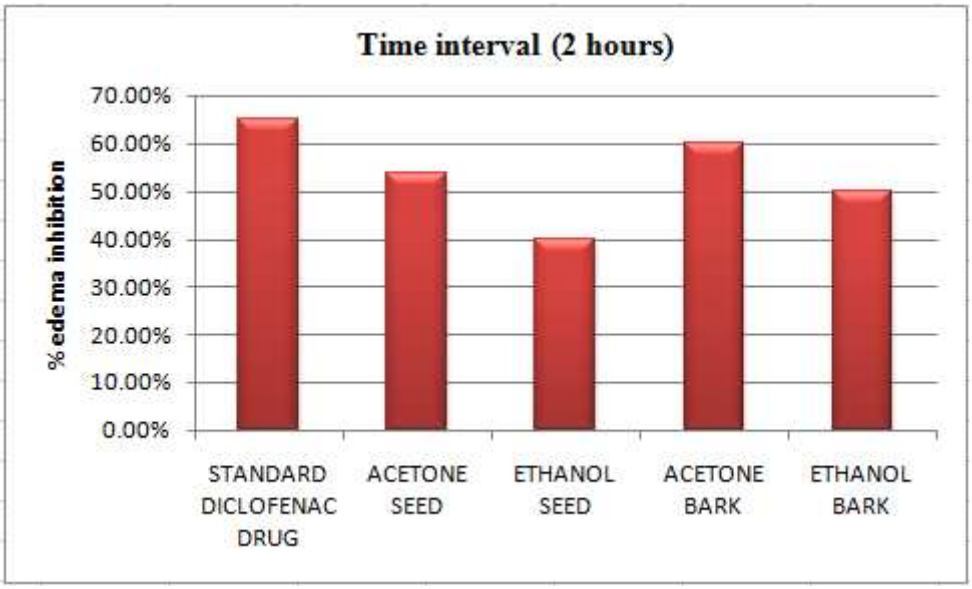

Figure 5.4 % edema inhibition at 2 hours interval

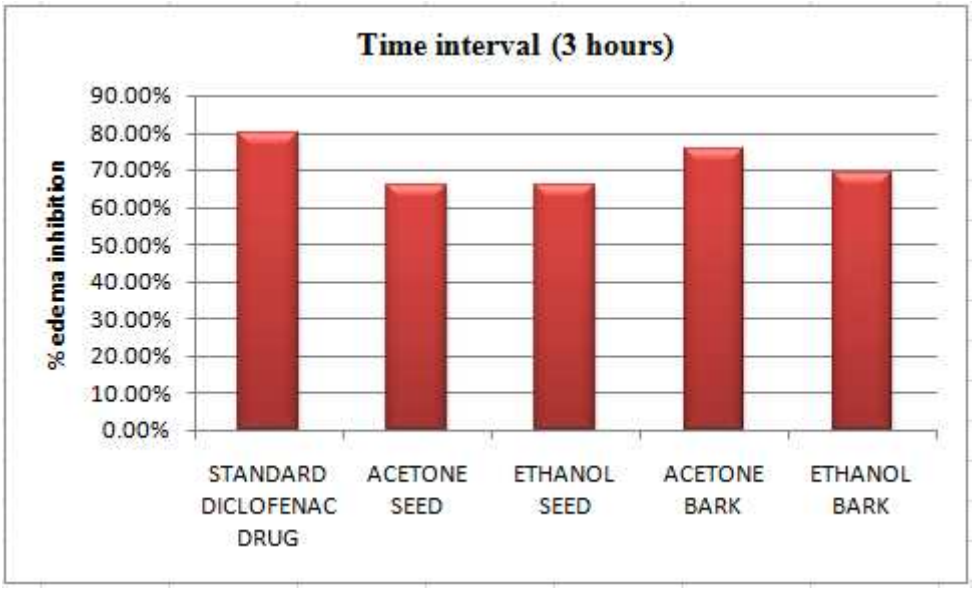

Figure 5.5 % edema inhibition at 3 hours interval

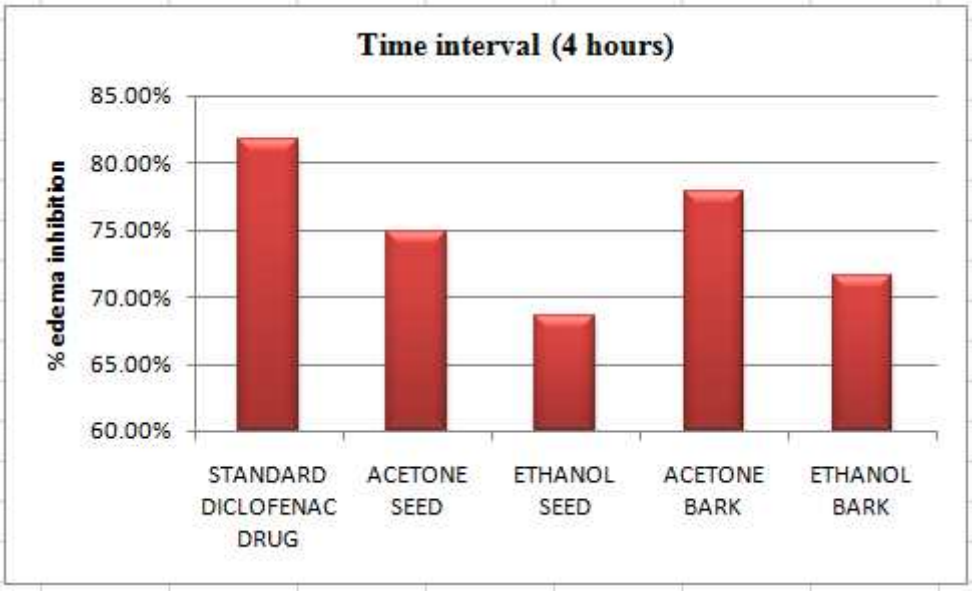

Figure 5.6 % edema inhibition at 4 hours interval

Rajendran A *et al*., (2010), has studied about the anti-inflammatory activity of the flowers of *Acacia arabica*. Fresh flowers of Acacia Arabica willd were extracted with 80% alcohol and the concentrated extract was fractionated in the usual way. The ethyl acetate

fraction was found to contain isoquercetin. The structure was characterized by UV, NMR, paper chromatographic and chemical studies. The yellow pigment was found to contain promising results with respect to acute and chronic anti-inflammatory studies.

Acacia nilotica (*Acacia arabica*) is known for high amounts of tannins, flavonoids, polysaccharides, and organic acids (Sotohy*et al*., 1995; Dafallah and Al Mustafa, 1996). Dafallah and Al Mustafa (1996) suggested that these metabolites are the main factors responsible of *in vivo* anti inflammatory, analgesic, and antipyretic activities of *Acacia nilotica* (*Acacia arabica*) extracts. More recently, an anti-inflammatory androstene steroid was isolated from the aerial parts of *Acacia nilotica* (Chuabal*et al*., 2003).

Anti-Oxidant activity:

The antioxidant effect of ethanol and acetone extracts of the seeds and bark of *Acacia arabica* were investigated in this study. The results were tabulated in the table 5.4

Table 5.4: Anti-oxidant effect of seed and barkof*Acacia Arabica*

Concentration (µg/ml)	**Percentage of activity (Mean ± SEM)**				
	Standard (vitamin C)	**Acetone seed extract**	**Ethanol seed extract**	**Acetone bark extract**	**Ethanol bark extract**
20	0.529±0.001 (45.10%)	0.932±0.001 (4.27%)	0.893±0.001 (8.22%)	0.956±0.001 (3.14%)	0.825±0.003 (15.11%)
40	0.410±0.002 (58.45%)	0.912±0.001 (6.29%)	0.809±0.001 (16.73%)	0.928±0.001 (4.67%)	0.706±0.002 (27.17%)
60	0.329±0.001 (66.66%)	0.892±0.002 (9.50%)	0.704±0.002 (27.37%0	0.904±0.002 (8.40%)	0.625±0.001 (35.37%)
80	0.236±0.002 (74.78%)	0.875±0.002 (11.34%)	0.617±0.002 (36.18%)	0.878±0.002 (9.74%)	0.434±0.003 (54.72%)
100	0.166±0.002 (83.18%)	0.845±0.002 (13.08%)	0.423±0.002 (57.14%)	0.852±0.002 (13.67%)	0.255±0.003 (74.16%)

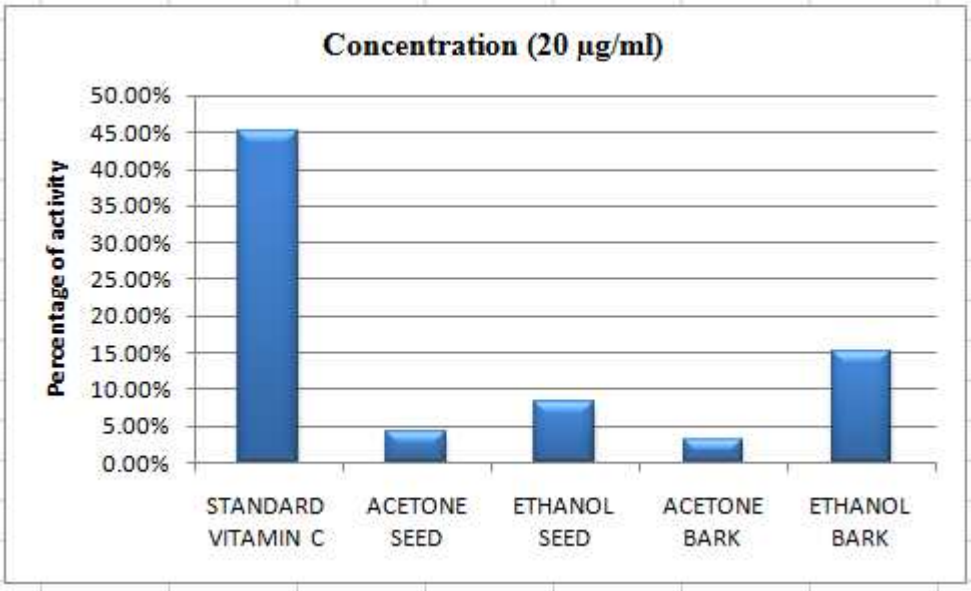

Figure 5.7 Percentage of activity at 20 µg/ml

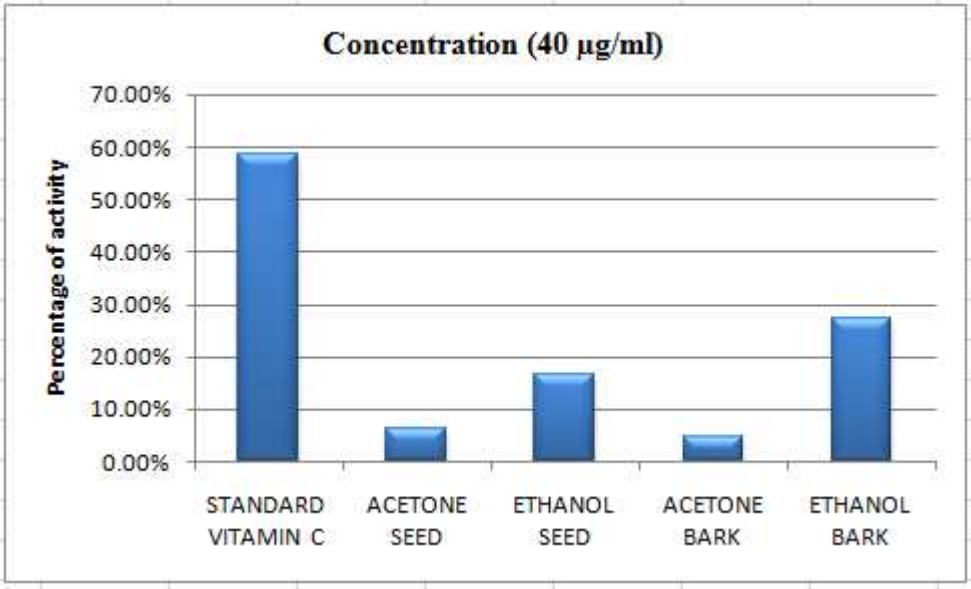

Figure 5.8 Percentage of activity at 40 µg/ml

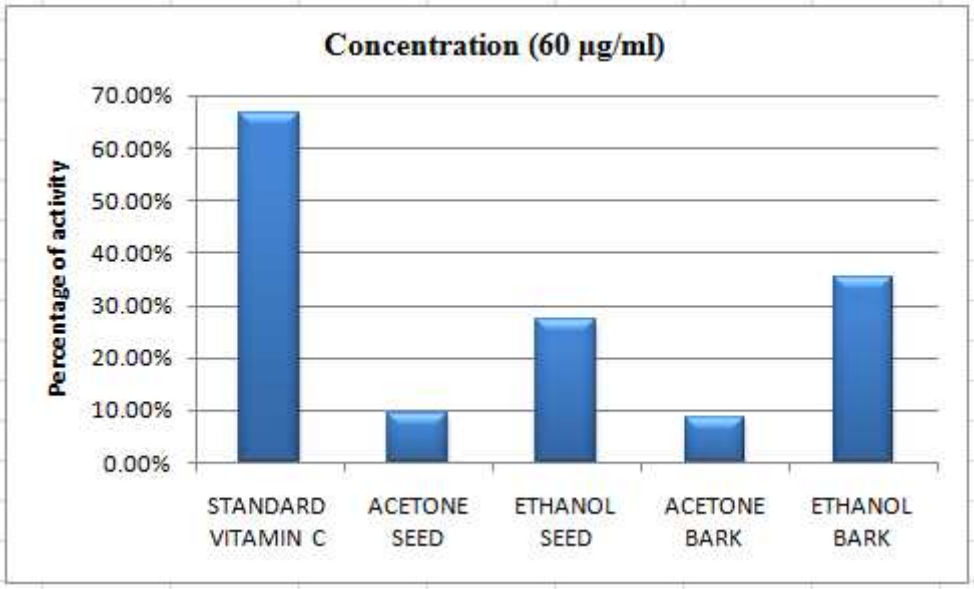

Figure 5.9 Percentage of activity at 60 µg/ml

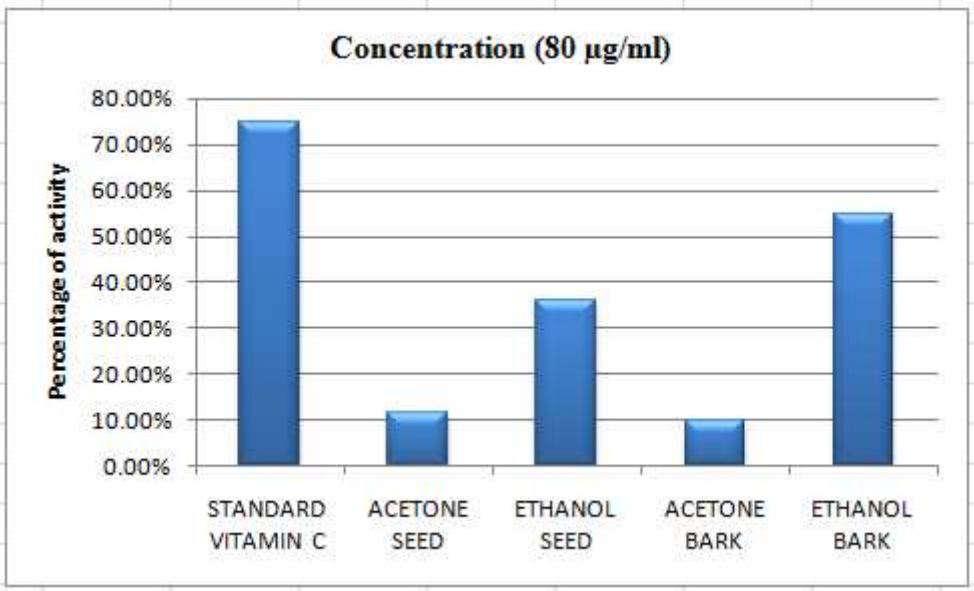

Figure 5.10 Percentage of activity at 80 µg/ml

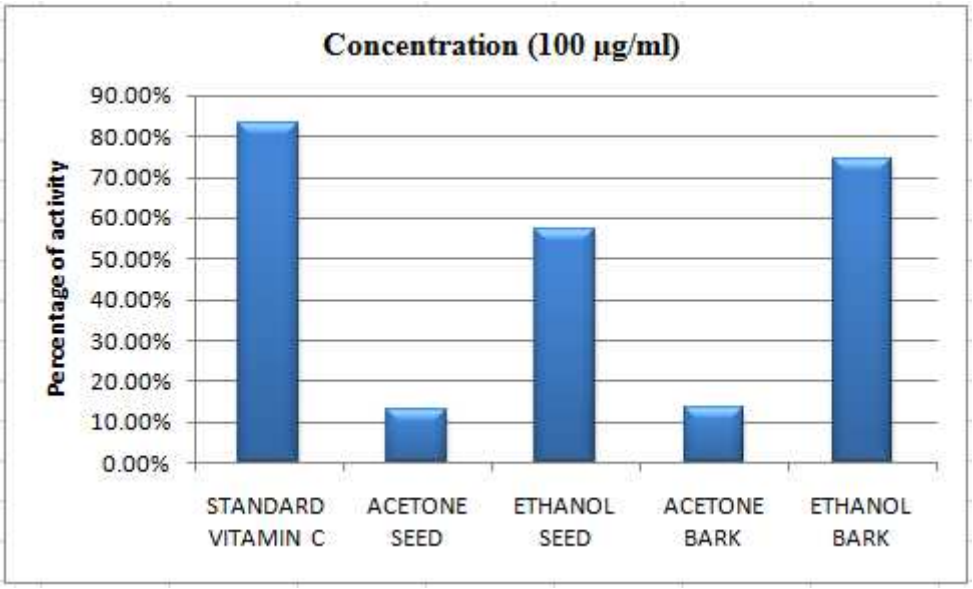

Figure 5.11 Percentage of activity at 100 µg/ml

The method used in this study was the free radical scavenging activity of DPPH. It was obvious from the result that the ethanol bark extract showed highest antioxidant activity than the ethanol seed extract of *Acacia arabica*. As the concentration of the extracts increases the percentage of activity against the DPPH radicals also increases. At 100 µg/ml of extracts, the ethanol extract of bark showed maximum activity (74.16%) which is nearly to the standard value (83.18 %) followed by ethanol extract of seed (57.14%). But the acetone extracts of seed and bark showed very low activity (13.08%, 13.67%) against the DPPH radicals. It reveals that the ethanol extract showed very significant antioxidant activity than the acetone extracts of *Acacia arabica*.

Katiyar S *et al*., (2013), reported that the antioxidant activity of ethyl acetate soluble fraction of *A. arabica* bark by *in vitro* lipid peroxidation model was carried out by tertiary butyl hydroperoxide induced lipid peroxidation and the most active fraction were identified by TLC and in vivo experiment in most active fraction were carried out with 50, 100 and 150 mg/kg oral dose in carbon tetra chloride induced hepatotoxicity in rats and it is hypothesized that flash chromatographic fraction of ethyl acetate extract exhibited maximum activity with in vitro lipid peroxidation and 150 mg/kg dose of carbon tetra chloride shows marked liver protection in in vitro model.

DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Brand-Williams *et al*., 1995). Different researches have showed that the electron donation capacity of plant extract is associated with antioxidant activity. *Acacia* species are a rich source of polyphenolic compounds, known to have strong antioxidant properties (Dhabai K *et al.,* 2012). Kalaivani T and Mathew L (2010) reported that the high scavenging property of *Acacia nilotica* (*Acacia arabica*) maybe due to the hydroxyl groups existing in the phenolic compounds that can scavenge the free radicals. Flavonoids and other phenolic compounds (polyphenols) of plant origin have been reported to be good scavengers of free radicals (Formica and Regelson 1995).

From this study it is evident that the bark of *Acacia arabica* has significant antioxidant activities and this could be an added pharmacological use of the plant.

6. CONCLUSION:

Ethanol and acetone extracts of seeds and bark of *Acacia arabica* at 5% concentration were found to be an effective antimicrobial agent. From the results, it is concluded that the acetone extracts showed significant antimicrobial activity. All test organisms showed sensitivity towards the extracts. The phytochemical compounds found in *Acacia arabica* may be responsible for the antimicrobial activity of the plant.

The anti-inflammatory activity of *Acacia arabica* carried out with the ethanol and acetone extracts of seeds and bark at 10% concentration showed a significant evidence for the use of these plant materials in the treatment of inflammation. Among the studied extracts, bark extract showed highest activity than the seed. Acetone extracts showed maximum activity than ethanol extracts.

The present study reported the antioxidant activity of *Acacia arabica* extracts made with ethanol and acetone at 10% concentration. In order to realize the health benefits from potential plant sources, it is important to measure the antioxidant activity using various radicals and oxidation systems. The ethanol bark extract showed maximum antioxidant activity than the leaf extract against the DPPH radicals. Ethanol extracts showed maximum activity then acetone extracts. The present study clearly established that the bark of *Acacia arabica* extract has high promise as sources of natural antioxidants.

7. SUMMARY:

Medicinal plants play vital role in the sustainability of the human race in this planet Earth. Medicinal plants are a rich source of novel drugs that forms the ingredients in traditional systems of medicine, nutraceuticals, food supplements, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. The present study confirms the different medicinal uses of *Acacia arabica* seeds and bark in traditional medicines as well as in various other beneficiary uses.

In this study, seeds and bark of *Acacia arabica* collected from Jagaveerapandiapuram, Thoothukudi district, was used to perform antimicrobial, anti-inflammatory and anti-oxidant activity. Seeds and bark were shade dried for 7 days. The dried materials were grinded to fine powder. 1.75 g (5%) and 2.5 g (10%) of seed and bark powder each was weighed and soaked in 25 ml of solvents like acetone and ethanol. After 7 days of soaking, the solvents were allowed to evaporate and the crude extracts were stored for further use.

Extracts of *Acacia arabica* at 5% concentration was taken to perform antimicrobial test. *Escherichia coli* and *Staphylococcus aureus* were isolated from sewage sample and throat sample. The culture was swabbed on Mueller - hinton agar plates and well was cut to perform well diffusion technique and the extract solution was added to the well and the plates were incubated. Control was maintained. Zone of inhibition was observed. Both the organisms showed sensitivity to all extracts. Extracts made with acetone was more effective comparatively.

Extracts at 10% concentration was taken to study the anti-inflammatory activity of *Acacia arabica*. Inflammation was induced in Wistar albino rat model to perform anti-inflammatory activity. Seed and bark extracts, normal saline (control) and standard drug were given at 1 hour intervals. The size of paw edema was measured. Percentage inhibition of paw edema was calculated by comparing the control for each dose at different hours. Acetone bark extract at 4 hour interval has showed maximum anti-inflammatory activity than acetone seed extract. Overall, acetone extracts showed maximum anti-inflammatory activity than ethanol extracts. The study indicates that *Acacia arabica* extracts has a potential anti-inflammatory activity.

Extracts at 10% concentration was taken to determine the anti-oxidant activity of *Acacia arabica*. Antioxidant activity was performed by DPPH assay. To the methanolic solution of DPPH, seed and bark extract was added. Control was maintained. Absorbance was recorded at 517 nm. Scavenging of DPPH radicals by the extract was calculated using formula and plot graph in compared to standard(ascorbic acid). Ethanol bark extract at high concentration showed maximum anti-oxidant activity than other extracts. Overall ethanol extracts showed maximum anti-oxidant activity than acetone extracts. The present study clearly established that *Acacia arabica*extract has promise as sources of natural antioxidants.

This study paves the way for further attention and research to identify the bioactive compounds which are responsible for the plant's pharmaceutical activity. Further studies should be undertaken to identify the exact mechanism of action by which extracts exert their antimicrobial, anti-inflammatory and anti-oxidant effect which can be use in drug development program for safe health care services.

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EXPLORING THE POTENT BIOLOGICAL ACTIVITY OF *Acalypha indica* LEAVES, A COMMON WEED

A DISSERTATION SUBMITTED TO

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

Affiliated to Manonmaniam Sundaranar University,

in partial fulfilment of the requirements for the award of degree of

BACHELOR OF SCIENCE IN MICROBIOLOGY

SUBMITTED BY

A. MUTHU CATHRIN KEPSHI (REG. NO: 18SUMB23)

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APRIL 2021

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"EXPLORING THE POTENT BIOLOGICAL ACTIVITY OF *Acalypha indica* LEAVES, A COMMON WEED" is a bonafide record of the original work completed by A. Muthu Cathrin Kepshi (Reg. No: 18SUMB23), P. Muthu Kanaga Lakshmi (Reg. No:18SUMB24), P. Muthu Lakshmi (Reg. No: 18SUMB25), S. Nanthini (Reg. No: 18SUMB26), P. Pon Lakshmi (Reg. No: 18SUMB28) during this academic year 2020-2021 in St. Mary's college (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirement for the award of the Degree of Bachelor of science in Microbiology prescribed by the Manonmaniam Sundaranar University.**

Signature of the Guide

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Self Supporting Course

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Lucia Rose

Signature of the Principal

Principal

St. Mary's College (Autonomous)

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15/04/21.

Signature of the External Examiner

EXPLORING THE POTENT BIOLOGICAL ACTIVITY OF *Acalypha indica* LEAVES, A COMMON WEED

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Signature of the Guide **Signature of the H.O.D**

Signature of the Director **Signature of the Principal**

Signature of the External Examiner

DECLARATION

We hereby declare that the dissertation work entitled **"EXPLORING THE POTENT BIOLOGICAL ACTIVITY OF *Acalypha indica* LEAVES, A COMMON WEED"** is a bonafide record of the original work completed by us during this academic year 2020 - 2021 in St. Mary's college (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirement for the award of the degree of Bachelor of Science in Microbiology prescribed by Manonmaniam Sundaranar University. We also affirm that this is an original work done by us under the supervision of **A. Maria Heartina Adlin Vaz M.Sc., SET,** Assistant professor, Department of Microbiology, St. Mary's college (Autonomous), Thoothukudi.

Signature of the student **Signature of the Guide**

Place: Thoothukudi

Date:

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Last but not least we express our hearty thanks to our lovable family members.

A. MUTHU CATHRIN KEPSHI,

P. MUTHU KANAGA LAKSHMI,

P. MUTHU LAKSHMI,

S. NANTHINI,

P.PON LAKSHMI

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ABBREVATION

MAPs-Mean Arterial Pressure

DPPH - 2,2-diphenyl-1-picryl-hydrazyl-hydrate

DMSO -Dimethyl sulfoxide

EDTA- Ethylenediamine tetra acetic acid

TBA-Tertiary butyl alcohol

TCA- Trichloroacetic acid

INTRODUCTION

Wild plants which grow naturally and aggressively in a cultivation field are known as weeds. They inhibit the growth of useful plants in agriculture, gardens etc. They depend on other plants for their nutrients, space, water and light. They are one of the main reasons for the reduction of crop production and quality. But some weeds have certain beneficial characteristics as well such as *Centella asiatica* (Indian pennywort), *Alternanthera sessilis* (Dwarf copper leaf) are edible, *Abutilon indicum* (Indian abutilon) and *Leucas zeylanica* (Ceylon slitwort) are used to treat haemorrhoid's and worm infestations respectively. In some countries like India and Sri Lanka, some of the weeds are used in their traditional medicine. Different parts of the plant (leaves, stems, roots) or the entire plant is used in various preparations to treat different diseases (Hrckova 2013).

Herbal plants play an important role in human life. Medicinal plants are commonly used in china and India. This treatment, also known as conventional treatment. It was the main source of medical treatment during the early period of time. The civilization has modified and with it has come the introduction of more advanced techniques and methods, it is very helpful for future generations to tend to choose advanced treatment over conventional treatments. The information related to conventional treatments are gradually vanishing since the previous generations are getting older and dying with failure. This knowledge is passed on to the next generation, through experiments and observation and oral teaching. (Sudhakar chekuri *et al*., 2020). It is crucial to have proper documentation from the extant practitioners since conventional treatments are an alternative path to curing various types of human diseases (Martin *et al.,* 1995). The traditional methods practiced in India such as Ayurveda, Unani and Siddha. It cures a various type of diseases.

Plants have lot of bioactive constituents like alkaloids tannins, phenolic compound and flavonoids. These substances are found in several part of plants such as root, leaf, shoot and bark. (Sudhakar chekuri *et al*., 2016). India officially recognizes over 3500 plants for their medicinal uses. It is generally estimated that over 6000 plants in India are in use in folk, traditional and herbal medicines. (Sudhakar chekuri *et al*., 2020). India has a richest flora that is widely distributed throughout the country. Scientific experiments done on the antimicrobial properties and their component have been documented in the late 19th century.

Now a days, nearly 88% of the global populations use the medicinal plant drug as their defends for maintaining good health and dangerous diseases. One hundred and nineteen secondary plant metabolites derived from herbal plants are used globally as drugs ;15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived component were discovered (Govindarajan *et al*., 2008). The large proportion of the word population depend on traditional medicine plant (Suresh *et al.,* 2011). During recent year considerable attention of medicinal plant product as botanical pesticides for control of different microbial infestations. (Varma *et*

*al.,*2002). The medicinal plants play an important role in the development and improvement of new drug. It is effectiveness, less side effects and relatively low cost when compared to the synthetic drugs (Dubey *et al*., 2004). The increase unsuccess of antibiotic resistance and chemotherapeutics exhibited by pathogenic microbial infectious agents that led to screening of many medicinal plants of their potential antimicrobial activity (Colombo and Bosisio 1996). The number of publications describing bioactive plant derived compounds in the last few years are anti- tumor drugs, anti-inflammatory drug, immunomodulators, kidney protectors and drugs for psychiatric use (Muniappan Ayyanar and Savarimuthu Ignacimuthu 2008). Medicinal plant drugs are the sources of natural pesticides that made a successful lead for new pesticides development (Vinisha *et al.,* 2017).

Natural products derived from medicinal plants have wide range of pharmacological significance. Bioactive compounds contain therapeutic and their complex nature will able to interact with mammalian cell targets. Phytochemicals naturally isolated from the herbal plants (MAPs) are used specifically in drug industries. These Phytochemicals' have certain limitations of low absorption, high toxicity, and other side effects, bioavailability and efficacy. Irrespective of the advantages of synthetic, combinatorial chemistry and molecular modelling, they remain an important source for new drugs discovery (Lali lingfa *et al.,* 2020). An increasing interest in the medicinal based treatment for disease control observed in different countries and herbal remedies integrated into orthodox herbal practice (Wachtel-Galor and Benzieet, 2011). Some of the diseases such as malaria, epilepsy, infantile convulsion, diarrhea, cholera, dysentery, fungal and bacterial infections have been treated using medicinal plants (Sofowora, 1996). Medicinal plants are considered as a chemical factory, since it contains alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (Singh, 2005).

Sometimes, Synthetic drugs may have side effects and antibiotic resistant microorganisms also increasing every year. Hence, researchers are going toward the herbal drug discovery for the treatments of varies diseases (Esakkirajan *et al.,* 2014). The traditional medicinal plants-based treatment covers the basic health needs in the developing countries (Alavijeh *et al.,* 2012). India is one of the richest and different cultural traditions connected with the use of medicinal plants to cure different diseases.

Acalypha indica is a weed widely distributed throughout the Indian country. It also known as Indian acalypha is a weed that can be found in India, tropical Africa, sri Lanka and many other countries. It has been reported use in the treatment of constipation, skin disorder and snake bites on Sri Lanka and India (Perera *et al.,* 2016). It grows well in west and south of Africa northeast, Somalia, Ethiopia and other regions. The plant can also be found in most wet, tropical and temperate countries in Asia, Europe and both North and South American regions. It grows as a weed in bushes, backyards, alongside roads and other places such as home and crop premises (sivasankari *et al*., 2014) Many international manuscripts on *Acalypha indica* were published from Indian region because this plant has a close connection with Ayurveda, Siddha and Unani medicinal practices

executed by older Indian generations (Kirtikar *et al.,* 1918).

The leaves of *Acalypha indica* have sub obtuse crenate-serrate and base cuneate and glabrous thin. Their petiolate is usually longer than the slender, blade, and stipulate minute. The leaves are simple and arranged spirally; 0.02 – 12.00 cm petiole long; blade broadly ovate to ovate-lanceolate; 2 – 9 cm × 1 – 5 cm; base cuneate; apex acute; margins toothed; membranous; sparingly short hairs to almost glabrous is nature on both surfaces; hairier along the midrib; 5-veined at base and with 4 to 5 pairs of lateral veins. One month after germination, the stem starts to turn woody as it matures. (Shivaprasad panjala *et al.*, 2020). The branches are numerous, ascending, long and finely pubescent. The flower of the *Acalypha indica* is arranged in numerous erect, lax, elongated, clusters and auxiliary spikes near the summit of the spikes. The female is in white colour, scattered, and surrounded by a shortly pedunculate large leafy dentate cuneiform with many nerves bract that is approximately 6 to 8 mm in diameter (Sivasankari *et al.,* 2014).

The Arial parts contain a cyanogenic glycoside called acalyphin (a 3-cyanopyridone derivative) as well as flavonoids, such as kaempferol mauritianin, clitorin, nicotiflorin and biorobin, Tannins, β- sitosterol, acalyphamide, aurantiamide, succinamide, and flindersin (a pyranoquinolinone alkaloid) have also been isolated. The chemicals that attract cats are the iridoid compounds isodihydronepetalactone and isoiridomyrmecin. Leaves and twigs contain acalyphamide and other amides, quinone, sterols, cyanogenic glycoside (Jakka Chiranjeevi *et al*., 2020). The plant substance constituent a cyanogenetic glucoside, kaempferol, triacetoneamine, a base and acalyphine, an alkaloid. It too contains the acalyphamide, amide along with a few further 2-methylanthraquinone, amides, y-sitosterol, Beta sitosterol, tri-O-methyl ellagic acid and stigmasterol, Beta sitosterol glucoside, quinine, tannin, resin, n-octacosanol, and essential oil. Acalyphine is awfully cooperative within the action of sore gums. The Plant Contain Antimicrobial, antimutagenic, antidiabetic, ant tumorous, cytotoxic, antibiotic-chemotherapeutic, anti-teratogenic properties which make it important medicinal plant for Medicine purpose. (Aushi Nag *et al.,* 2018).

FIG 1: ***Acalypha indica***

Scientific name: *Acalypha indica*

Taxonomic Classification

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Order: Malpighiales

Family: Euphorbiaceous

Genus: Acalypha

Species: *Acalypha indica*

Name of the plant in different languages

Sanskrit: Arittamanjarie.

English: Indian acalypha.

Hindi: Kuppu; Khokali.

Telugu: Kuppichettu; Harita-manjiri;

Kuppinta or Muripindi.

Constituents: Alkaloids “acalypus” and “acalyphine

Name of the plant in other countries

Alcalifa: Brazil

Tie Xian: China

Baro, Berbere: Ethiopia

Kuppimeni: India

Ricinela: Spain

Kuppameniya: Sri Lanka

The root extract is treating as a stimulant, harsh and tough purgative (Silber bush *et al.,*2010). The leaves are laxative while a moisturizer for the diagnosis of facial paralysis (Li H *et al.,*2012). The leaves extract can be used in the diagnosis of jaundice, piles and also externally skin eruptions, ring worms. Leaves possess anti-periodic with laxative properties and the leaf extract can be applied to insect bites (Bourdy and Walter 1992). Root is of use during heart disease plus retain excretions. The 50 % ethanol extracts of pods reveal the anti-fertility activity in female albino rats. Cough can be easily cured by making burnt pods with little salt along with honey 3-4 times. Roots are worn during the cure of antipyretic, diabetes, helpful in coffer complaint, gorge plight, eyes defect along with the diagnosis of cardiac disorder, stiff situation, ulcer, wound, boils furthermore diverse crust disease. The extract of the root bark among alcohol be capable of exist use meant for back wart fever. The seed is slightly sweet and also possess laxative, carminative, cooling improves the appetite (Chopra *et al,.*2006). Leaf be use like painkiller the same as an antipyretic, it be a tonic used for malaria also fever. It is to apply inside anthrax, blood poisoning, along with antidysentery, anti-diabetic and leprosy intended for the elimination of abdominal obstacle (Khare et al.,2007) and they are also helpful in the treatment of asthma (Kirtikar and Basu 2006). The leaves of the plant contain too be report toward seize the superiority of contraceptive action (Vaishnav MM and Gupta KR 1996). The extract of the origin is to be able to condensed the blood sugar stage via 30% (Vaishnava *et al.,*1993). Seed powder is used in amoebiasis. The extract of the flower inhibits the ovarian utility with excite the utility of uterine in albino rats. The pulp of the fruit around the seed is used in curing diabetes. Outwardly it is of use used for the emigration in flatulent tummy ache, while salad dressing intended for gouty otherwise stiff joint. The fruit flesh is used for constipation, tummy ache, chlorosis along with urinary disorder. The bark seizes stimulant also antidysentery property helpful in administering of leprosy, jaundice and heart disease

It is useful for the treatment of pneumoniae, rheumatism asthma and several other diseases. The leaves have been reported to possess contraceptive activity. In the present study the plant extract against gram positive and gram-negative bacteria (Jebanesan *et al*., 2008). The leaf extracts reduce the mutagenicity in *Escherichia coli*. The root is prescribed as a tonic, febrifuge, astringent, and strong purgatives. Alcohol Extract of the root bark can be used externally as emollient; a poultice (a soft, moist mass of material, typically consisting of bran, flour, herbs, etc., applied to the body to relieve soreness and inflammation and kept in place sore with a cloth) is used for chilblains (a painful, itching swelling on a hand or foot, caused by poor circulation in the skin when exposed to cold), in insect bites, swelling rheumatism and facial paralysis. The roots are used in chest pain, joint pain, migraine, blood dysentery and the root extract lower the blood sugar level up to 30%. Methanol extract of the whole plant has potential analgesic and anti-inflammatory actions in rats and mice (Rahman *et al.,* 2010). The root extraction has potential Nitric oxide scavenging activity. (Balakrishnan et al., 2009). *Acalypha indica* has acaricidal activity Acaricides are pesticides which is kill the members of the arachnid subclass Acari, which includes ticks and mites. Acaricides are used in medicine and agriculture, although the selective toxicity differs between the two fields. This

medicinal herb has anti-cancerous activity against different types of cancers (Sanseera 2021)

Powder of the dry leaves is given to children to expell worms; also given in the form of decoction with little garlic. In homoeopathy, the plant is used in severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis. The plant contains kaempferol, a cyanogenetic glucoside, a base, triacetonamine an alkaloid, acalyphine. Amide, acalyphamide, 2-methylanthraquinone, tri-O-methyl ellagic acid, stigmasterol, n-octacosanol, quinine, tannin resin, and essential oil (Chandra Mohan *et al*., 2012). *Acalypha indica* contains acalyphine which is used in the treatment of sore gums (Bedon *et al*., 1982). *Acalypha indica* used as emetic, expectorant, laxative, diuretic bronchitis, pneumonia, asthma and pulmonary tuberculosis. In homeopathy, this plant is used for severe cough, bleeding from lungs, haemopytosis and incipient phthisis (Devi *et al.,* 2012).

Antibiotics are powerful medicines that fight bacterial infections. Used properly, antibiotics can save lives. They either kill bacteria or keep them from reproducing. Antibiotics provide the main basis for the therapy of microbial and bacterial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents. With increased knowledge of the causative agents of various infectious diseases, antibiotics has come to denote a broader range of antimicrobial compounds, including antibacterial, antifungal and another compound (Scott *et al.,* 1998). An antibacterial is a compound or substance that kills or slows down the growth of bacteria (Houghton *et al.,* 1995). With advances in medicinal chemistry most of today's antibacterial chemically are semisynthetic modifications of various natural compounds. These include, for example, the beta-lactam antibacterial, which include the *penicillin* (produced by fungi in the genus *Penicillium*), the cephalosporins and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterial for example, the sulfonamides, the quinolones, and the oxazolidinones are produced solely by chemical synthesis. In accordance with this, many antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semisynthetic and synthetic. Another classification system is based on biological activity, in this classification, antibacterial are divided into two broad groups according to their biological effect on microorganisms, bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth. In the recent years, the interest in medicinal plants has increased in a great deal. Apart from this people from the west have also taken this matter seriously by conducting various researches on plant-based medicines. Traditional medicines are used by about 60 per cent of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While the traditional medicines are derived from medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes, hence

this requires proper documentation and research. In western world also, the use of herbal medicines is steadily growing with approximately 40 percent of population reporting use of herb to treat medical illnesses within the past year. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine, against microbial infections (Warrier *et al.,* 2005). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents hassled to the screening of several medicinal plants for their potential antimicrobial activity. *Acalypha indica* Linn Is a member of Euphorbiaceous family. It is essentially a weed which grows in waste areas. It is an annual herb, about 80 cm high and commonly found in waste places or fields. Previous studies on *Acalypha indica* revealed that this plant has antibacterial activity against several gram-positive bacteria (Govindarajan *et al*., 2005) the petroleum ether and ethanol extract were found to have anti-implantation activity when they were given to female albino rats. This effect was reversible upon withdraw of the treatments with the extracts. This effect is due to some oestrogenic activity as evidenced by histological studies of the uterus. Studies found that *Acalypha indica* does have wound healing ability, however it is inferior to Heliotropism indicum which has better activity and tensile strength. *Acalypha indica* is a common weed while viper russelli is amongst the deadliest snakes in the world. Ethanol leaf extract of *Acalypha indica* possess potent snake venom neutralizing properties. A drug having a marked action on the alimentary canal and respiratory organs. It is indicated in incipient phthisis, with hard, racking cough, bloody expectoration, arterial haemorrhage, but no febrile disturbance. In congestive headache a piece of cotton saturated with the expressed juice of the plant or leaves and inserted into each nostril is said to relieve it by causing haemorrhage from the nose (Kanimozhi *et al*., 2012)

Anti-bacterial are the agents that interferes with the growth and reproduction of the bacteria. Anti-bacterial are used to dis infect surfaces and to potentiate harmful bacteria. Heat, chemicals such as chorine, phenol etc. and anti-bacterial drugs have anti-bacterial properties. The mechanism of Antibacterial agents are as follows Inhibitors of cell wall synthesis, Inhibitors of nucleic acid synthesis, Inhibitors of cell membrane function, Inhibitors of other metabolic processes, Inhibitors of protein synthesis**,** they follow any of the following mentioned mechanisms. The ethyl acetate, butyl alcohol and distilled water extracts from the leaves, stem and roots of *Acalypha indica* were tested for their antibacterial activities against *Bacillus subtilis, Staphylococcus sp, Escherichia coli, Pseudomonas sp*. The results indicated that all the extracts exhibited antibacterial activities against the bacteria. (Sudhakar chekuri *et al.,* 2016)

An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host. Fungi can be found throughout the world in all kinds of environments. Most fungi don't cause disease in people. But some species can infect humans and cause illness. Antifungal drugs target structures or functions that are necessary in fungal cells but not in human cells, so they can fight a fungal infection

without damaging your body's cells. In the present study *Acalypha indica*, a medicinal plant was used to screen its antifungal activity against *C*and*ida albicans.* Azoles are fungi static and not fungicidal, so it has given rise to both primary and secondary drug resistance. Therefore, there is a real need for a next generation of safer and more potent antifungal agents. One possible approach is to screen local medicinal plants to get the compound which can be directly used as antifungal agents or can serve as template for drug development. Vulvovaginal candidiasis is an important cause of morbidity in women of reproductive age and majority of the infections are caused by the species, *C*and*ida albicans*. In about five percentages of cases, the disease has a chronic course showing frequent and refractory episodes. (Sobel, *et al.,* 2003). The four extracts were also screened for antifungal activity in comparison with standard antibiotic Ketoconazole (10mg/ml) in well diffusion fusion method Lawn culture was prepared using the test organism on wells were made in those plates at prescribed distance. (Perez, *et al.*, 1999).

Parasitic earth worms cause substantial morbidity and mortality and morbidity in livestock animals globally, and considerable productivity losses to farmers. The control of these nematodes has relied largely on the use of a limited number of anthelmintics. However, resistance to many of these anthelmintics is now widespread, and, therefore, there is a need to find new drugs to ensure sustained and effective treatment and control into the future. (Shivkar and Kumar. 2008)

In the recent years interest in the study of antioxidant activity of plant extracts and isolation from plants has grown due to the fact that the free radicals have been related to degenerative diseases. Human cells are constantly exposed to reactive oxygen radicals generated by a number of biotic and a biotic factor such as irradiation, environmental factors, pollutants, stress or by products of metabolic processes. Free radical attacks biological molecules such as lipids, proteins, enzymes, DNA and RNA leading to cell or tissue injury associated with many diseases including ageing, atherosclerosis, heart diseases and carcinogenesis. Antioxdants are compounds which act as radical scavengers when added to the food products and prevent the radical chain reaction of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the processes of lipid peroxidation (Shanmugapriya et al., 2007).

AIM AND OBJECTIVES

- To collect *Acalypha indica* from our college premises.
- To prepare the leaf extracts of *Acalypha indica* using ethyl acetate, butyl alcohol and distilled water
- To study the preliminary phytochemical analysis of the three different leaves extract.
- To study the antimicrobial activity of the leaf extract of *Acalypha indica* extracts by Agar well diffusion method.
- To study the antihelminthic activity of the leaf extracts of *Acalypha indica* by using standard method.
- To study the antioxidant activity of the leaf extracts of *Acalypha indica* by using standard method
- To prepare herbal soap using fresh leaves the leave of *Acalypha indica*

REVIEW OF LITERATURE

Kirtikar *et al.,* (1918) are said many international manuscripts on *Acalypha indica* were published from Indian region because this plant has a close connection with Ayurveda, Siddha and Unani medicinal practices executed by older Indian generations.

Bedon *et al.,* (1982) reported that the *Acalypha indica* contains acalyphine which is used in the treatment of sore gums.

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Houghton *et al.,* (1995) reported that an antibacterial is a compound or substance that kills or slows down the growth of bacteria

Vaishnava and Gupta K (1996) observed the leaves of the plant contain too be report toward seize the superiority of contraceptive action

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Sofowora, (1996) reported that some of the diseases such as malaria, epilepsy, infantile convulsion, diarrhea, cholera, dysentery, fungal and bacterial infections have been treated using medicinal plants

Scott *et al*., (1998) stated Antibiotics are powerful medicines that fight bacterial infections. Used properly, antibiotics can save lives. They either kill bacteria or keep them from reproducing. Antibiotics provide the main basis for the therapy of microbial and bacterial infections. the discovery of these antibiotics and their uses as chemotherapeutic agents with increased knowledge of the causative agents of various infectious diseases, antibiotics has come to denote a broader range of antimicrobial compounds, including antibacterial, antifungal and another compound

Perez, *et al*., (1999) said that the four extracts were also screened for antifungal activity in comparison with standard antibiotic Ketoconazole (10mg/ml) invitwell diffusion fusion method Lawn culture was prepared using the test organism on wells were made in those plates at prescribed distance

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Dubey *et al*., (2004) identified that the medicinal plants play an important role in the development and improvement of new drug. It is effectiveness, less side effects and relatively low cost when compared to the synthetic drugs

Warrier *et al., (*2005) reported that Indian systems of medicine, most practitioners formulate and dispense their own recipes, hence this requires proper documentation and research. In western world also, the use of herbal medicines is steadily growing with approximately 40 per cent of population reporting use of herb to treat medical illnesses within the past year. In western world also, the use of herbal medicines is steadily growing with approximately 40 per cent of population reporting use of herb to treat medical illnesses within the past year.

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Jebanesan *et al.,* (2008) reported that it is useful for the treatment of pneumoniae, rheumatism asthma and several other diseases. The leaves have been reported to possess contraceptive activity. The bark seizes stimulant also antidysentery property helpful in administering of leprosy, jaundice and heart disease. The bark seizes stimulant also antidysentery property helpful in administering of leprosy, jaundice and heart disease

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Shivkar and Kumar. (2008) said that Parasitic earth worms cause substantial morbidity and mortality in livestock animals globally, and considerable productivity losses to farmers. The control of these nematodes has relied largely on the use of a limited number of anthelmintics. The extraction of *Acalypha indica* was evaluated for anthelmintic activity using adult earthworms.

Govindarajan *et al.*, (2008) stated that now a days, nearly 88% of the global populations use the medicinal plant drug as their defends for maintaining health and combating diseases. One hundred and nineteen secondary plant metabolites derived from herbal plants are used globally as drugs ;15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived component were discovered

Balakrishnan *et al*., (2009) reported the root extraction has potential Nitric oxide scavenging activity.

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Wachtel-Galor and Benzieet (2011) said that increasing interest in the medicinal based treatment for disease control observed in different countries and herbal remedies integrated into orthodox herbal practice

Li H *et al.*, (2012) described the leaves are laxative while a moisturizer for the diagnosis of facial paralysis

Chandra mohan *et al*., (2012) said that Powder of the dry leaves is given to children to expell worms; also given in the form of decoction with little garlic. In homoeopathy, the plant is used in severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis the plant contains kaempferol, a cyanogenetic glucoside, a base, triacetonamine an alkaloid, acalyphine. Amide, acalyphamide, 2-methylanthraquinone, tri-O-methyl ellagic acid, stigmasterol, n-octacosanol, quinine, tannin resin, and essential oil

Devi *et al*., (2012) reported that *Acalypha indica* used as emetic, expectorant, laxative, diuretic bronchitis, pneumonia, asthma and pulmonary tuberculosis. In homeopathy, this plant is used for severe cough, bleeding from lungs, haemopytosis and incipient phthisis

Kanimozhi *et al.,* (2012) reported *Acalypha indica* is a common weed while viper russelli is amongst the deadliest snakes in the world. Ethanol leaf extract of *Acalypha indica* possess potent snake venom neutralizing properties. A drug having a marked action on the alimentary canal and respiratory organs. It is indicated in incipient phthisis, with hard, racking cough, bloody expectoration, arterial haemorrhage, but no febrile disturbance. That in congestive headache a piece of cotton saturated with the expressed juice of the plant or leaves and inserted into each nostril is said to relieve it by causing haemorrhage from the nose

Alavijeh *et al*., (2012) said that traditional medicinal plants-based treatment covers the basic health needs in the developing countries

Hrckova (2013) identified but some weeds have certain beneficial characteristics as well such as Centella asiatica (Indian pennywort), Alternanthera sessilis (Dwarf copper leaf) are edible, Abutilon indicum (Indian abutilon) and

Esakkirajan *et al*., (2014) observed Synthetic drugs may have side effects and antibiotic resistant microorganisms also increasing every year. Hence, researchers are going toward the herbal drug discovery for the treatments of varies diseases

Sivasankari *et al., (*2014) said this plant grow well in west and south of Africa northeast, Somalia, Ethiopia and another region. plant can also be found in most wet, tropical and temperate countries in Asia, Europe and both North and South American regions. It grows as a weed in bushes, backyards, alongside roads and other places such as home and crop premises

Sivasankari *et al*., (2014) said that the branches are numerous, ascending, long and finely pubescent. The flower of the *Acalypha indica* is arranged in numerous erect, lax, elongated, clusters and auxiliary spikes near the summit of the spikes. female is in white colour, scattered, and surrounded by a shortly pedunculate large leafy dentate cuneiform with many nerves bract that is approximately 6 to 8 mm in diameter

Sudhakar chekuri *et al*., (2016) reported that the ethyl acetate, butyl alcohol and distilled water extracts from the leaves, stem and roots of *Acalypha indica* were tested for their antibacterial activities against *Bacillus subtilis, Staphylococcus sps, Escherichia coli* & *Pseudomonas sp* all the extracts exhibited antibacterial activities against the bacteria.

Sudhakar chekuri *et al.,* (2016) observed the bioactive constituents of plants are alkaloids tannins, phenolic compound and flavonoids. These substances are found in several part of plants such as root, leaf, shoot and bark.

Perera *et al.,* (2016) reported that *Acalypha indica* is a weed widely distributed throughout the Indian country. It also known as Indian acalypha is a weed that can be found in India, tropical Africa, sri Lanka and many other countries and use in the treatment of constipation, skin disorder and snake bites on Sri Lanka and India

Vinisha *et al*., (2017) described that the medicinal plant drugs are the sources of natural pesticides that made a successful lead for new pesticides development

Aushi nag *et al.,* (2018) said that plant Contain Antimicrobial, antimutagenic, antidiabetic, ant tumorous, cytotoxic, antibiotic-chemotherapeutic, anti-teratogenic properties which make it important medicinal plant for Medicine purpose.

Somchit MN *et al*., (2018) said that Acalypha *indica* is an uncultivated tropical plant commonly found in Asia, Africa and Central America and has been used since ancient times to treat various diseases and health disorders, including diseases affecting the respiratory system their medicinal uses. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicines.

Lali lingfa *et al*., (2020) said that Irrespective of the advantages of synthetic, combinatorial chemistry and molecular modelling, they remain an important source for new drugs discovery

Shivaprasad panjala *et al.,* (2020) said the leaves are simple and arranged spirally; 0.02 – 12.00 cm petiole long; blade broadly ovate to ovate-lanceolate; 2 – 9 cm × 1 – 5 cm; base cuneate; apex acute; margins toothed; membranous; sparingly short hairs to almost glabrous is nature on both surfaces; hairier along the midrib; 5-veined at base and with 4 to 5 pairs of lateral veins. One month after germination, the stem starts to turn woody as it matures

Jakka Chiranjeevi *et al.,* (2020) identified that leaves and twigs contain acalyphamide and other amides, quinone, sterols, cyanogenic glycoside

Sanseera (2021) stated this medicinal herb *Acalypha indica* has anti-cancerous activity against different types of cancers.

Methodology

Collection of sample:

The fully matured *Acalypha indica* weeds were collected from our college premises St. Mary's college (Autonomous) Thoothukudi in the month of January. The leaves of the plants were collected and washed two to three times with tap water to remove dust and wash with sterile water, shade dried at room temperature on sterile blotting paper for 15 to 20 days after complete drying the plant materials were powder using the Blender and stored in separate air tight container the powders were used for further analysis

FIG 2: ***Acalypha indica***

FIG3: ***Acalypha indica*** **powder**

Solvent extraction:

10 g of the dried powder of the leaves of *Acalypha indica* were dissolved in 100 µl of three different solvents such as ethyl acetate, butyl alcohol, and distilled water respectively. The extraction was carried out for 48 hours. After extraction the sample was filtered with Whatman No;1 filtered paper of the filtrates were stored in refrigerator for further use.

FIG 4: SOLVENT EXTRACTS

Test microbe:

The pure culture of *Escheriachi coli, Staphylococcus aureus, Rhizopus, Penicillium* the organisms were obtained from our microbiology laboratory and used for antibacterial and antifungal assay. The test organisms obtainted were maintained on the nutrient agar slant under refrigerator.

FIG5: ESCHERICHIA COLI

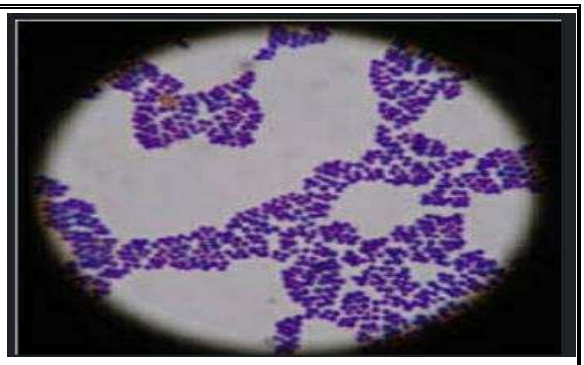

FIG6: STAPHYLOCOCCUS AUREUS

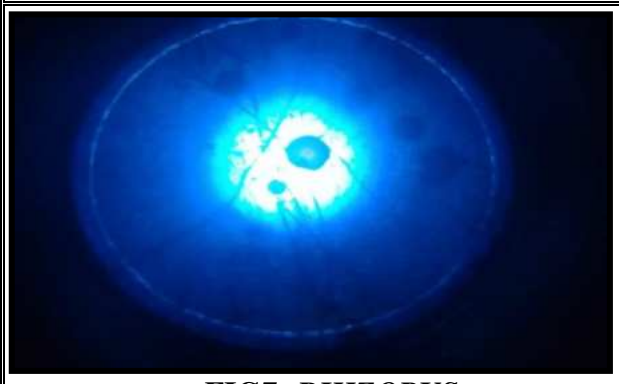

FIG7: *RHIZOPUS*

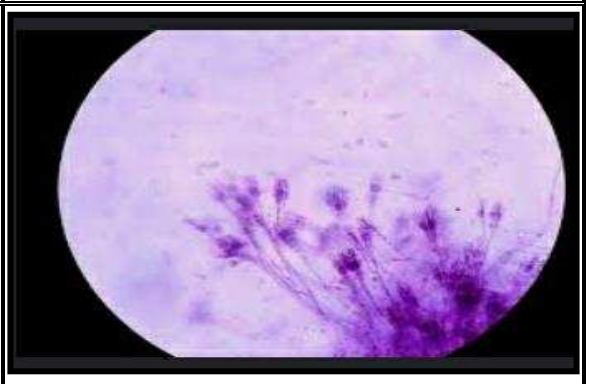

FIG8: *PENICILLIUM*

Anti-bacterial Assay:

Anti-bacterial assay was carried out using agar well diffusion method against *Escheriachia coli* and *Staphylococcus aureus.* Mueller Hinton agar was prepared, poured and allowed to solidify. 24 hours culture of *Escherichia coli* and *Staphylococcus aureus* were swabbed in respective plates. Well, was made using well cutter and the samples were loaded in different concentration such as 10 µl, 30 µl and 50 µl. All the plates were incubated at 37 degrees Celsius for 24 hours. The zone of inhibition was measured after incubation.

Anti-fungal assay:

Antifungal assay for Butyl alcohol, Ethyl Acetate extract and distilled water was performed by Agar well diffusion method. Mueller Hinton agar was prepared, poured and allowed to solidify. The cultures of *Rhizopus and Penicillium* were swabbed in respective plates. Well was made using well cutter and the samples were loaded in different concentration such as 10 µl, 30 µl and 50 µl. All the plates were incubated at room temperature for 5 days. The zone of inhibition was measured after incubation.

QUALITATIVE PHYTOCEMICAL ANALYSIS

The extracts obtained were analysed for preliminary phytochemical screening using the following phytochemical protocol

TEST FOR CARBOHYDRATES:

The 2ml of extract was taken to this 5ml of Benedict's solution was added and boiled for 5 minutes. The observation of brick red coloured precipitate indicates presence of carbohydrates.

TEST FOR FLAVANOIDS:

The extract was treated with a few drops of lead acetate solution. The observation of yellow colour precipitate indicates presence of flavonoid.

TEST FOR SAPONINS:

To the 5ml of diluted extract were taken in a test tube and shaken vigorously and kept for 5 minutes. Formation of foamy layer indicate presence saponins.

TEST FOR PHENOL

Ferric Chloride Test Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols

TEST FOR PHYTOSTEROLS:

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand Appearance of golden yellow colour indicates the presence of triterpenes.

TEST FOR PROTEIN:

The small quantity of extract was treated with few drops of Biuret reagent. The blue reagent turned into violet in the presence of protein.

TEST FOR GLUCOSIDES:

The small quantity of extract was treated with few drops of dilute HCL. The colour change means positive result.

ANTIHELMINTHIC ACTIVITY OF LEAVES OF *ACALYPHA INDICA* Linn.

Ethyl acetate, butyl alcohol and distilled water extracts at 0.1g/ml from the *Acalypha indica* leaves were investigated for their anthelmintic activity against *Eisenia fetida*. Albendazole at 0.01g/ml was used for positive control and distilled water for negative control. Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms neither moved when shaken vigorously.

FIG 9: *Eisenia fetida*

ANTIOXIDATION DETERMINATION ASSAY:

DPPH radical scavenging assay:

The 1, 1-diphenyl 1-2 picrylhydrazyl (DPPH) radical scavenging activity of chitosan concentration at 1mg/ml was determined described. The sample dissolved in 500 IL of 99.5% ethanol and 125 IL of 0.02% DPPH in 99.5% ethanol. This mixture to be incubated for 60 min in the dark at room temperature and then the DPPH radical was reduced. It was to be measured at 517 nm using an UV-visible spectrophotometer. In its radical formation is DPPH has an absorption band at 517 nm, then disappears up to reduction by an antiradical compound. The reaction mixture absorbance of lower absorbance indicated higher DPPH – free radical -scavenging activity was calculated as follows:

$$\text{Radical -scavenging activity} = \frac{\text{Absorbance of control- absorbance of sample}}{\text{Absorbance of control}} \times 100$$

The absorbance of control was conducted in the same manner. Distilled water is expected using instead if sample. BHA is positive standard, finally the test carried out in triplicate.

RESULT & DISCUSSION

Qualitative phytochemical analysis:

The ethyl acetate shows the presence of saponins and phenol and absence of carbohydrates, Flavanoids, phytosterol, protein and glucoside. (Sudhakar chekuri *et al*., 2016) The butyl alcohol show presences on Flavanoids and phenol and absence of saponin and glycosides. (Ashwini and asha, 2017). The distilled water the presence of phenol, saponins and carbohydrates and absences of Flavanoids and alkaloids. (Teklani and Perera, 2016). The extract ethyl acetate, butyl alcohol and distilled water of *Acalypha indica* Leaves contain phytochemicals. The present study test shows present of phenol, carbohydrates and phytosterol. And complete absence of such phytochemical in a leaf extracts are glucoside, flavonoids, proteins and saponins. These compounds are known to be biologically active because they protect the plant against infection. The study was investigation the presence or absence of this component in this plant leaf materials of *Acalypha indica*

TABLE 1: Phytochemical analysis of ethyl acetate extract

S.NO	Phytochemical	Ethyl acetate extract of *Acalypha indica*
1	Carbohydrates	+
2	Flavanoids	-
3	Saponins	-
4	Phenol	+
5	Phystosterol	+
6	Protein	-
7	Glucoside	-

TABLE 2: Phytochemical analysis of butyl alcohol:

S. no	Phytochemical	Butyl alcohol extract of *Acalypha indica*
1	Carbohydrates	+
2	Flavanoids	-
3	Saponins	-
4	Phenol	+
5	Phystosterol	+
6	Protein	-
7	Glucoside	-

TABLE 3: Phytochemical analysis of distilled water:

S. no	Phytochemical	Distilled water extract of *Acalypha indica*
1	Carbohydrates	+
2	Flavanoids	-
3	Saponins	-
4	Phenol	+
5	Phystosterol	-
6	Protein	-
7	Glucoside	-

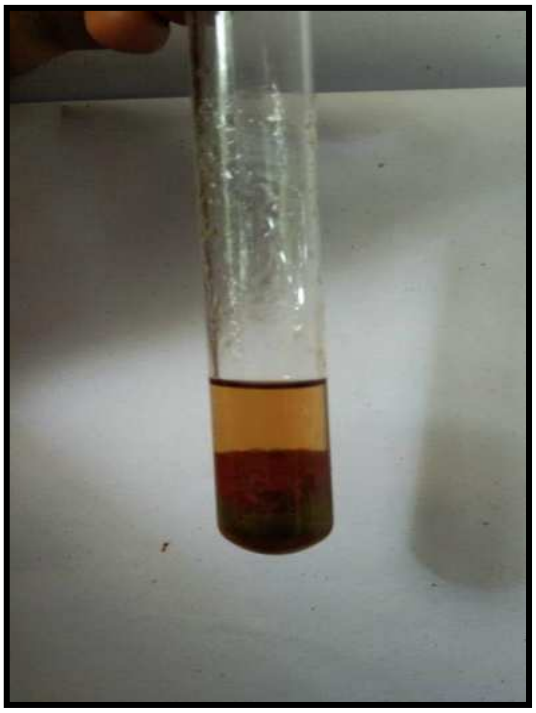

PLATE: 1.2CARBOHYDRATES

(ETHYL ACETATE EXTRACT)

PLATE: 1.2CARBOHYDRATES

(BUTYL ALCOHOL EXTRACT)

PLATE: 1.3 CARBOHYDRATE

(DISTILLED WATER EXTRACT)

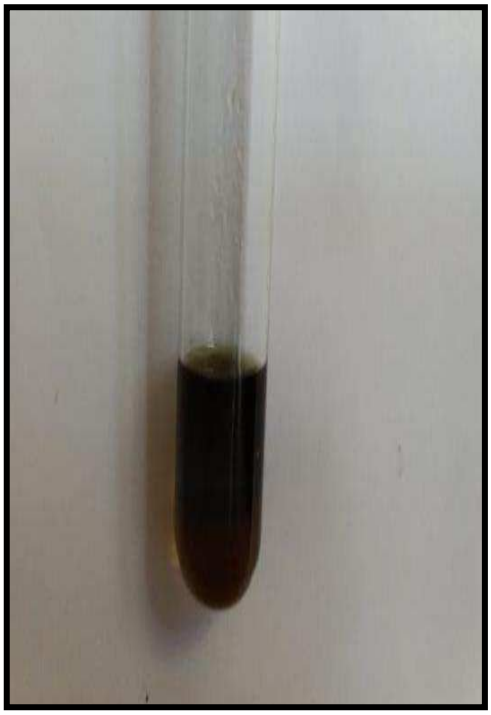

PLATE: 1.4 PHENOL
(ETHYL ACETATE EXTRACT)

PLATE: 1.5 PHENOL
(BUTYL ALCOHOL EXTRACT)

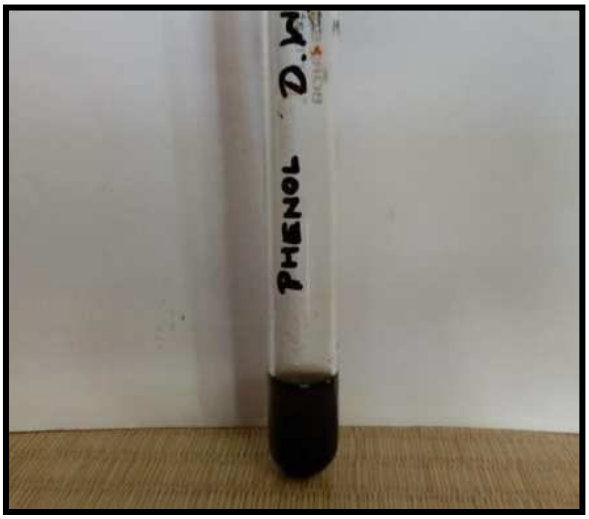

PLATE: 1.6 PHENOL

(DISTILLED WATER EXTRACT)

PLATE: 1.7 PHYSTOSTEROL (ETHYL ACETATE EXTRACT)

PLATE: 1.8 PHYTOSTEROL (BUTYL ALCOHOL EXTRACT)

Antimicrobial activity

In the present study the anti-bacterial and anti-fungal activities of *Acalypha indica* where recorder against the bacterial strain includes *Escherichia coli* and *Staphylococcus aureus* and the fungus strains like *Rhizopus* and *Penicillium*. The extract of plants shows variable activities.

Antibacterial activity

The antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of Acalypha indica against nine pathogenic bacterial isolates viz., *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae and Pseudomonas aeruginosa.* The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of Acalypha indica ethanol extract was tested by using Agar well diffusion method. The ethanol extract of Acalypha indica (100 mg/ml) showed maximum zone of inhibition (30 mm) against *Pseudomonas aeruginosa, Escherichia coli and Bacillus subtilis. Staphylococcus aureus* showed less zone of inhibition (12 mm). The ethyl acetate extract of Acalypha indica (100 mg/ml) showed maximum zone of inhibition (23 mm) against *Escherichia coli*. (Saranraj *et al.,* 2010). The ethyl acetate shows the inhibition zone at different concentration 10µl is 1mm,25 µl is 3mm and 50 µlis 4mm.agaist *Escherichia coli* and also against *staphylococcus aureus* 10 µl is 1mm,25 µl is 1mm and 50 µl is 4mm. (Sudhakar chekuri *et al*., 2016. The

distilled water shows the inhibition against *staphylococcus aureus.* (teklani and Perera 2016)

In this present study shows that the extraction of *Acalypha indica* was evaluated in different concentration (10µl, 30µl and 50µl) for antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* indicating the difference zone of Inhibition. our results revealed that Ethyl Acetate, Butyl alcohol and Distilled water of *Acalypha indica* show signification higher inhibitory activities against *E. coli* and *Staphylococcus aureus*. The butyl alcohol extract of *Acalypha indica* shows the maximum zone of inhibition 16mm against *Staphylococcus aureus* and 15mm against *Escherichia coli.*

TABLE 4:

ETHYL ACETATE EXTRACT OF *Acalypha indica* LEAF:

MICROBIAL CULTURE	**CONCENTRATION OF PLANT EXTRACTION**		
	10µl	30µl	50µl
Staphylococcus aureus	**8mm**	**9mm**	**12mm**

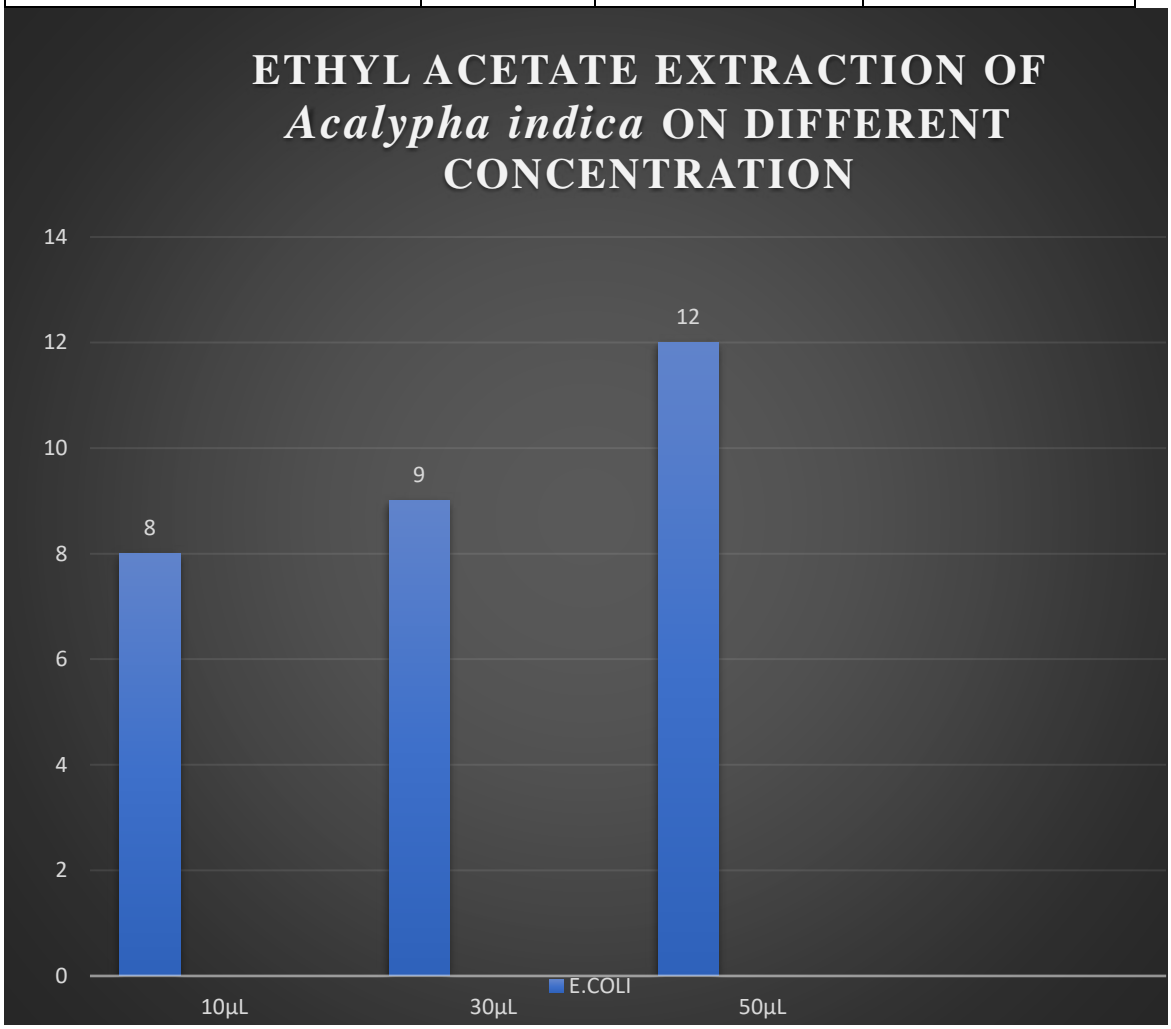

FIG 10: ETHYL ACETATE EXTRACTION OF *Acalypha indica* ON DIFFERENT CONCENTRATION

TABLE5:

BUTYL ALCOHOL EXTRACT OF *Acalypha indica* LEAF:

MICROBIAL CULTURE	CONCENTRATION OF PLANT EXTRACTION		
	10µl	30µl	50µl
Escherichia coli	12mm	13mm	15mm
Staphylococcus aureus	12mm	13mm	16mm

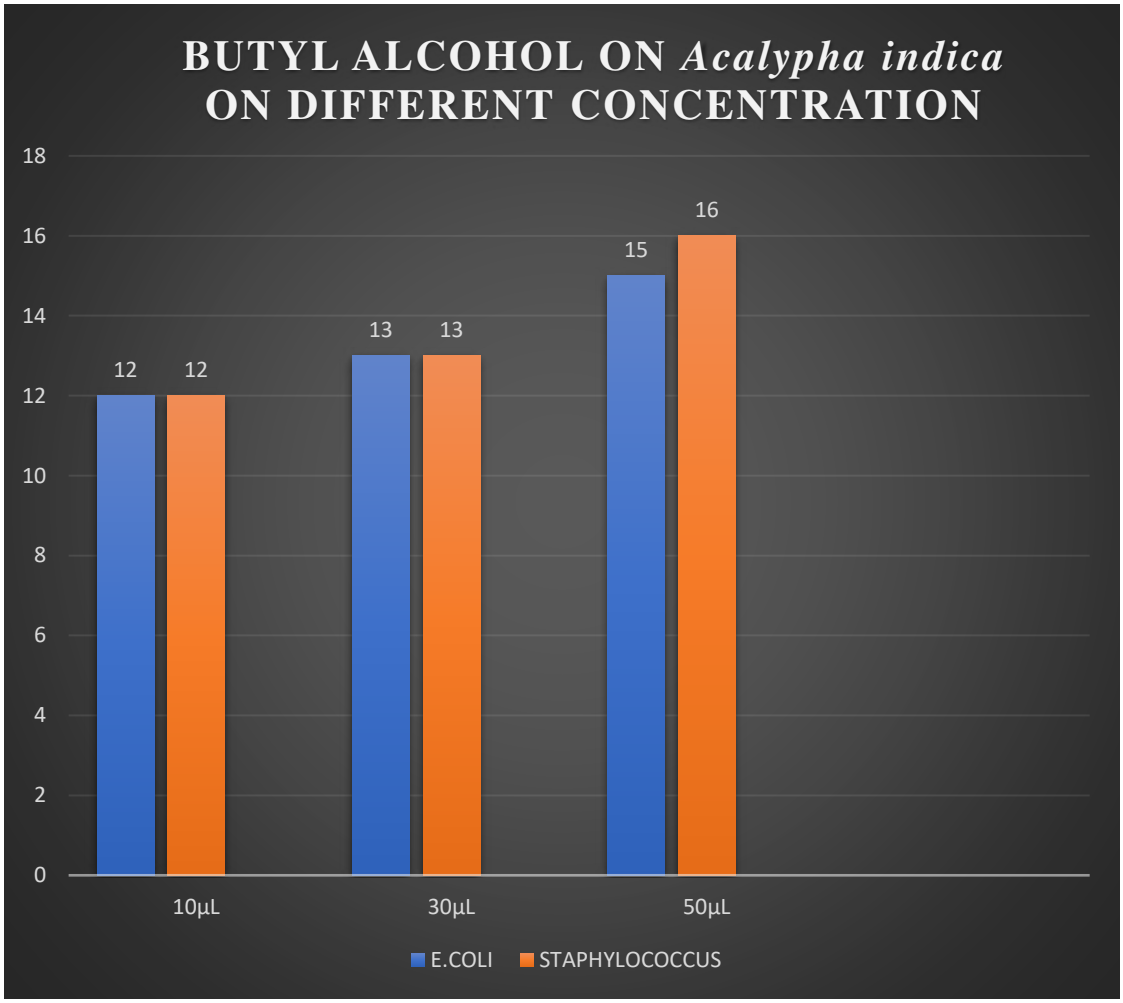

FIG 11: BUTYL ALCOHOL ON *Acalypha indica* ON DIFFERENT CONCENTRATION

TABLE 6:

DISTILLED WATER EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION:

MICROBIAL CULTURE	CONCENTRATION OF PLANT EXTRACTION		
	10µl	30µl	50µl
Staphylococcus aureus	10mm	11mm	13mm

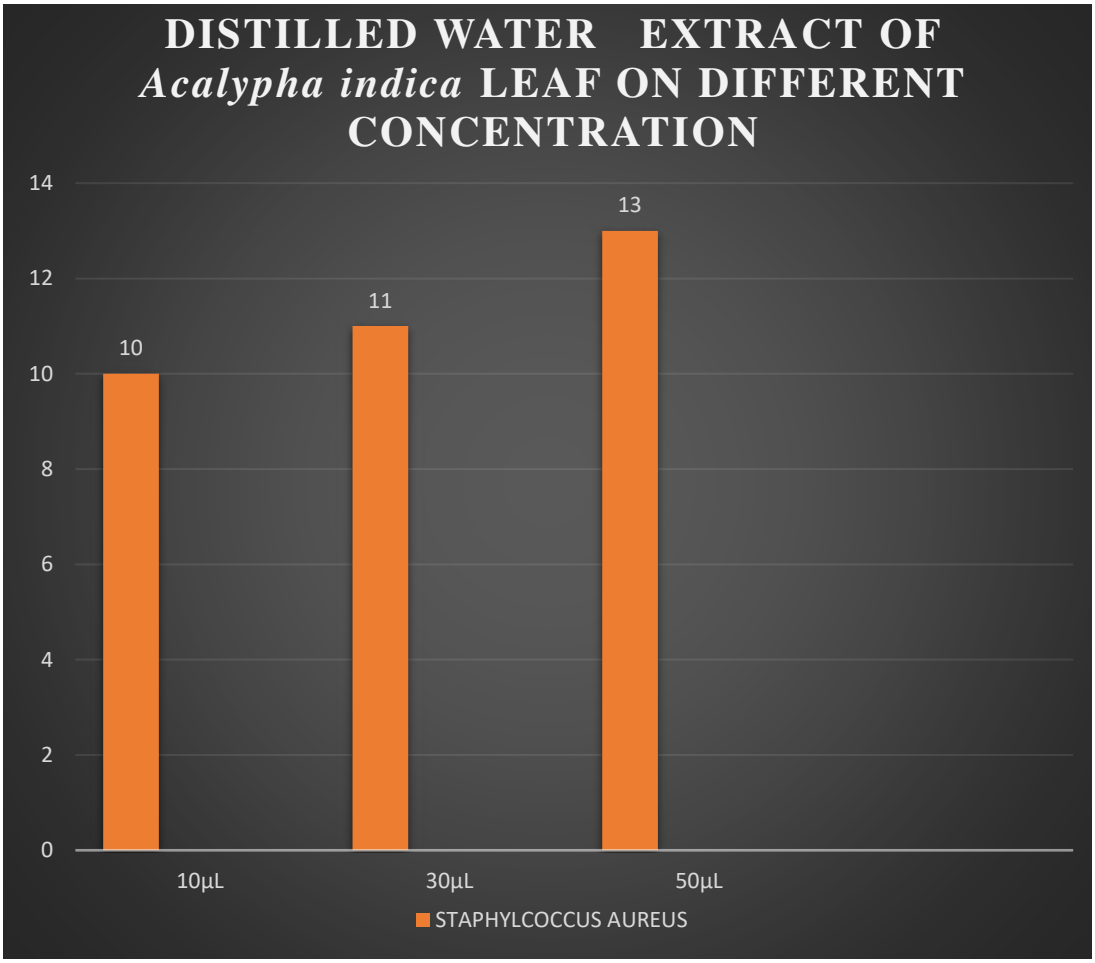

FIG 12: DISTILLED WATER EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION

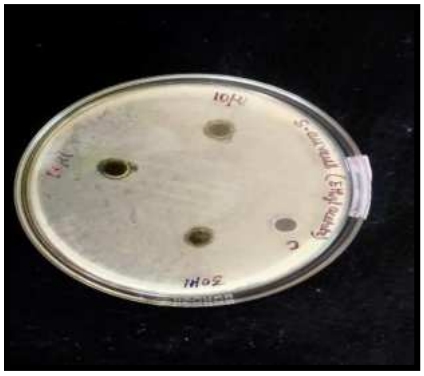

PLATE 2: ***Staphylococcus aureus***

(ETHYL ACETATE EXTRACT)

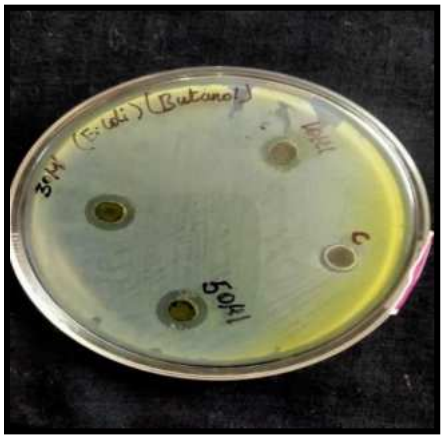

PLATE 3:

Escherichia coli

(BUTYL ALCOHOL)

PLATE 3.1:

Staphylococcus aureus

(BUTYL ALCOHOL)

PLATE4: ***Staphylococcus aureus***

(DISTILLED WATER EXTRACT)

Antifungal activity:

The pharmacological activity of the ethanol and ethyl acetate extract of *Acalypha indica* for its antifungal activity against pathogenic fungi. Six different fungal isolates viz., *Candida albicans, Candida glabrata, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* and *Penicillium chrysogenum* were tested for its antifungal activity. The collected leaf samples were powdered and the bioactive compounds were extracted by using ethanol and ethyl acetate. The antifungal activity was determined by using Well diffusion method. Ethanol and ethyl acetate extracts with different concentrations (100 mg/ml, 200 mg/ml and 300 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. The inhibitory effect of ethanol extract was relatively high when compared to ethyl acetate extract. The extract of *Acalypha indica* showed zone of inhibition against fungal pathogens when compared to *Acalypha indica*. (Siva Sakthi et al., 2011). The present study shows the extraction of *Acalypha indica* was evaluated in different concentration (10µl, 30µl and 50µl) for antifungal activities against *Rhizopus* and *Penicillium* indicating the difference zone of Inhibition. our results revealed that Ethyl Acetate, Butyl alcohol and Distilled water of *Acalypha indica* show signification higher inhibitory activities against *Rhizopus* and *penicillium.*The Butyl alcohol extract of *Acalypha indica* shows the maximum zone of inhibition 19mm against *Penicillium.* The Ethyl acetate extract of *Acalypha indica* shows the maximum zone of inhibition 12mm against *Penicillium* and 12mm against *Rhizopus.*

TABLE 7:

ETHYL ACETATE EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION:

MICROBIAL CULTURE	CONCENTRATION OF PLANT EXTRACTION		
	10µl	30µl	50µl
Penicillium	9mm	11mm	12mm

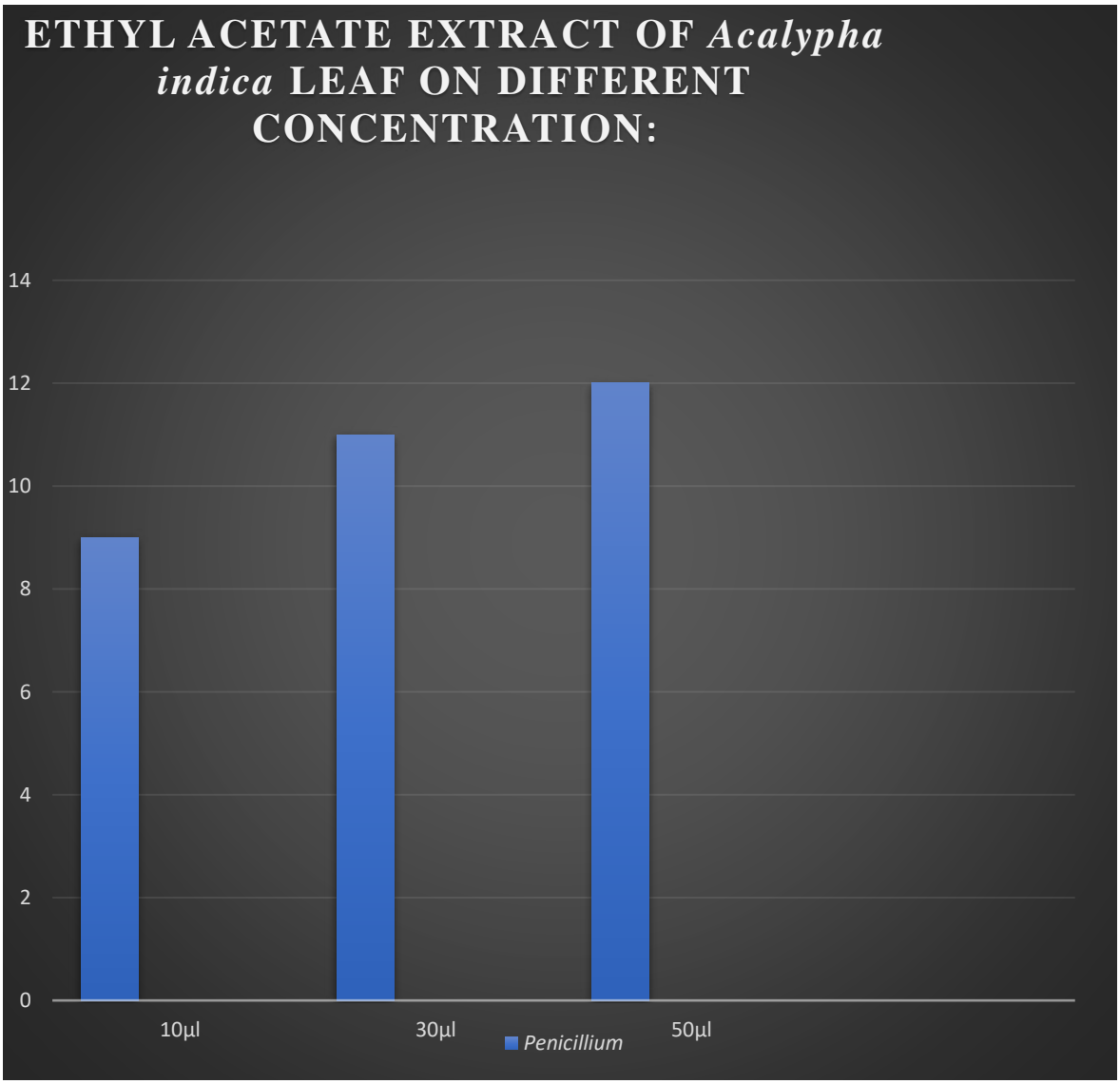

FIG 13: ETHYL ACETATE OF *Acalypha indica* ON DIFFERENT CONCENTRATION

TABLE 8:

BUTYL ALCOHOL EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION:

MICROBIAL CULTURE	CONCENTRATION OF PLANT EXTRACTION		
	10µl	30µl	50µl
Rhizopus	7mm	9mm	10mm
Penicillium	10mm	11mm	13mm

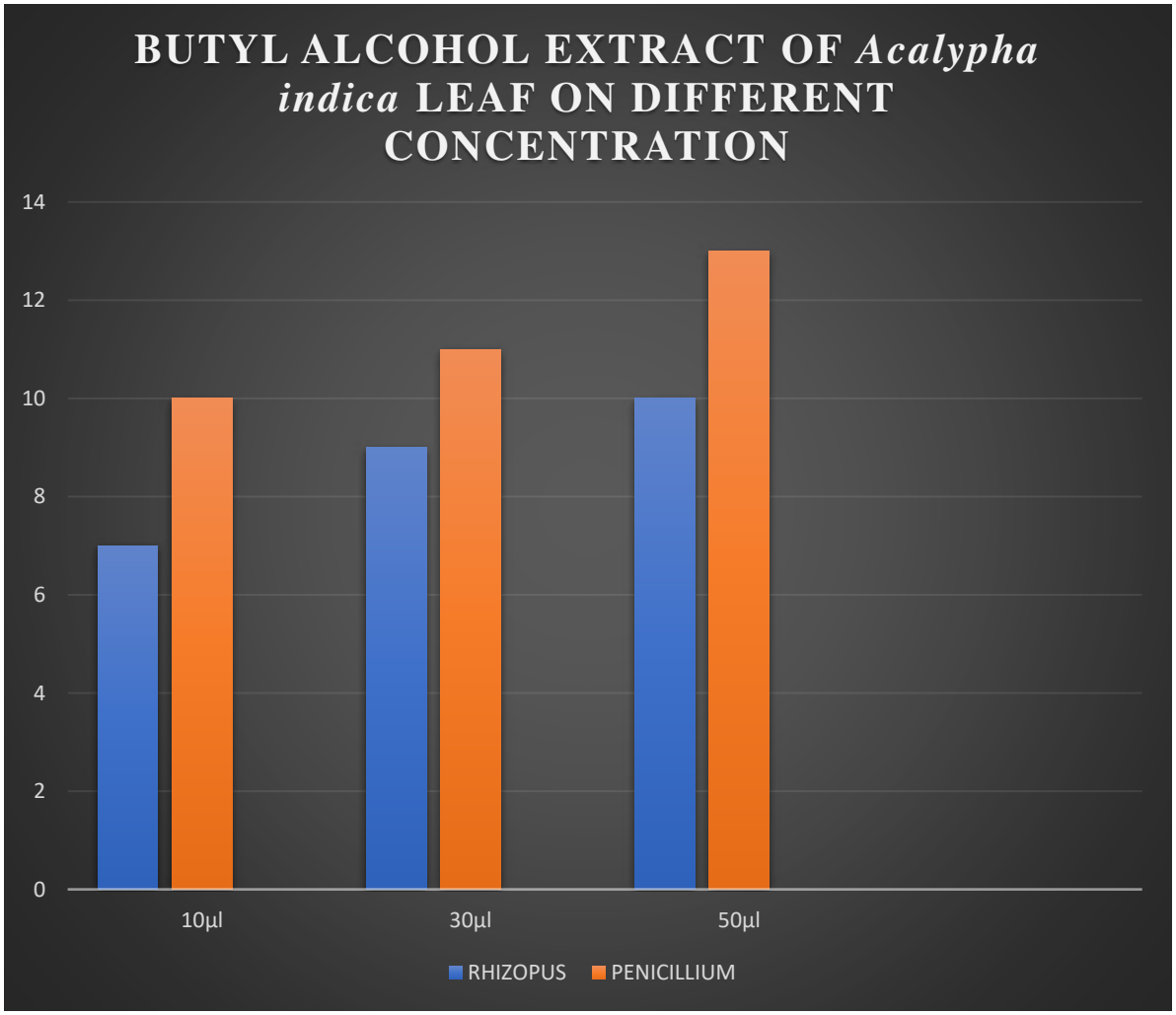

FIG 14: BUTYL ALCOHOL EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION

TABLE 9:

DISTILLED WATER EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION:

MICROBIAL CULTURE	CONCENTRATION OF PLANT EXTRACTION		
	10µl	30µl	50µl
Rhizopus	9mm	10mm	12mm

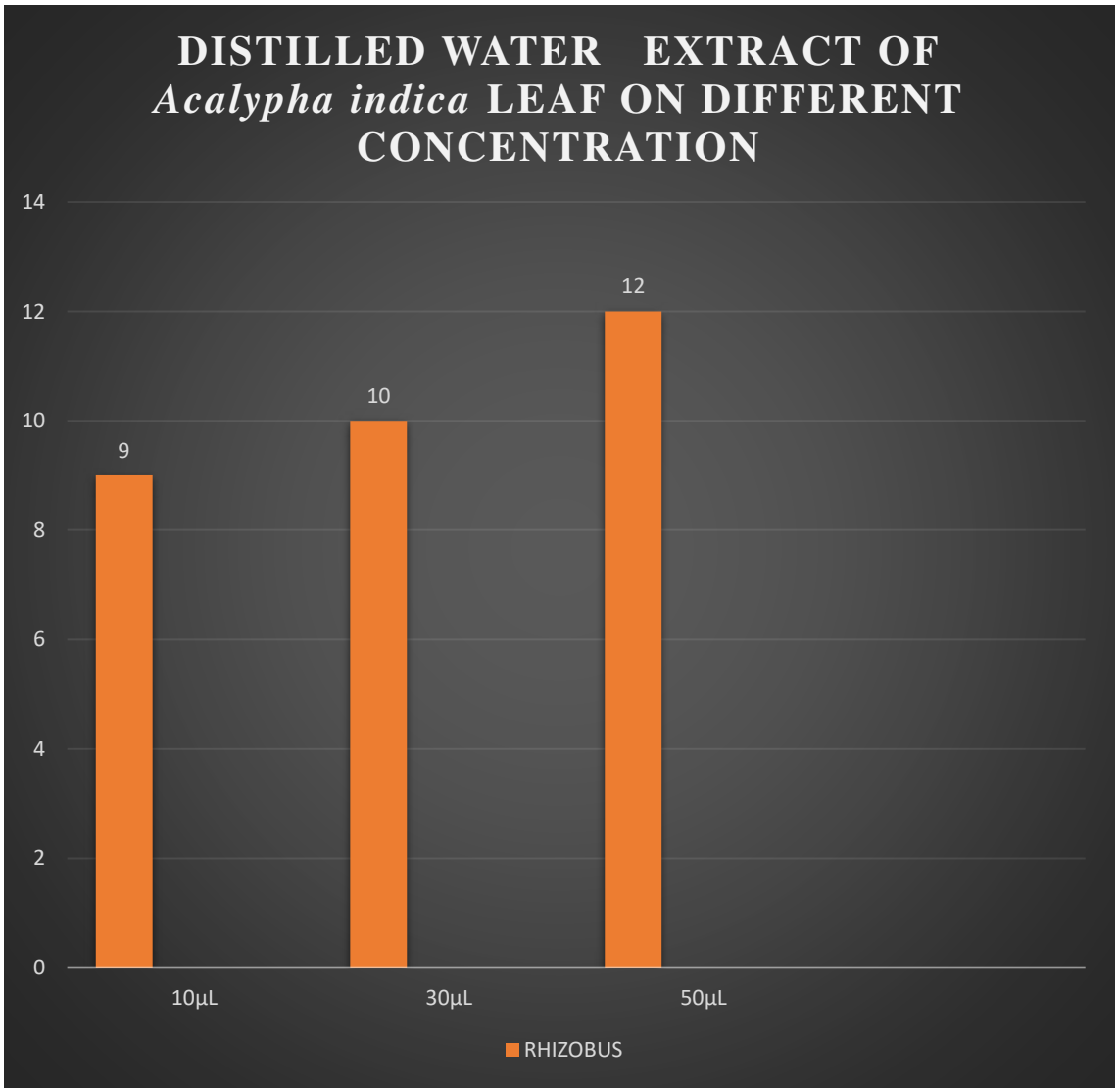

FIG 15: DISTILLED WATER EXTRACT OF *Acalypha indica* LEAF ON DIFFERENT CONCENTRATION

PLATE 5: *Penicillium* (ETHYL ACETATE EXTRACT)

PLATE6: *Rhizopus* (BUTYL ALCOHOL EXTRACT) EXTRACT)

PLATE6: *Penicillium* (BUTYL ALCOHOL

PLATE 7: *Rhizobus* (DISTILLED WATER EXTRACT)

ANTIHELMINTHIC ACTIVITY

The leaves extracts of *Acalypha indica* displayed a significant anthelmintic activity ($p<0.05$) in dose dependent manner. The anthelmintic activity of both the ethanolic and aqueous extracts was comparable with that of standard drug at 100mg/ml. The predominant effect of Piperazine citrate on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Both the extracts demonstrated paralysis (Aqueous 12 min, Ethanol 10 min) as well as death (Aqueous 32 min, Ethanol 29 min) of worms at a time comparable to Piperazine citrate * (P 08 min and D20 min) especially at higher concentration of 100 mg/ml. (Garai Ranju et al., 2011).

The present study showed that the Ethyl Acetate, butyl alcohol and distalled water of *Acalypha Indica* leaves exhibits Antihelminthic activities extraction dependent manner. The Ethyl acetate extraction of *Acalypha indica l*eaves at 0.1g/ml caused paralysis 5 minute and death in 7minutes against earth worm. The butyl alcohol extraction of *Acalypha indica* leaves at 0.1g/ml caused paralysis 4minute and death in 6 minutes against earth worm. The distilled water extraction of *Acalypha indica* leaves at 0.1g/ml caused paralysis 61minute and death in 80 minutes against earthworm. The Albendozole solution of at 0.01g/ml caused paralysis 200 minutes and death in 210 minutes against earthworm.The distilled water caused no death. *Acalypha indica* extraction is more powerful than albendozole.

TABLE 10

ANTIHELMINTIC ACTIVITY ON *Acalypha indica* ON DIFFERENT EXTRACTION:

S.NO	DIFFERENT EXTRACTION	PARALYSIS (in min)	DEATH (in min)
1	NEGATIVECONTROL (DISTILLED WATER)	0	0
2	POSITIVECONTROL (ALBENDAZOLE)	200	210
3	ETHYL ACETATE	5	5
4	BUTYL ALCOHOL	7	8
5	DISTILLED WATER	60	80

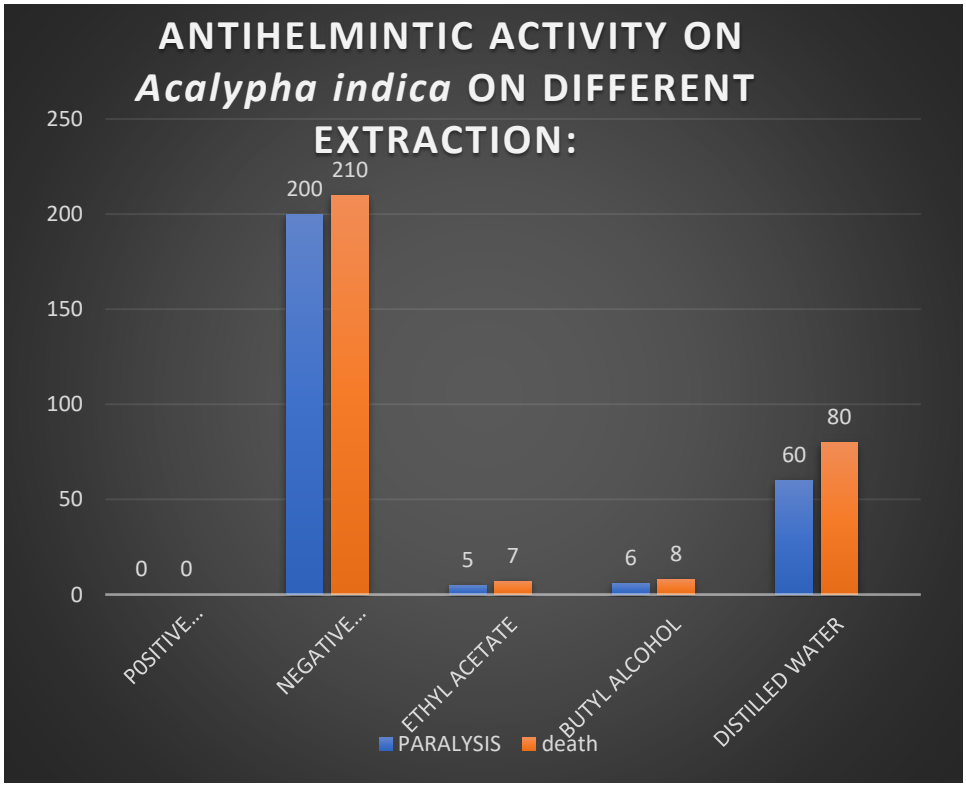

FIG 16: ANTIHELMINTIC ACTIVITY ON *Acalypha indica* ON DIFFERENT EXTRACTION:

PLATE8: *Eisenia fetida*

(NEGATIVE CONTROL

– DISITILLED WATER)

PLATE9: *Eisenia fetida*

(POSITIVE CONTROL

-ALBENDOZOLE)

PLATE 10: *Eisenia fetida*

ETHYL ACETATE EXTRACT

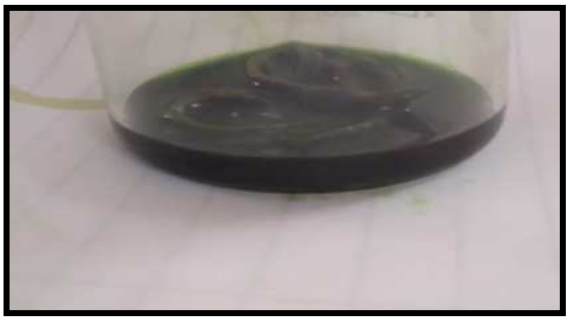

PLATE 11: *Eisenia fetida*

(BUTYL ALCOHOL EXTRACT)

PLATE12: *Eisenia fetida*

(DISTILLED WATER EXTRACT)

DPPH Radical Scavenging Activity

In vitro antioxidant assay of MPE revealed the presence of antioxidant potential. The percentage of inhibition was observed in all the antioxidant models that free radicals were scavenged by the extract in a concentration dependent manner up to the given concentration. The percentage inhibition of scavenging activities of the MPE for DPPH in Ethyl Acetate showed 45.30% DPPH inhibition at 400 μg/mL concentrations. Antioxidant activity depends on the presence of amount of total polyphenolic compounds (Figure)

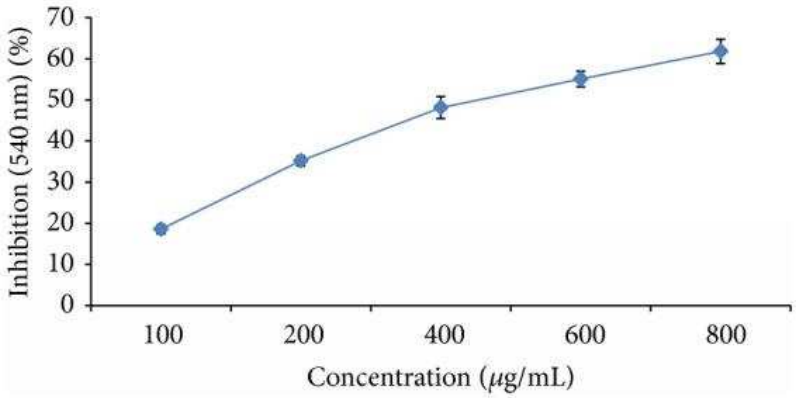

FIG 17: scavenging activities of the MPE for DPPH in ethyl acetate

In vitro antioxidant assay of MPE revealed the presence of antioxidant potential. The percentage of inhibition was observed in all the antioxidant models that free radicals were scavenged by the extract in a concentration dependent manner up to the given concentration. The percentage inhibition of scavenging activities of the MPE for DPPH in Butyl alcohol and Distilled water showed 60.30% & 35.02 DPPH inhibition at 0.2 μg/mL and 0.6 μg/mL concentrations. Antioxidant activity depends on the presence of amount of total polyphenolic compounds (Figure)

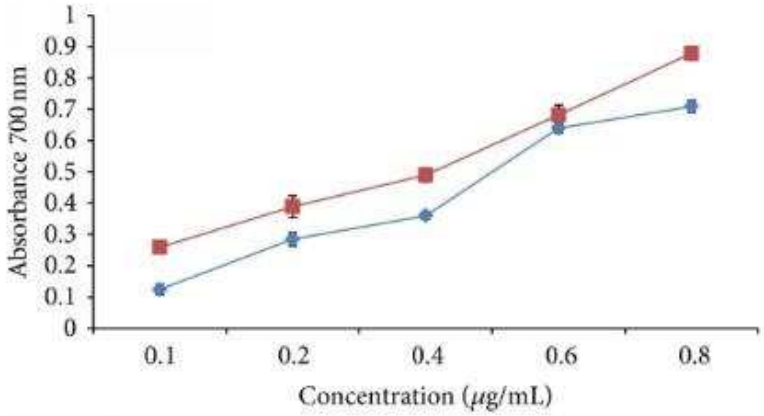

FIG 18: Scavenging activities of the MPE for DPPH in butyl alcohol and distilled water.

PREPARATION OF SOAP

Acalypha indica leaves were collected. The leaves were washed with running water 3-4 times. The leaves were blended with water. The mixture was strained and the juice was extracted. Next the soap base was melted using double boil method. The leaf juice was added with the soap and boiled well. Some fragment essential oil was added for good smell. Poured it in the soap mould. Allow to cooled. After the hardening of the soap, it is ready to use.

FIG 19: *Acalypha indica* soap

SUMMARY:

The present study was carried out using a weed, *Acalypha indica* to determine its potent biological ability to fight against various disease. And this study was designed to check the antimicrobial, antioxidant, anthelminthic activity of *Acalypha indica* leaves. Among this which has the comparatively high potential to fight against pathogenic microbes. The phytochemical analysis was carried out using leaf extract in different solvent such as butyl alcohol, ethyl acetate and distilled water. And the results shows that the presence of alkaloids, sterols, phenols and carbohydrates.

The comparative antimicrobial activity of the acalypha indica plants extracts were tested against test microorganisms (Bacteria – *Escherichia coli* and *staphylococcus,* Fungi – *Rhizopus* and *Penicillium*) in extract were inoculated in these different concentrations such as 10µl, 30µl, 50µl. In this plant leaf extract shows maximum to moderate zone of inhibition. From this we concluded that these medicinal plants have ability to inhibit the test microbes. The present study justified the importance of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes.

The anthelminthic of *Acalypha indica* plant extract were tested against earthworm (*Eisenia fetida*). The ethyl acetate, butyl alcohol and distilled water extract were prepared from the dried powder leaf in the form of 0.1g/ml. Albendazole is used as a negative control in the form of 0.01g/ml. Distilled water is used as a positive control. Note the time when it is paralysed and death.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reaction can from free radicals and these start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reaction.

In this study the DPPH is treated with the sample and place it in the dark room for 60 mins and observed on the spectrophotometer. And the hydroxyl radical scavenging assay was assessed by the method reaction mixture composed *Acalypha indica* solvent, it was incubated with deoxyribose, H_2O, $Fecl_3$, EDTA, and ascorbic acid, in potassium phosphate buffer for 60 minutes at 37. The reaction was terminated then adding 1ml of TBA and 1 ml TCA. The tubes are heating in boiling water bath for 15 min. The absorbance of the reaction mixture was measured.

CONCLUSION

Plants are a great source of medicinal property and it can be used widely to treat and cure lot of human diseases and ailments. Since we are in need of new medicines to treat the emerging diseases globally, medicinal plants can be effectively used to formulate pharmaceutical products. India is a country with rich diversity of flora and they are used as medicine in Indian traditional food varieties also. This present study entitled "Exploring the potent biological activity of *Acalypha indica* leaves, a common weed" suggests that the common weed *Acalypha indica* has a lot of potential biological activity in it. *Acalypha indica* is a very common weed we can see everywhere. Owing to its abundance in nature it can be used to formulate lot of medications. The plant contains kaempferol, a cyanogenetic glucoside, a base, triacetonamine and an alkaloid, acalyphine. The plant is reported to have a post-coital antifertility effect, antivenom properties, wound healing effects, antioxidant activities, anti-inflammatory effects acaricidal effects, diuretic effects and anti-bacterial activities.

This plant shows biological activities such as presence of phytochemicals. The phytochemical analysis of leaves of *Acalypha indica* in ethyl acetate and butyl alcohol extract showed the presence of carbohydrates, phenol and phytosterol and distilled water extract showed the presence of carbohydrate and phenol. The antibacterial activity was done with *Escherichia coli* and *Staphylococcus aureus.* The ethyl acetate extract showed the inhibition against *Escherichia coli.* The butyl alcohol extract showed inhibition against *Escherichia coli* and *Staphylococcus aureus.* The distilled water extract showed inhibition against the *Staphylococcus aureus.* The antifungal activity done with *Rhizopus* and *Penicillium*. The ethyl acetate extract showed inhibition against *Penicillium.* The butyl alcohol extract showed the inhibition against *Rhizopus* and *Penicillium*. The distilled water extract showed inhibition against *Rhizopus.* The plant is having both anti-bacterial and anti-fungal activity.

The antihelminthic activity was done with *Eisenia fetida.* The ethyl acetate and butyl alcohol extract showed quick result. The distilled water extract took 80 mins to kill the earth worm. The negative control was distilled water. The positive control was albendazole. In vitro antioxidant assay of MPE of the 3 extracts of *Acalypha indica* revealed the presence of antioxidant potential. The percentage inhibition of scavenging activities of the MPE for DPPH in Ethyl Acetate showed 45.30% DPPH inhibition at 400 μg/mL concentrations. The percentage inhibition of scavenging activities of the MPE for DPPH in Butyl alcohol and Distilled water showed 60.30% & 35.02 DPPH inhibitions at 0.2μg/mL and 0.6 μg/mL concentrations respectively. Also a herbal soap was prepared from this plant intended to be used externally.

We conclude this study by the quote by 'Robin Rose Bennet', "Mother Earth's medicine chest is full of healing herbs of incomparable worth". *Acalypha indica* also called as 'Kuppaimeni' is also a weed of incomparable worth to mankind. Many herbal formulations both for internal and external use can be prepared from golden plant to meet the demand of the growing global population.

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ANTIBACTERIAL, ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *HIBISCUS ROSA-SINENSIS* AND FORMULATION OF HERBAL SOAP

A Dissertation Submitted to

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI.

Affiliated by Manonmaniam Sundaranar University,

in partial fulfillment of the requirements for the award of degree of

BACHELOR OF SCIENCE IN MICROBIOLOGY

Submitted by

P.PRINCY	-18SUMB29
P.RAMA LAKSHMI	-18SUMB30
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APRIL-2021

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled. **"ANTIBACTERIAL. ANTIDANDRUFF AND ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *HIBISCUS ROSA-SINENSIS* AND FORMULATION OF HERBAL SOAP"** is a bonafide record of the original work completed by P. Princy (Reg No: 18SUMB29), P. Rama Lakshmi (Reg No: 18SUMB30), S. Rama Pushpam (Reg No: 18SUMB31), S. Rinolaa (Reg No: 18SUMB33), S. Salomi Sellammal (Reg No: 18SUMB34) as a group project during the academic year 2020 – 2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirements for the award of the degree of BACHELOR OF SCIENCE IN MICROBIOLOGY prescribed by the Manonmaniam Sundaranar University.

A. Alaiyan 09/04/2021

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Signature of the Guide

Signature of the H.O.D

Signature of the Director

Signature of the Principal

Signature of External Examiner

DECLARATION

Hereby we declare that the project entitled "**ANTIBACTERIAL, ANTIDANDRUFF AND ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *HIBISCUS ROSA-SINENSIS* AND FORMULATION OF HERBAL SOAP**" is a bonafide record of the original work completed by us during the academic year 2020-2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as the partial fulfilment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by the Manonmaniam Sundaranar University. We also affirm that this is an original work done by us under the supervision of **A. Maria Heartina Adlin Vaz M.Sc., SET**, Assistant professor, Department of Microbiology, St. Mary's college (Autonomous), Thoothukudi.

Signature of the Students Signature of the Guide

Place: Thoothukudi

Date :

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ABBREVIATION

ft - Feet

in - Inch

cm - Centimeter

bp - Base pair

RNA Ribo nucleic acid

gm- Gram

ml- Milliliter

HCl- HydroChloric acid

w/v -Weight by Volume

mm- Millimeter

µl- Micro liter

DPPH- 2,2-diphenyl-1-picrylhydrazyl

BHA- beta hydroxy acid.

TBA -Tertiary butyl alcohol

TCA - Tricholoroacetic acid

MPE- Maximum Permissible Number

AHA - Alpha Hydroxy Acids

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from natural source, many based on their use in traditional medicines or phytomedicines. Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for aliments of both microbial and non-microbial origins. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Some antibiotics have become almost archaic because of drug resistant and consequently new drugs must be sought, for which herbal treatment is one possible way to treat diseases caused by multi drug resistant bacteria.

It is well known that plants, through lacking the typical immune response, have an in-built system for production against biotic and abiotic, stress conditions. Since plants have co-evolved with pathogens, they understandably have also developed the chemical protection pathways against the parasitic organisms. Therefore, it is reasonable to expect a verity of plant compounds with specific as well as general antimicrobial activity and antibacterial potential. The plants *Hibiscus rosa-sinensis* (*H. rosa- sinensis*) belongs to the family Malvaceae. Traditionally the flowers can be used as anti asthmatic agents. Many chemical constituents such as cyanidin, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin and ascorbic acids have been isolated from this plant. Resistance towards reveling antibiotics having become widespread among bacteria and fungi, new class of antimicrobial substances are urgently required. There are several studies which reveal the presence of such compounds with antimicrobial properties in various plant parts. The petals have some protective mechanism against microbial attack in most of the plants. The *H. rosa-sinensis* flower petals of a large number of plant species growing in the vicinity of our environment were screened for their antibacterial activity (Ruban *et al.,*2012).

Nature has bestowed us with rich botanical wealth and large number of diverse type of plants grown in different parts of country (Vastrad *et al.,*2014). Plants are the source of great economic value all over the world.

Plants range in size from diminutive duck weeds only a few millimetres in length to the giant sequoias of California that reach 90 meters (300 feet) or more in height. There are an estimated 390,900 different species of plants known to science, and new species are continually being described, particularly from previously unexplored tropical areas of the world. Plants evolved from aquatic ancestors and have subsequently migrated over the entire surface of Earth, inhabiting tropical, Arctic, desert, and Alpine regions. Some plants have returned to an aquatic habitat in either fresh or salt water (Vivek Abhinev, 2013).

Plants are autotrophs; they produce their own food. They do so via photosynthesis, which is the process of making nutrients such as sugars from light energy and carbon dioxide. Photosynthesis occurs in cell organelles called chloroplasts, which contain chlorophyll and carotenoids, molecules that absorb light energy and change it into a usable form. Heterotrophs, on the other hand, are organisms that cannot make their own food and must eat other organisms to survive. Many heterotrophs eat plants. Other heterotrophs eat animals that have eaten plants. Plants are primary producers in many ecosystems, giving them a vital role in the survival of many other organisms. In addition, oxygen is a byproduct of photosynthesis, and many organisms depend on oxygen to survive. We couldn't live without plants (BD editors, 2017).

Traditionally, several plants and their products have been used in foods (as herbs or spices) as a mode of natural preservative, flavoring agent as well as a remedy to treat some of the common ailments in humans. This property of curing is attributed mainly to their antimicrobial activities. Use of natural plant derived antimicrobials can be highly effective in reducing the dependence on antibiotics, minimize the chances of antibiotic resistance in food borne pathogenic microorganisms as well as help in controlling cross-contaminations by food-borne pathogens (Voon *et al.,* 2012). In addition to the antioxidant and antimicrobial activities exhibited by plants or their extracts, they can also be used as natural colorants of foodstuffs; as in most of the cases, they are believed to be safe, and non-toxic to humans (Rymbai *et al.,* 2011).

Plant, (kingdom Plantae), any multicellular eukaryotic life-form characterized by

1. Photosynthetic nutrition (a characteristic possessed by all plants except some parasitic plants and underground orchids), in which chemical energy is produced from water, minerals, and carbon dioxide with the aid of pigments and the radiant energy of the Sun,
2. Essentially unlimited growth at localized regions,
3. Cells that contain cellulose in their walls and are therefore to some extent rigid,
4. The absence of organs of locomotion, resulting in a more or less stationary existence,
5. The absence of nervous systems, and
6. Life histories that show an alteration of haploid and diploid generations, with the dominance of one over the other being taxonomically significant.

Flowering plant (PHYLUM ANGIOSPERMOPHYTA) are divided into two classes monocotyledons (class Monocotyledoneae) and dicotyledons (class Dicotyledoneae) . Typically monocotyledons have seeds with one cotyledon (seed leaf) ; their foliage leaves are narrow with parallel veins; the flower components occur in multiples of three; sepals and petals are indistinguishable and are known as tepals; vascular (transport) tissue are scattered in random bundles throughout the stem ; and , since they lack stem cambium (actively dividing cells that produce wood) most monocotyledons are herbaceous. Dicotyledons have seeds with two cotyledons; leaves are broad with a central midrib and branched veins; flower parts occur in multiplies of four or five; sepals are generally small and green; petals are large and colourful ; vascular bundles are arranged in a ring around the edge of the stem; and, because many dicotyledons possess wood- producing stem cambium, there are woody forms as well as herbaceous ones. Example: *Hibiscus rosa-sinensis.*

The plants *Hibiscus rosa-sinensis* belongs to the family Malvaceae. With attractive and colorful flowers, plants of Hibiscus are widely planted as ornamentals and are used in traditional medicine. Hibiscus species have been used as a folk

remedy for the treatment of skin diseases, as an anti fertility agent, antiseptic and carminative. *Hibiscus rosa-sinensis* possesses many biological activities such as antipyretic, analgesic and anti-inflammatory activities. It has also been reported that the plant's flower possesses anti - spermatogenic, androgenic, anti-tumor and anticonvulsant properties, in addition, the leaves and flowers have been found to be aid in the healing of ulcers. Infusion of the petals is given as refrigerant and demulcent.

Kingdom **Plantae** – Plants

Sub kingdom **Tracheobionta** – Vascular plants

Super division **Spermatophyta** – Seed plants

Division **Magnoliophyta** – Flowering plants

Class **Magnoliopsida** – Dicotyledons

Subclass **Dilleniidae**

Order **Malvales**

Family **Malvaceae** – Mallow family

Sub family**Malvoideae**

Genus **Hibiscus L.** – rose mallow

Species **Hibiscus rosa-sinensis L.** – shoe black plant

Hibiscus rosa-sinensis is a bushy, evergreen shrub or small tree growing 2.5–5 m (8–16 ft) tall and 1.5–3 m (5–10 ft) wide, with glossy leaves and solitary, brilliant red flowers in summer and autumn. The 5-petaled flowers are 10 cm (4 in) in diameter, with prominent orange-tipped red anthers. The flowers are large, conspicuous, trumpet-shaped, with five petals and their colors can be white to pink, red, orange, peach, and yellow or purple that are 4–18 cm broad. The flowers from various cultivars and hybrids can be either a single flower or a double flower.

At the bottom of every hibiscus bud is the calyx which is green in color. The pointed ends of the calyx are the sepals. When the hibiscus begins to bloom, the petals begin to grow which contains multiple petals and multiple colors. The ovary and other female parts of the flower lie in the main structure of the hibiscus, the pistil, which is long and tubular. The hibiscus has both male and female parts on the same flower. The five hairy red spots on the top of the flower is the stigma (female part) of the flower. The stigma is located at the end of the style branch. At the top of the pistil is known as the stigma, where pollen is collected, and in the middle is the style, which is the section that the pollen travels down to the ovary. The ovary lies at the bottom of the blossom and the hibiscus has only one ovary which is superior.

The male part (stamen) of the flower consists of stem-like filaments and each filament ends with the pollen-producing anther. The anthers, which release the pollen, sits on the filament and these two organs make up the stamen, the male part of the flower. Together, these organs make up the male part of the flower known as the stamen. The hibiscus has hundreds of stamens.. Overall, the hibiscus is a dicot, with solitary (axillary), complete, perfect flowers, which have a superior ovary, regular symmetry, and axile placentation. They have five carpels, five locules, five sepals, and the number of stamens may vary (Royal Horticultural Society, 2020).

Rosa-sinensis chloroplast genome was 160,951 bp, comprising of large single copy (89,509 bp) and small single copy (20,246 bp) regions, separated by IRa and IRb (25,598 bp each). The genome contained 130 genes including 85 protein-coding genes, 37 transfer RNAs and 8 ribosomal RNAs. Comparative analyses of chloroplast genomes revealed similar structure among 12 species within family Malvaceae.

Evolutionary rates of 77 protein-coding genes showed 95% similarities. Analyses of codon usage, amino acid frequency, putative RNA editing sites, and repeats showed a great extent of similarities between *Hibiscus rosa-sinensis* (Jheng *et al.,* 2012).

There may be some competition in the niche that the *Hibiscus rosa-sinensis* occupies. The *Hibiscus rosa- sinensis* is a plant that has secondary growth, meaning that it grows out instead of up. This may cause a problem for other plants in the niche. They may have to compete for space, nutrients, and sunlight with the *Hibiscus rosa- sinensis.* The plant may deprive many young trees from having the ability to develop in the tropical regions because the *Hibiscus rosa-sinensis* spreads anywhere from five to eight feet in width making it hard for the young trees to establish a root system. However, the *Hibiscus rosa-sinensis* may benefit some forms of ground cover that require shade to successfully grow. The prevalence of the *Hibiscus rosa-sinensis* may affect the carrying capacity on organisms such as the aphids, mites, and white flies. Even though white flies, aphids, and mites feed on other organisms, the decrease in population of the *Hibiscus rosa-sinensis* will affect these organisms drastically. If there is decrease in the *Hibiscus rosa-sinensis* when there is a relatively high population of these organisms it can lead to a major die off of the organism.

Hibiscus is easily grown over a wide range of conditions. Their diverse habitats range from wetlands to savannahs and woodlands. The genus is widely distributed throughout the north temperate zone. Their habitats are varied, ranging from cold and montane regions to the grassy slopes, meadow-lands and river bank of Europe, the middle east and northern Africa, Asia and across North America (Mohana Chandran). *Hibiscus rosa-sinensis* is the national flower of Malaysia, called Bunga Raya in Malay. Introduced into the Malay Peninsula in the 12th century, it was nominated as the national flower in the year 1958 by the Ministry of Agriculture amongst a few other flowers, namely ylang ylang, jasmine, lotus, rose, magnolia, and medlar. On 28 July 1960, it was declared by the government of Malaysia that *Hibiscus rosa-sinensis* would be the national flower (Ramesh menon, 2015).

Local/ Vernacular name in India: (Jadhav *et al*., 2009)

Tamil : Semparutti

Hindi : Jasum

English : Chinese Hibiscus

Gujarat : Jasvua

Sanskrit : Japa

Orissa : Mondaro.

Telugu : Dasanamu

Name in various countries:

Arabic : Angharachindi

Burma : Kaungyan

China : Hong can

French : Rose de china

The *Hibiscus rosa-sinensis*, like all angiosperms is sporophyte dominant, meaning that the multicellular diploid is the most prevalent. The life cycle for the *Hibiscus rosa- sinensis* follows the alternation of generations. Which means the sporophyte undergoes meiosis to produce haploid cells. The haploid cell then develops into a multicellular haploid, which is called the gametophyte. The gametophyte then undergoes mitosis to produce gametes. One of the gametes is then fertilized and forms a zygote. The zygote undergoes mitosis to produce what we see as the flower or the sporophyte portion of the plant.

The *Hibiscus rosa- sinensis* is a bisexual plant, meaning that the plant contains both male and female reproductive anatomy. The male reproductive structure is referred to as the stamen and the female reproductive structure is referred to as the ovary. If a part of the stamen is removed as well as the petals of the flower, the flower cannot take part in any form of genetic crosses with other species of the *Hibiscus rosa- sinensis*. The pollen on the *Hibiscus rosa- sinensis* has a high fertility rate of over 60%. The stalks of the *Hibiscus rosa- sinensis* only contain one flower. The *Hibiscus rosa-sinensis* is also heterosporous, meaning that the plant contains

microspores and megaspores that are produced via meiosis. The microspores make male gametophytes and the megaspores make female gametophytes.

The *Hibiscus rosa-sinensis* is an angiosperm meaning that it has a seed covering. The seed covering on this plant tends to be dry and hard. When the plant produces fruit it tends to be in an oval shape. The flowers tend to be 4 to 8 inches and the plant flowers year around when it is in the right habitat. The *Hibiscus rosa-sinensis* requires frequent watering and a lot of fertilizer when it is first planted. The *Hibiscus rosa- sinensis* also requires full to partial sun light (Anderson,2007).

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades therez has been an exponential growth in the field of herbal medicine (Palanisamy Hariprasad *et al.,* 2011). Hibiscus, also called a Botox plant, is considered a powerful anti-aging plant with its astounding ability to hold in the activity of the enzyme called elastase. Elastase breaks down skin's elastin, which is responsible for helping the skin return to its original form after being pinched or poked. The herb *Hibiscus rosa-sinensis* Linn (Malvaceae) is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers . In medicine , however the red flowered variety is preferred. The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers. Flowers have been found to be effective in the treatment of arterial hypertension and to have significant anti-fertility effect (Kadam*et al.,*2009).

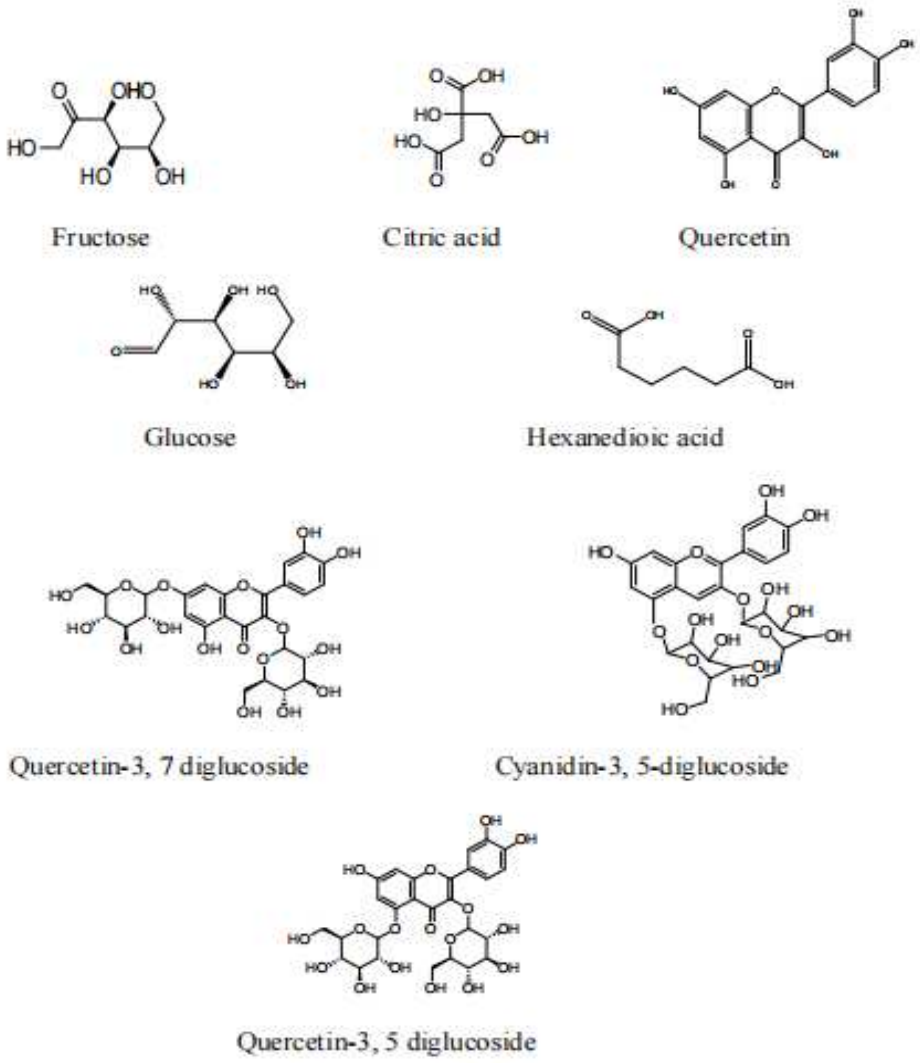
Hibiscus has been used by different cultures as a remedy for several conditions. Egyptians used hibiscus tea to lower body temperature, treat heart and nerve diseases, and as a diuretic to increase urine production. In Africa, tea was used to treat constipation, cancer, liver disease, and cold symptoms. Pulp made from the leaves was applied to the skin to heal wounds. In Iran, drinking sour tea is still a common treatment for high blood pressure. Today, hibiscus is popular for its potential to reduce high blood pressure. Modern studies show promise for both the tea and hibiscus plant extract to lower blood pressure and cholesterol levels. Although more research is still needed, this could be good news for the future of heart disease treatment. Hibiscus shows potential for cancer treatment and as a weight loss aid,

along with other uses. There aren't many studies in these areas, but some research Trusted Source suggests that anthocyanins may hold the key to hibiscus' anticancer properties.

It is used in traditional medicine for the treatment of skin disease, as an anti-fertility agent, antiseptic, carminative, antimicrobial, antioxidant, antiviral, hypotensive, depressant, anti-inflammatory, analgesic, antipyretic, antitumor, and anti-implantation. Similarly, flowers also have medicinal value as emollient, demulcent, refrigerant, and aphrodisiac and have cosmetic use to stimulate hair growth, prevent hair loss, premature graying, etc. Flower preparation proves the antibacterial potential of *H. rosa-sinensis* L. Many chemical constituents have been reported. Another recent study Trusted Source found that hibiscus extract might have an effect on metabolism, preventing obesity and fat buildup in the liver. The tropical plant has even been used successfully Trusted Source as part of an herbal extract mixture to treat head lice (Debra Rose Wilson, 2018).

Each part of *H. rosa-sinensis* contains a wide range of compounds. It was reported that phlobatannins, glycosides, saponins, flavonoids, terpenoids including other compounds such as thiamine, riboflavin and niacin are present in leaves, flowers, stem and roots. According to Patel and Adhav, whose their study was conducted on four different morphotypes of H. rosa sinensis, glucosides, flavonoids, phytosterols, terpenoids, tannins, and phenolic compounds contributed to the pharmacological effects of the plant as they were present in all of them. This suggested that although the flower color differed, the phytochemical constitions were very similar. These findings also correlates with those of another study carried out by thin layer chromatographic analysis .Generally, the edible flowers contain moisture, nitrogen, fat, crude fibre calcium, phosphorus, and iron. The yellow flowers contain several flavones such as cyanidin-3,5-diglucoside, cyaniding-3-sophoroside3-5- glucoside quercetin-3,5-diglucoside, and quercetin-3,7diglucoside. Including the mentioned compounds, kaempferol-3-xylosylglucoside isolate can be found in white flowers. In addition to fatty acids, fatty alcohols, hydrocarbons, the leaves also contain about 7.34 mg / 100 gm of carotene, as well as gentisic acid, mucilage, and catalase. On the other hand, cyclopropenoids can be found in root barks. Although flowers, stems, and

leaves contain minor amounts of cyanin and cyanidin chorides, quercetin can be found in all parts of *Hibiscus rosa sinensis*. However, ß-sitosterol, teraxeryl acetate, and malvalic acids can be found only in stems and leaves (Asmaa Missoum,2018).



Compounds present in flower of *Hibiscus rosa sinensis*

Phytochemicals produced in plants which are divided into two groups, namely primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while the secondary constituents consists of alkaloids, terpenoids and phenolic compounds and many more such as flavonoids, tannins and so on. Many secondary metabolites of plant are commercially important, responsible for pharmacological

activities and find use in a number of pharmaceutical compounds (Krishnaiah *et al.,* 2009).

Bacteria that cause bacterial infections and disease are called pathogenic bacteria. They cause diseases and infections when they get into the body and begin to reproduce and crowd out healthy bacteria or to grow into tissues that are normally sterile. To cure infectious diseases, researchers discovered antibacterial agents, which are considered to be the most promising chemotherapeutic agents. Keeping in mind the resistance phenomenon developing against antibacterial agents, new drugs are frequently entering into the market along with the existing drugs. In this chapter, we discussed a revised classification and function of the antibacterial agent based on a literature survey. The antibacterial agents can be classified into five major groups, i.e. type of action, source, spectrum of activity, chemical structure, and function (Hamid Ullah *et al.,*2017).

Antibacterial activity was evaluated by using disc and agar diffusion methods. The protein was run through polyacrylmide gel electrophoresis to view their protein profile (Ruban *et al.,* 2012). Antibacterial: Anything that destroys bacteria or suppresses their growth or their ability to reproduce (William *et al.,*2018).

Antibacterial as well as antiviral activity of a molecule is completely associated with the compounds that provincially kill bacteria and virus or slow down their rate of growth, without being extensively toxic to nearby tissues. Most recently discovered antimicrobial agents are modified natural compounds and this modification is done through chemical mode, for example, b-lactams (penicillins), carbapenems, or cephalosporin. Pure natural products, such as aminoglycosides, and other entirely synthetic antibiotics, for example, sulfonamides, are also frequently used. The antimicrobial agents could be classified as the agents that can either be bactericidal, which kill bacteria, or bacteriostatic, which slow down the growth of bacteria. Antibacterial agents are the most important in fighting infectious diseases. But, with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry. Resistance is most commonly based on developmental processes taking

place, for example, antibiotic therapy, that leads to inheritable resistance (Kushagri Singh *et al.,* 2019)

According to the U.S. Food and Drug Administration (FDA), there isn't enough science to show that over-the-counter (OTC) antibacterial soaps are better at preventing illness than washing with plain soap and water. To date, the benefits of using antibacterial hand soap haven't been proven. In addition, the wide use of these products over a long time has raised the question of potential negative effects on your health.

Antibacterial soaps (sometimes called antimicrobial or antiseptic soaps) contain certain chemicals not found in plain soaps. Those ingredients are added to many consumer products with the intent of reducing or preventing bacterial infection.

REVIEW OF LITERATURE

Vaishav *et al.,* (1993) reported that the extracts of the origin is to be able to condencer the blood Sugar stage via 30%.

Houghton *et al,* 1995) reported that an antibacterial is a compound or substance that kills or slows down the growth of bacteria.

Anderson*et al.,* (2007) identified the *Hibiscus rosa-sinensis* requires frequent watering and a lot of fertilizer when it first planted.The *Hibiscus rosa-sinensis* also requires full to the partial sun.

Mohana Chandran (2007) reported that the genus is widely distributed throughout the north temperate zone. Their habitats are varied, ranging from cold and mountain regions to the grassy slopes, meadow-lands and riverbank of Europe, the middle east and northern Africa, Asia, and across North America.

Varsha Jadhav *et al.,* (2009) suggested that flowers have been found to be effective in the treatment of articles hypertension and to have a significant anti fertility effect.

Krishnaiah D *et al.,* (2009) reported that many secondary metabolites of plants are commercially important,responsible for pharmacological activities and find use in a number of pharmaceutical compounds

Rymbai *et al.,*(2011) reported to in addition to the antioxidant and antimicrobial activities exhibited by plants or their extracts, they can also be used as natural colorants of food stuffs; as in most of the cases they are believed to be safe, and nontoxic to human's.

Ruban *et al.,* (2012) reported on *Hibiscus rosa-sinensis* flowers petals of large number of plants species growing in the vicinity of our environment were screened for their anti bacterial activity.

Dorling Kindersley (2012) reported that Dicotyledons have seeds with two cotyledons; leaves are broad with a central midrib and branched veins; flower parts occur in multiplies of four or five; sepals are generally small and green; petals are large and colorful; vascular bundles are arranged in a ring around the edge of the stem; and, because many dicotyledons possess wood- producing stem cambium, there are woody forms as well as herbaceous one.

Boo *et al.,* (2012) believed that the extracts of flowers to be safe, and non-toxic to humans.

Jheng *et al.,* (2012) reported that Evolutionary rates of 77 protein-coding genes showed 95% similarities.

Gupta *et al.,* (2012) reported that the antioxidant and antimicrobial activities exhibited by plants

Voon *et al.,* (2012) suggested the use of natural plants derived antimicrobial can be highly effective in reducing the dependence on antibiotics minimize The chances of antibiotics resistance in food borne pathogenic Microorganisms as well as help in controlling cross contamination by food borne pathogens.

Vivek Abhinav *et al.,* (2013) reported on plants evolved from aquatic ancestors and have subsequently migrated over the entire surface of Earth, inhabiting tropical, Arctic,desert, and Alpine regions.

Vastrd *et al.,* (2014) reviewed that plants range in size from diminutive duck weeks only a few millimeters in length to the giant sequoias of California that reach 90meters (3000feet)or more in height.

Ramesh Menon *et al.,* (2015) reported on declaration by the government of Malaysia that *Hibiscus rosa-sinensis* would be the National flowers.

Debra Rose Wilson *et al.,* (2018) reported on tropical plants has been used successfully as part of an herbal extracts mixture to treat head like.

Asmae Missoum *et al.,* (2018) identified that cyclopropenoids can be found in flowers, stems and leaves contains minor amounts of cyanine and cyanidine chloride, quercetin can be found in all parts of *Hibiscus rosa-sinensis.*

Kushari Singh *et al.,* (2019) reported that resistance is most commonly based on developmental processes taking place for example, antibiotics therapy, that leads to inheritable.

AIM AND OBJECTIVES

1. Collection and authentication of flower part of *Hibiscus rosa-sinensis.*
2. Extraction of the flower of Hibiscus species by cold extraction method.
3. Qualitative screening of phytochemicals and vitamins in butanolic, aqueous and chloroform flower extracts of *Hibiscus rosa-sinensis.*
4. Quantitative analysis of phytochemicals and vitamins in flower of Hibiscus species.
5. Antimicrobial activity in butanolic, aqueous and chloroform flower extracts of *Hibiscus rosa-sinensis*.
6. Antidandruff activity in butanolic, aqueous and chloroform flower extracts of *Hibiscus rosa-sinensis*.
7. Antioxidant activity in butanolic, aqueous and chloroform flower extracts of *Hibiscus rosa-sinensis*.

METHODOLOGY

Collection of plant materials:

The plant species namely *Hibiscus rosa-sinensis* were collected from the Millerpuram in Thoothukudi, Tamilnadu, India and from St. Mary's College (Autonomous) Botanical Garden, Thoothukudi, Tamilnadu, India.

Fig A: Collected Sample of *Hibiscus rosa-sinensis*

Preparation of plant powder:

The plant flowers were shade dried for about 25-30 days. Then the dried materials were grinded to fine powder using an electric grinder and stored in airtight bottles.

Fig B: Dried Sample of *Hibiscus rosa-sinensis*

Fig C: Powdered Sample of *Hibiscus rosa-sinensis*

Solvent extraction:

Dried flowers of *Hibiscus rosa-sinensis* were weighed and soaked in butanol, aqueous and chloroform, about 10g of flower extract powder was added to 100 ml of the solvent. After 48hrs of soaking, the powders with the solvents were filtered using

filter paper and glass funnel. After filtration, the extract was collected and stored for further analysis.

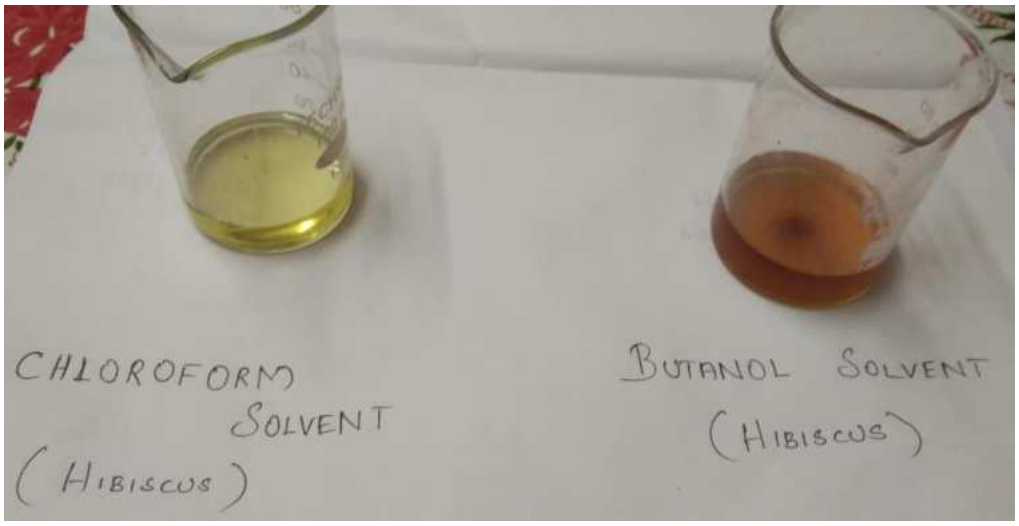


Fig D: Extracts of *Hibiscus rosa-sinensis* flower

Qualitative analysis of phytochemical

The butanol, aqueous and chloroform extract of *Hibiscus rosa-sinensis* flowers were subjected to phytochemical testing to detect the presence of different chemical compounds. Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick and inexpensive procedure that give the researcher a quick answer to the various types of phytochemicals in a mixture (Sasidharan *et al.,* 2011). A brief summary of the experimental procedures for the various phytochemical screening methods for the secondary metabolites is shown. After obtaining the extract or active fraction from plant materials, phytochemical screening can be performed with the appropriate tests as shown. to get an idea regarding the type of phytochemicals existing in the filtrate.

Phytochemicals:

Detection of Carbohydrates:

Fehling's test: Filtrates were hydrolyzed with dil. HCL, neutralized with alkaline and heated with fehling's A&B solution. Formation of Red precipitate indicates the presence of reducing sugar.

Detection of Saponins:

1ml sample was taken and then 20 ml of distill water was added , shake vigorously. Foam formation indicates the presence of saponins.

Detection of proteins:

Ninhydrin test: To the extract 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of protein.

Detection of Glycosides:

To the 1ml of sample 3 drops of diluted HCL was added. Pink colour formation indicates the presence of glycosides.

Detection of flavanoids:

Alkaline reagent test: Extracts were trated with few drops of lead acetate solution. Formation of yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavanoids.

Detection of phenol:

Ferric chloride test: Extracts were treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of phytosterols:

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of reddish brown colouration at the interface indicate the presence of terpenoids .

Detection of benedicts:

Benedict's test: Filtrate was treated with benedict's reagent and heated gently.Orange red precipitate indicate the presence of reducing sugars.

BACTERIAL STRAINS:

A total of two bacterial strains including both gram-positive and gram negative bacteria *Escherichia coli* and *Staphylococcus aureus* were selected .The bacterial cultures were maintained in nutrient agar slants at 37°C Each of the micro organisms were freshly cultured prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Bacterial strains used in the present study.

S.No	Bacterial Strain	Gram (+/-)
1	*Escherichia coli*	-
2	*Staphylococcus auerus*	+

Antimicrobial Activity:

In the present study the antibacterial and antidandruff activities of *Hibiscus-rosa sinensis* were recorded against the bacterial strain includes *Escherichia coli* and *Staphylococcus aureus* and the fungus strain like *Malassezia furfur*.The extract of plant shows variable activities.

Anti-bacterial Activity:

The present study show that the extract of *Hibiscus-rosa-sinensis* evaluated in different concentration (10µl, 30µl, and 50µl) for the anti-bacterial activities against *Escherichia coli* and *Staphylococcus auerus* indicating the different zone of inhibition .Our result revealed that Ethyl Acetate, Butyl alcohol and Distilled water of *Hibiscus rosa-sinensis* show signification higher inhibitory activities against *Escherichia coli* and *Staphylococcus aureus*.The Butyl alcohol extract of *Hibiscus rosa-sinensis* shows the maximum zone of inhibiting 15mm aganinst *Escherichia coli* and 11mm against *Staphylococcus.*

ANTIOXIDATION DETERMINATION ASSAY:

DPPH radical scavenging assay:

The 1, 1-diphenyl 1-2 picrylhydrazyl (DPPH) radical scavenging activity of chitosan concentration at 1mg/ml was determined described. The sample dissolved in 500 IL of 99.5% ethanol and 125 IL of 0.02% DPPH in 99.5% ethanol. This mixture to be incubated for 60 min in the dark at room temperature and then the DPPH radical was reduced. It was to be measured at 517 nm using an uv-visible spectrophotometer. In it's radical formation is DPPH has an absorption band at 517 nm ,then disappears upto reduction by an antiradical compound. The reaction mixture absorbance of lower absorbance indicated higher DPPH – free radical -scavenging activity was calculated as follows:

Radical -scavenging activity = Absorbance of control- Absorbance of sample

---------- × 100

Absorbance of control

The absorbance of control was conducted in the same manner. Distilled water is expected using instead if sample. BHA is positive standard, finally the test carried out in triplicate.

ANTIDANDRUFF DETERMINATION

***Malassezia* Species Collection:**

We collected sample *Malassezia* species from the scalp of student who have a dandruff. Using sharp and sterile epilation forceps to detach the hair samples from the infected region were taken by scraping with the epilation forceps holding at an angle of 90° on the head to paper. The specimen was transferred into sterilized bottle to prevent exposure to the sunlight (Crespo *et al.,*2020). Then, sample was transported to Microbiology Laboratory for analysis.

Culturing *Malassezia* Species:

The Petri dishes were autoclaved for 15 min at 121°C for sterilization and allow cooling down in clean water bath. The collected samples were cultured on sabouraud dextrose agar (SDA) with olive oil. Small amounts of the sample scalps were introduced into Petri dishes containing the media using sterile forceps. The Petri dishes were then labeled and incubated at 32°C for 3–7 days for any developing colonies (Kindo *et al.,* 2004).

Direct Microscope Examination:

A drop of Lactophenol cotton blue was introduced on to a slide containing scalp with coverslip. The sample was placed over Bunsen burner flame to remove bubbles. The slide was viewed using ×10 and ×40 and ×100 objective lens so that the hyphae (spores) can be seen. Direct microscope that shows the typical *Malassezia* species and pseudomycelia coupled with mycelia in the form of yeast like and identify the *Malassezia* species from the other species of fungi. Direct microscopic examination of *Malassezia* species based on morphological structure they are appeared like psedomycelia coupled with mycelia in the form of yeast like structures differed from the other species of fungi (Crespo *et al.,* 2020).

The highest zone of inhibition was obtained in ethanol extract (14 mm). Besides, 12 mm, 11 mm, 10 mm, and 9 mm were obtained for aqueous extract, ethyl acetate extract, chloroform extract, and diethyl ether extract, respectively, and positive control ketoconazole shown that the highest 17 mm and 16 mm zone of inhibition growth were obtained. The study shows antidandruff activity of both polar and non-polar extracts for all concentrations of extracts used.The inhibition zones in ethanol extract were obtained 7-14 mm (8.57 ± 3.4 mm) and 6-12 mm (7.57 ± 2.6 mm) in water. Besides, 5-11 mm (7.28 ± 2.9 mm), 4-10 mm (7.00 ± 2.5 mm), 2-9 mm (5.85 ± 2.0 mm) were obtained in ethyl acetate, chloroform and diethyl ether, respectively. The result of the current study shows less (9.85 ± 3.5 mm and 9.57 ± 3.7 mm) inhibition zone than the control group (ketoconazole) (Prabha *et al.,*2012).

SOAP PREPARATION

Materials Required

- ¾ lb goat milk soap base
- 1 teaspoon hibiscus flower powder
- Flower silicone soap mould
- Soap knife
- Heat resistant spatula
- Large glass measuring cup

Procedure:

1. Prepare a flower silicone soap mold by cleaning, drying
2. Then carefully slice ¾ pound of goat milk soap base into cubes that are approximately ½" to 1" in size using a soap knife. And equally, divide sliced goat milk soap base. 3.
3. Scoop one half of the sliced soap base into a large glass measuring cup for safe melting and easy pouring. Place it in the microwave oven and melt the soap base in 30 seconds intervals, stirring intermittently to avoid burning. 4.
4. Promptly remove the melted soap base from the microwave oven and quickly, distribute evenly between 4 of the prepared flower molds. Let stand for 2 to 3 minutes.

5. Then scoop the second half of the sliced soap base into the large glass measuring cup. Place it in the microwave and melt the soap base in 30 seconds intervals, stirring intermittently.
6. Promptly remove the melted soap base from the microwave oven and add ½ teaspoon of rose water and 1 teaspoon hibiscus flower powder. Stir together using a non-stick heat resistant spatula to scent and color. Add more hibiscus flower powder as needed to create your ideal pink color.
7. Working quickly, pour scented, pink soap into each soap mold over the white layer, avoiding overflow, before the soap begins to harden.
8. If needed, spritz with rubbing alcohol to remove bubbles in the soap. Allow hibiscus soap to cool in the mold for one to two hours or until solid before removing.All together, lathering up with hibiscus can result in soft, younger longer looking skin. And it's ideal for all skin types.

Fig E Melting of goat milk soap base

Fig F Hibiscus soap in the mould.

RESULT AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The butanol extract shows the presence of Carbohydrate and Benedicts. The Aqueous extract shows the presence of Saponin, Carbohydrate, Flavanoid, Phenol, Terpenoids and Benedicts. The Chloroform extract shows the presence of Saponin, Carbohydrate and Flavanoid. And complete absence of such phytochemical in a flower extracts are Protein and Glycosides. These compounds are known to be biologically active because they protect the plant against infection. The study was investigation the presence or absence of this component in this plant flower material of *Hibiscus rosa-sinensis.*

Table 1 -PHYTOCHEMICAL TEST OF DIFFERENT SOLVENTS OF *HIBISCUS ROSA- SINENSIS*

S.No	Compounds	Butanol	Aqueous	Chloroform
1	Saponin	-	+	+
2	Carbohydrate	+	+	+
3	Protein	-	-	-
4	Glycosides	-	-	-
5	Flavanoid	-	+	+
6	Phenol	-	+	-
7	Terpenoids	-	+	-
8	Benedicts	+	+	-

Fig 1.1 Saponin result for Aqueous extract of *Hibiscus rosa-sinensis*

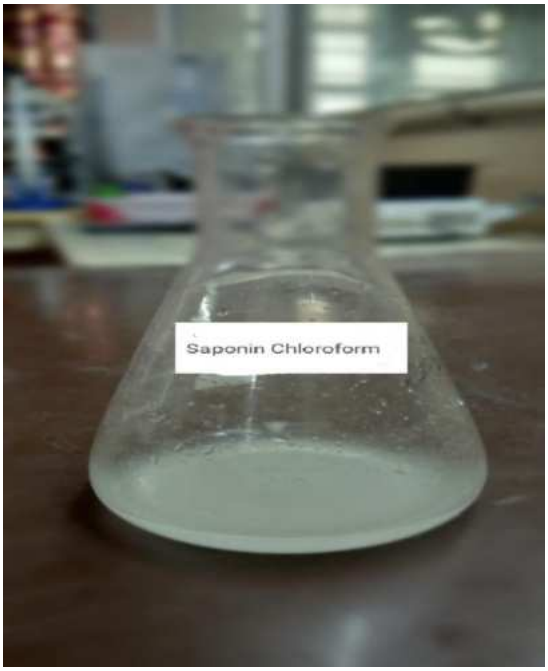


Fig 1.2 Saponin result for Chloroform extract of *Hibiscus rosa-sinensis*

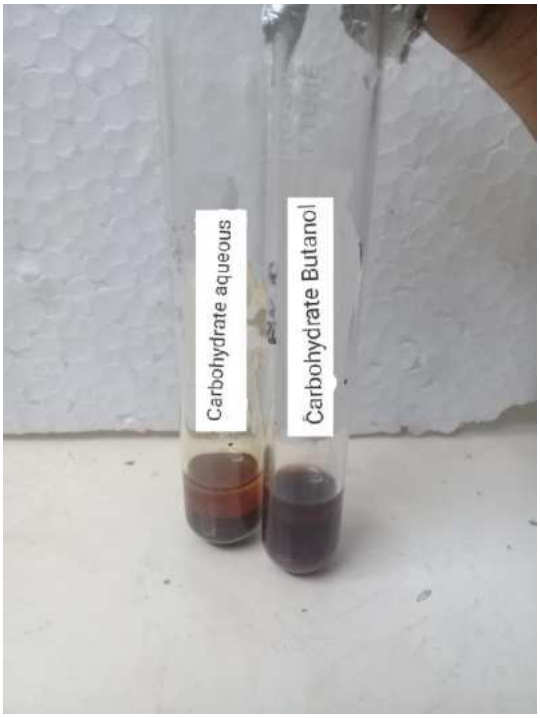


Fig 1.3 Carbohydrate result for Aqueous and Butanol extract of *Hibiscus rosa-sinensis*

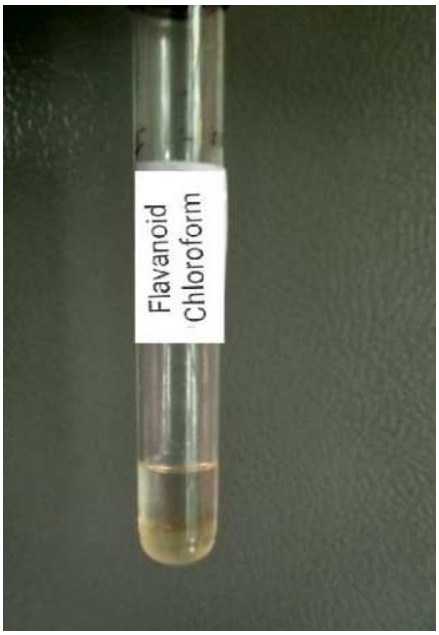


Fig 1.4 Flavanoid result for Chloroform extract of *Hibiscus rosa-sinensis*

Fig 1.5 Phenol result for Aqueous extract of *Hibiscus rosa-sinensis*

Fig 1.6 Terpenoids result for Aqueous extract

Fig 1.7 Benedict's result for Aqueous extract of *Hibiscus rosa-sinensis*

ANTIMICROBIAL ACTIVITY

In the present study the anti-bacterial, anti-Dandruff and antioxidant activities of *Hibiscus rosa-sinensis* where recorded against the bacterial strain includes *Escherichia coli* and *Staphylococcus aureus* and the Dandruff strain like *Malassezia furfur*. The extract of plants shows variable activities.

ANTIBACTERIAL ACTIVITY

The anti-bacterial potentially of the butanol and Chloroform solvent extracts of mature flowers of *Hibiscus-rosa sinensis* against mine pathogenic bacterial isolates viz., *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereu*s, *Escherichia coli*, *Salmonella typhi, Shigella flexneri*, *Klebsiella pneumoniae*, *vibrio cholerae* and *Pseudomonas aeruginosa*.The turbidity of the bacterial inoculums was compared with 0.5Mc Garland standards and the antibacterial potential of *Hibiscus rosa-sinensis* butanol extract was tesed by using Agar well difussion method. The butanol extract of *Hibiscusrosa-sinensis* (100mg/ml) showed maximum zone of inhibition (30mm) against *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. *Staphylococcus auerus* showed less zone of inhibiting (12mm).The Choroform extract of *Hibiscusrosa-sinensis* (100mg/mm)showed maximum zone of inhibition (23mm)against *Escherichia coli* (Saran raj *et al.*,2010).The Chloroform shows the inhibition zone at different concentration of 10µl is 1mm,25µl is 3mm and 50µl is 4mm against *Escherichia coli* and also against *Staphylococcus aureus* 10µl is 1mm, 25µl in 1mm and 50µl in 4mm. (Sudhakar chekuri *et al.,*2016) The distilled water show the inhibition against *Staphylococcus aureus*. (taklani *et al.,*2016).

In this present study shows that the extraction of *Hibiscus rosa-sinensis*was evaluated in different concentration (10µl, 30µl and 50µl) for antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* indicating the difference zone of Inhibition. Our results revealed that Butanol, Chloroform and Aqueous of *Hibiscus rosa-sinensis* show signification higher inhibitory activities against *E. coli* and *Staphylococcus aureus*. The butanol extract of *Hibiscus rosa-sinensis* shows the maximum zone of inhibition 21mm against *Staphylococcus aureus* and 20mm against *Escherichia coli.*

Table 2

ANTIBACTERIAL ACTIVITY OF BUTANOL EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER

MICROBIAL CULTURE	**CONCENTRATION OF PLANT EXTRACTION**		
	10µl	30µl	50µl
Staphylococcus aureus	10mm	11mm	21mm
Escherichia coli	12mm	15mm	20mm

Fig 2.1 ANTIBACTERIAL ACTIVITY OFBUTANOL EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER - *ESCHERICHIA COLI*

Fig 2.2 ANTIBACTERIAL ACTIVITY OF BUTANOL EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER-*STAPHYLOCOCCUS AUREUS*

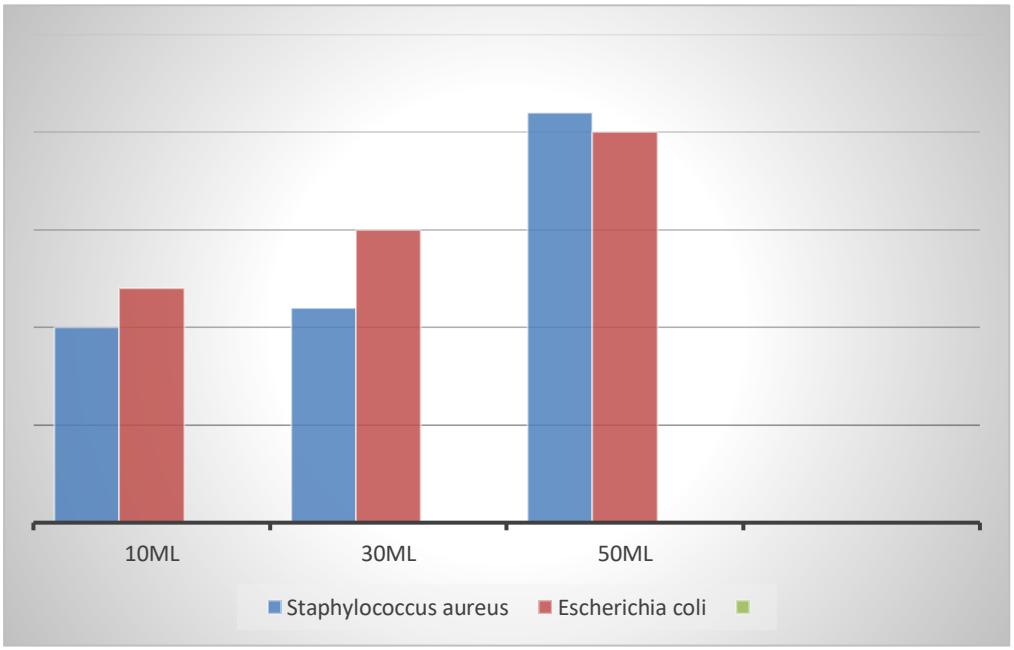


Chart 1 **ANTIBACTERIAL ACTIVITY OF BUTANOL EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER**

Table 3

ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER

MICROBIAL CULTURE	**CONCENTRATION OF PLANT EXTRACTION**		
	10µl	30µl	50µl
Staphylococcus aureus	10mm	11mm	13mm
Escherichia coli	7mm	8mm	9mm

Fig 3.1 ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACTOF *HIBISCUS ROSA-SINENSIS* FLOWER -*STAPHYLOCOCCUS AUREUS*

Fig 3.2 ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER - *ESCHERICHIA COLI*

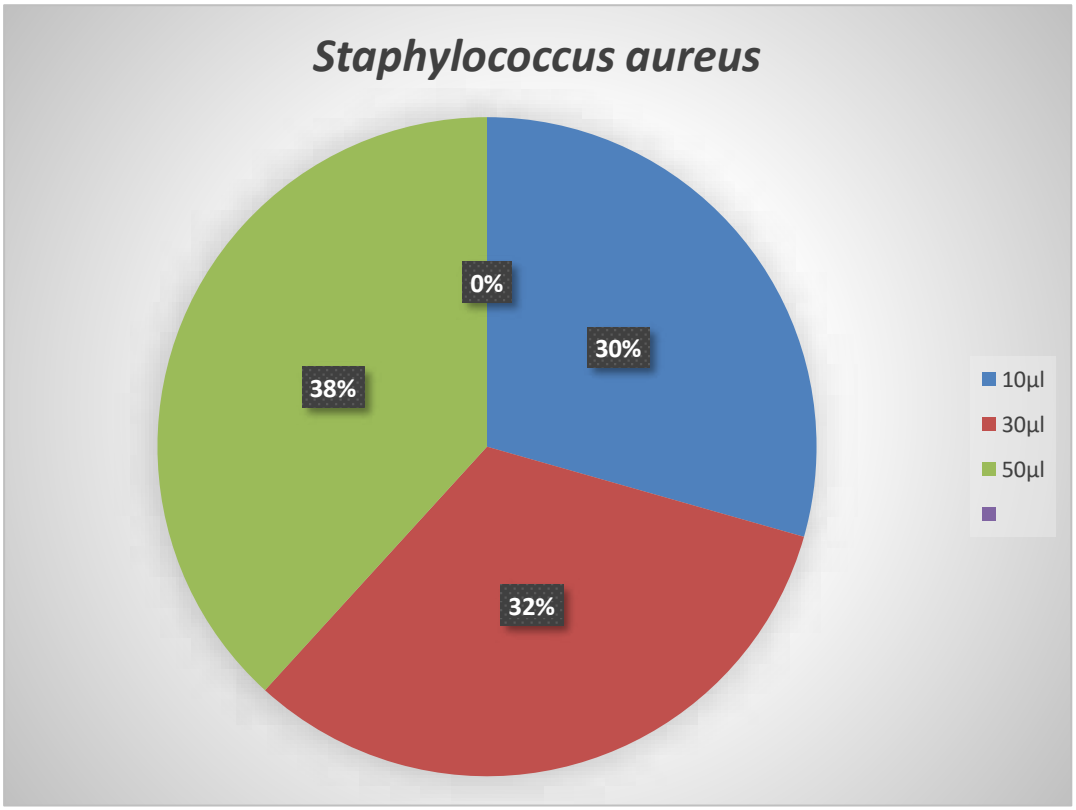


Chart 2 **ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER- *STAPHYLOCOCCUS AUREUS***

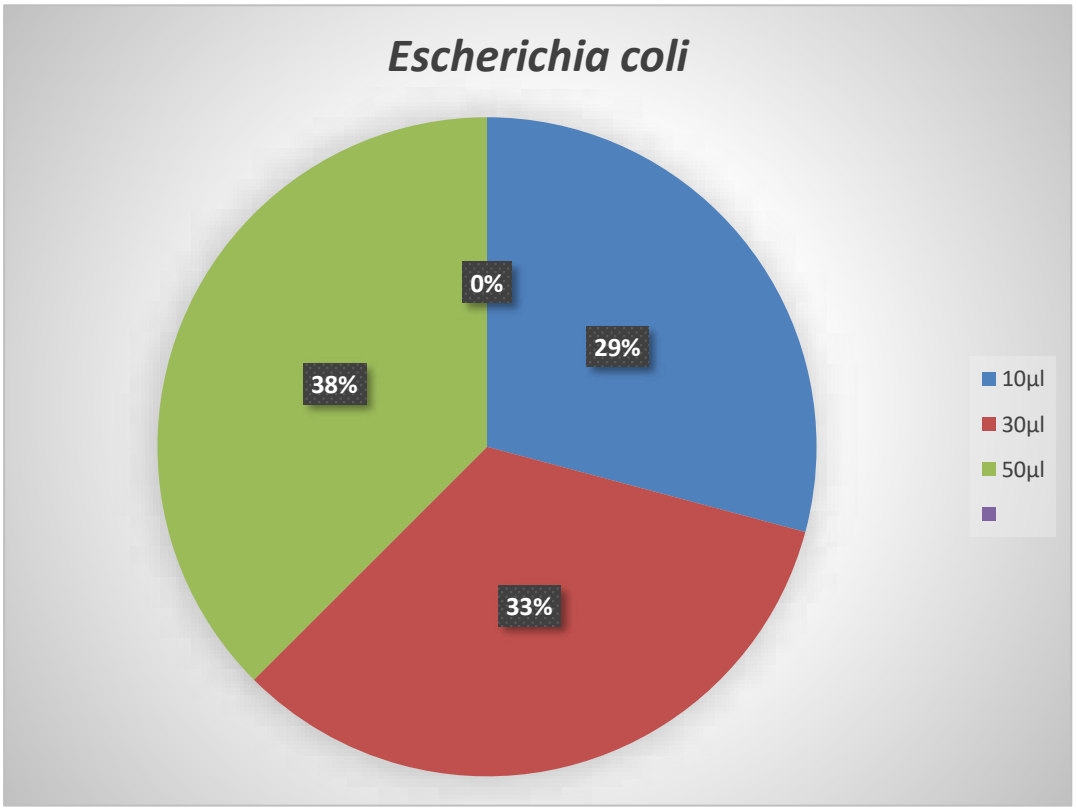


Chart 3**ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER- *ESCHERICHIA COLI***

Table 4

ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER:

MICROBIAL CULTURE	**CONCENTRATION OF PLANT EXTRACTION**		
	10µl	30µl	50µl
Staphylococcus aureus	6mm	9mm	7mm
Escherichia coli	Nil	Nil	Nil

Fig 4.1ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER - *STAPHYLOCOCCUS AUREUS*

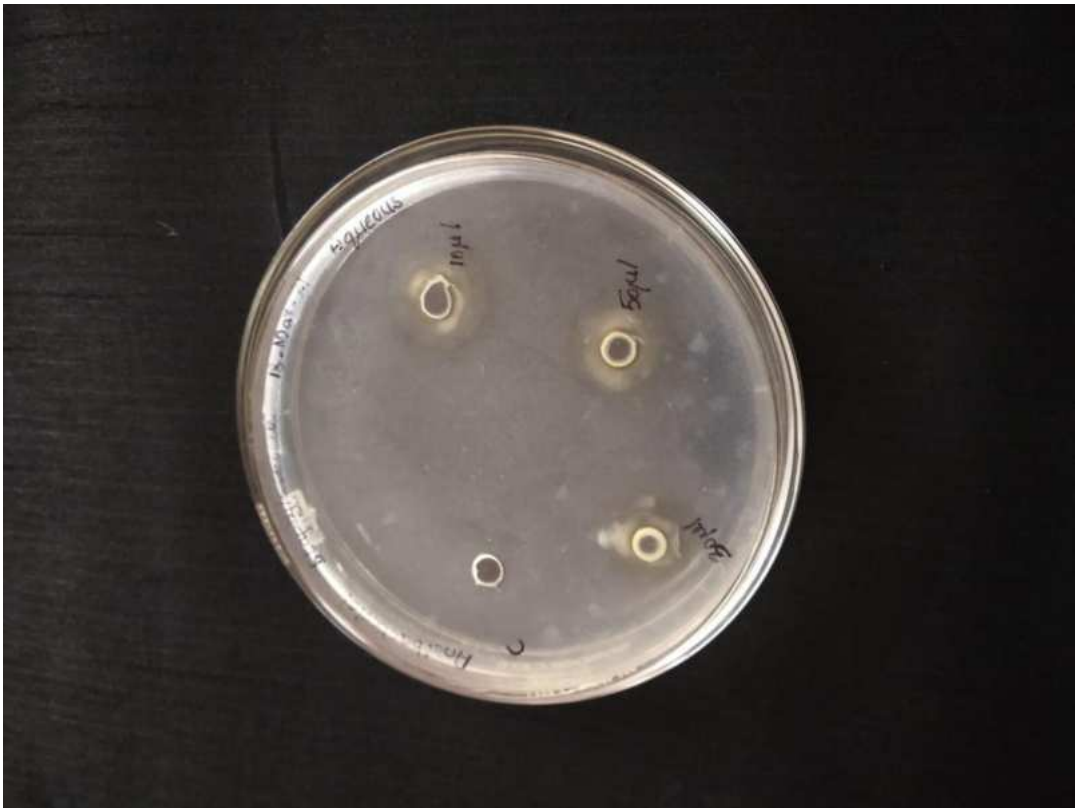


Fig 4.2 ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER - *ESCHERICHIA COLI*

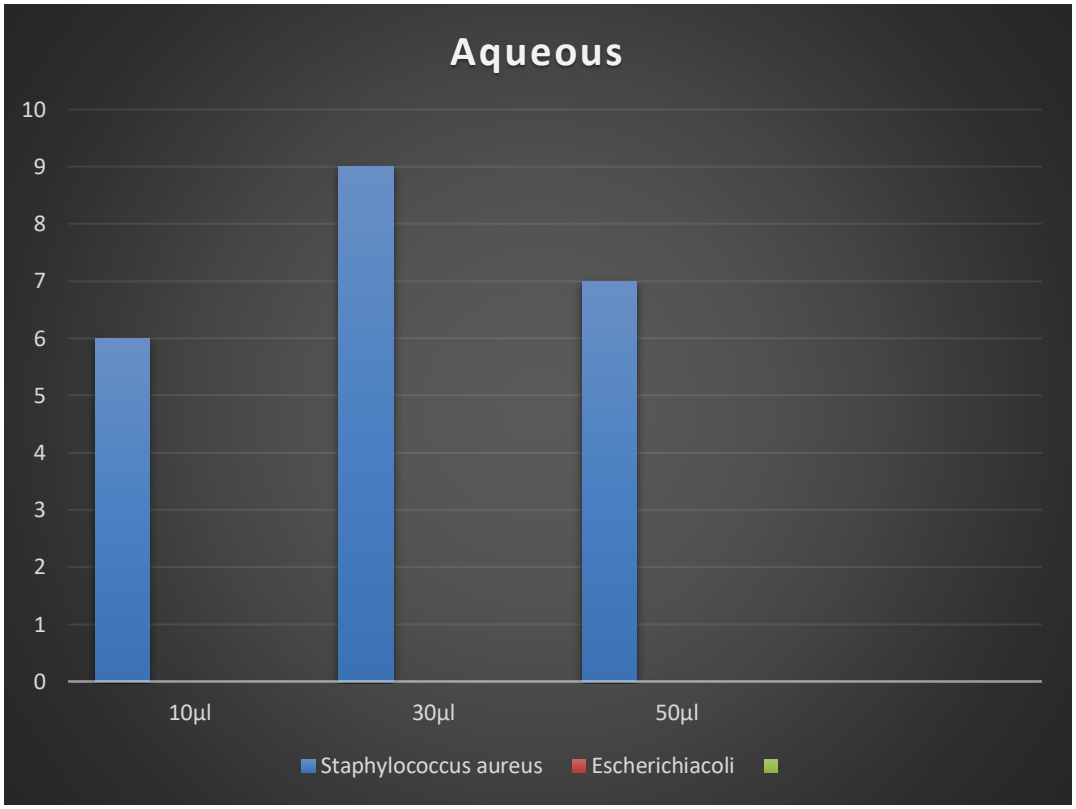


Chart 4 **ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER**

ANTIDANDRUFF ASSAY

Antidandruff activity has been carried out for ethanolic extracts of *Hibiscus rosa- sinensis*. The extracts demonstrated a significant antidandruff activity towards *Malassezia furfur*. Dandruffis a common disease caused by *Malassezia furfur*. The lipophilic nature of these organisms induces hydrolysis of human sebum tri-glycerides into free fatty acids that cause both hair loss and prompt turnover of scalp cells (DeAngelis YM,*et al., 2005)*. Thus the isolates grew well on Sabouraud‟s agar medium enriched with olive oil.

Malassezia furfur grew as a white to tan cream coloured colony on Sabouraud‟s media. All the plant extracts showed a reasonable inhibitory zone on *Malassezia furfur*. It shows the Minimum Fungicidal Concentration and zone of inhibition of the plant extracts.

In this present study shows that the extraction of *Hibiscus rosa-sinensis*was evaluated in different concentration (10µl, 30µl and 50µl) for antidandruff activity against *Malassezia furfur*indicating the difference zone of Inhibition. Our results

revealed that Butanol, Chloroform and Aqueous of *Hibiscus rosa-sinensis* show signification Minimum inhibitory activities against *Malassezia furfur.* The butanol extract of *Hibiscus rosa-sinensis* shows the maximum zone of inhibition 7mm against *Malassezia furfur.*

Table 5

ANTIDANDRUFF ACTIVITY OF *HIBISCUS ROSA-SINENSIS* FLOWER:

MICROBIAL CULTURE *Malassezia furfur.*	**CONCENTRATION OF PLANT EXTRACTION**		
	10µl	30µl	50µl
Butanol	6mm	7mm	7mm
Chloroform	5mm	5mm	5mm
Aqueous	2mm	3mm	3mm

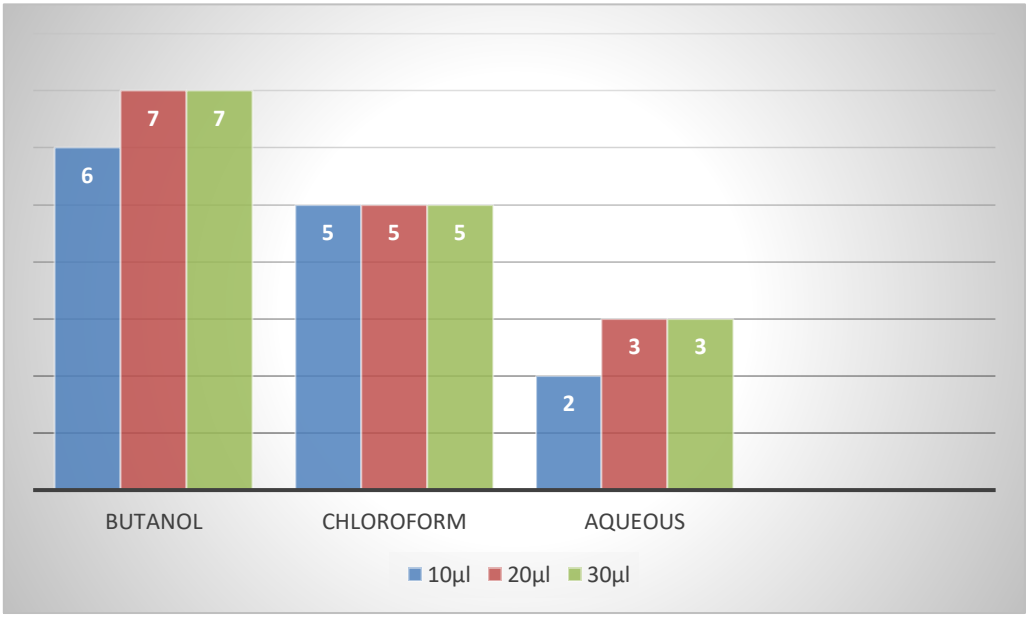


Chart 5 **ANTIDANDRUFF ACTIVITY OF *HIBISCUS ROSA-SINENSIS* FLOWER**

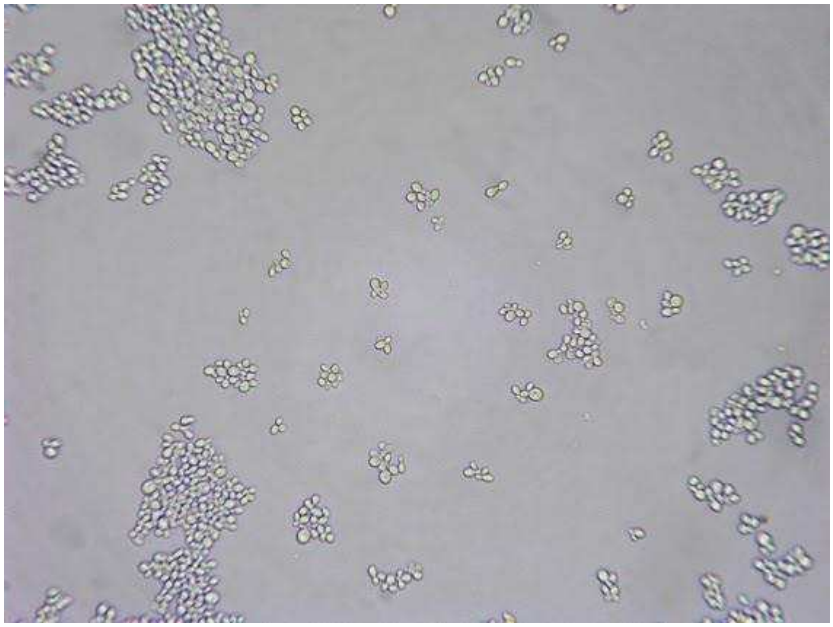


Fig 5 Microscopic examination of *MALASSEZIA FURFUR*

Fig 5.1 ANTIDANDRUFF ACTIVITY OF CHLOROFORM EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER

Fig 5.2 ANTIDANDRUFF ACTIVITY OF AQUEOUS EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER

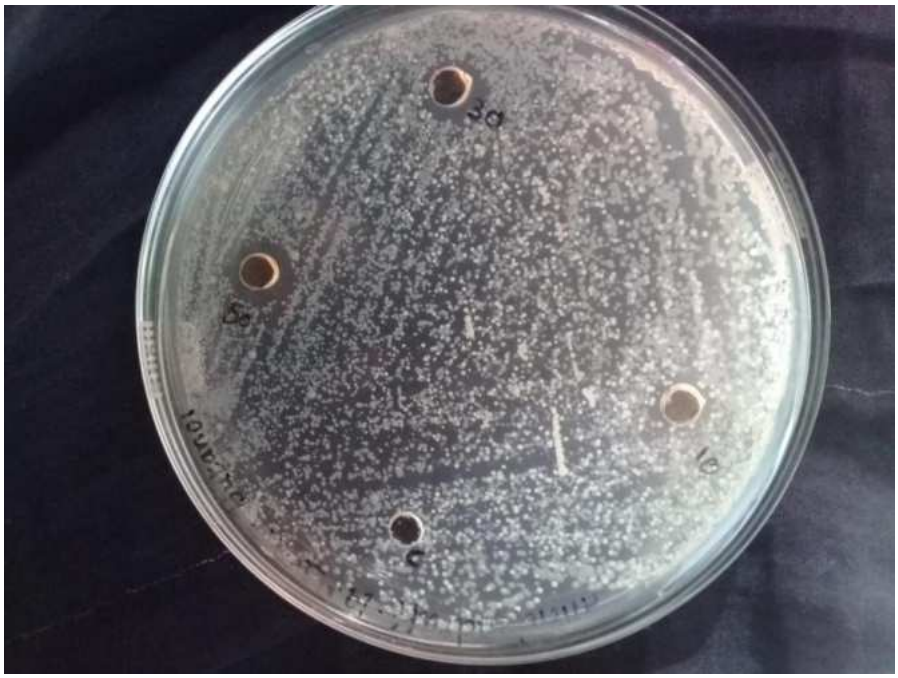
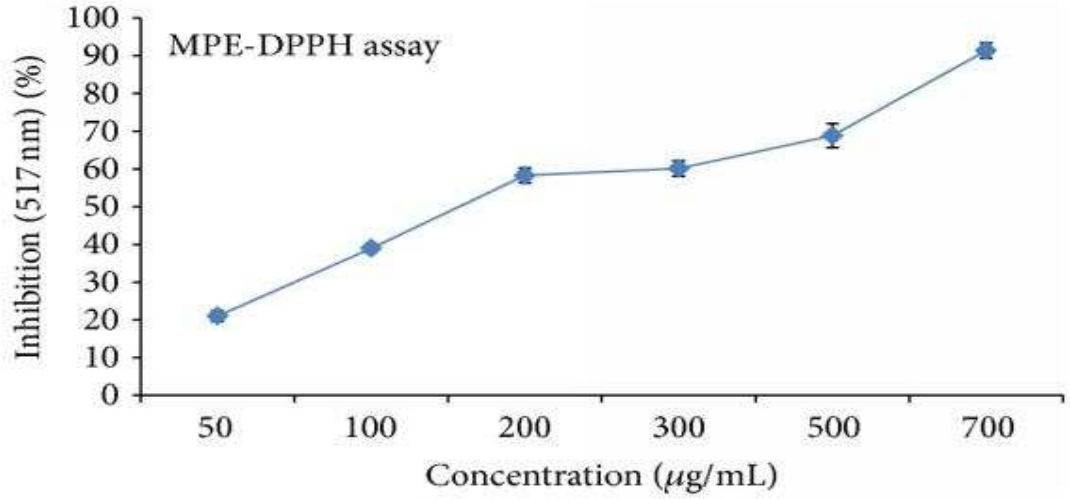


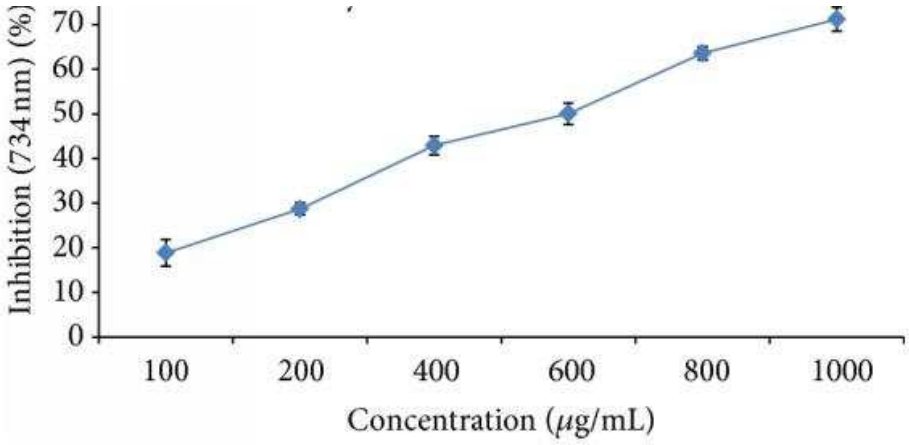
Fig 5.3 ANTIDANDRUFF ACTIVITY OF BUTANOL EXTRACT OF *HIBISCUS ROSA-SINENSIS* FLOWER

DPPH Radical Scavenging Activity

In vitro antioxidant assay of MPE revealed the presence of antioxidant potential. The percentage of inhibition was observed in all the antioxidant models that free radicals were scavenged by the extract in a concentration dependent manner up to the given concentration. The percentage inhibition of scavenging activities of the MPE for DPPH in chloroform showed 58.30% DPPH inhibition at 200 μg/mL concentrations. Antioxidant activity depends on the presence of amount of total polyphenolic compounds (Figure)



In vitro antioxidant assay of MPE revealed the presence of antioxidant potential. The percentage of inhibition was observed in all the antioxidant models that free radicals were scavenged by the extract in a concentration dependent manner up to the given concentration. The percentage inhibition of scavenging activities of the MPE for DPPH in butanol showed 47.10% DPPH inhibition at 600 μg/mL concentrations. Antioxidant activity depends on the presence of amount of total polyphenolic compounds (Figure)



In vitro antioxidant assay of MPE revealed the presence of antioxidant potential. The percentage of inhibition was observed in all the antioxidant models that free radicals were scavenged by the extract in a concentration dependent manner up to the given concentration. The percentage inhibition of scavenging activities of the MPE for DPPH in Distilled water showed 22.10% DPPH inhibition at 200 μg/mL concentrations. Antioxidant activity depends on the presence of amount of total polyphenolic compounds (Figure)

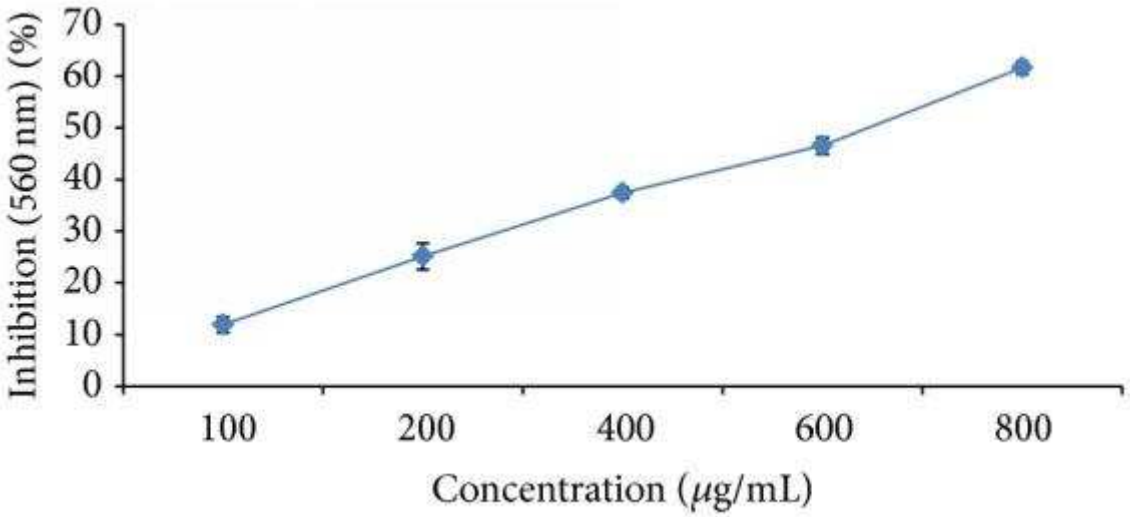


Fig 6 HIBISCUS SOAP

BENEFITS OF HIBISCUS SOAP

- Natural source of alpha-hydroxy acids (AHAs)
- Helps speed up cell turnover
- Unclogs pores helping to prevent blackheads
- Anti-bacterial cleansing properties help control acne
- High antioxidant levels protect from free radical damage
- Exfoliates and dissolves dead skin cells
- Improves elasticity, tightening wrinkles and fine lines
- Antioxidants help reduce the damage of UV rays
- AHAs aid in improving skin tone
- Anti-inflammatory properties help soothe skin
- Boosts collagen production with Vitamin C
- Alpha-hydroxy acids aid in reducing dark spots
- All together, lathering up with hibiscus can result in softer, younger longer looking skin. And it's ideal for all skin types

SUMMARY

Medicinal plants containing inherent active ingredients used to cure or prevent the diseases. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. So, the identification of bio active compounds in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important.

Phytochemical tests were carried out for alkaloids, flavonoids, sterols, saponins, terpenoids, phenols, carbohydrates and proteins by the standard methods. On qualitative screening of phytochemicals in flower of Hibiscus species showed the presence of carbohydrate, flavonoids, saponins, benedicts, terpenoids, phenol. The active compounds from the flowers of *Hibiscus rosa sinensis,* were extracted by using butanol, chloroform and aqueous and their physical properties were calculated and the extractive value was presented in the following order,

Aqueous > Butanol > Chloroform

It was identified that aqueous has a stronger extraction capacity which could have produced greater number of polar active constituents. So, in the present study, the further evaluations were made by using aqueous extract of flowers of Hibiscus species. The data obtained from antimicrobial activity, the butanolic flower extract of *Hibiscus rosa-sinensis* showed superior antibacterial and anti-dandruff activity due to the presence of the high content of flavonoids and phenolics.

The present study justified the importance of flowers in the traditional system of medicine to treat various infectious disease caused by the microbes.

CONCLUSION

Hibiscus rosa-sinensis has a long history of use as a medicinal herb and it is believed to be effective in the treatment of various health conditions such as decrease body temperature, treat heart disease, and sooth a sore throat, used to treat high blood pressure and high cholesterol. Flavonoids, tannins, terpenoids, saponins, and alkaloids are the main phytochemicals as they are present in different extracts, and are more likely responsible for their biological activities.

In this study, extracts obtained from *Hibiscus rosa-sinensis* were screened for antibacterial and anti-dandruff properties. Investigations were carried out to evaluate the phytochemicals of chloroform, butanolic and aqueous extracts of *Hibiscus rosa-sinensis* .The result indicated that the crude extract at various concentrations showed significant amount of phytochemicals such as flavonoids, tannins, terpenoids, saponins, and alkaloids. However the amount of phytochemicals in aqueous extract was considerably more compared to its alcoholic extracts. From the study it can be concluded that both aqueous and alcoholic extracts of *Hibiscus rosa-sinensis* showed remarkable antioxidant activities and its constituent could be used as an easily accessible source of antioxidant. Therefore, it is suggested that, further work could be done in the purification of the antioxidant components of *Hibiscus rosa-sinensis* and its constituent. Hibiscus soap with the goodness of hibiscus flowers has a luxurious appearance and a rich lather bursting with benefits for the skin. Dozen reasons to start using hibiscus soap, as this flower petal herbal soap has natural Botox benefits.

Thus, our findings provide evidence that the butanolic, chloroform and aqueous extracts of *Hibiscus rosa-sinensis* shows antibacterial and anti-dandruff properties and are potential source of natural antioxidants and some extent validated its medicinal potential. The phytochemicals in the flower makes it a pharmacologically effect antioxidant.

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A STUDY ON ABILITY OF HUMAN URINE ODOUR REDUCTION OF BACTERIA ISOLATED FROM COW'S SHELTER SOIL

A DISSERTATION SUBMITTED TO

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

AFFILIATED TO Manonmaniam Sundaranar University,

In partial fulfilment of the requirements for the award of the degree of

BACHELOR OF SCIENCE IN MICROBIOLOGY

SUBMITTED BY

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APRIL 2021

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"A STUDY ON ABILITY OF HUMAN URINE ODOUR REDUCTION OF BACTERIA ISOLATED FROM COW'S SHELTER SOIL"** is a bonafide record of the original work completed by **Ms. K. SANTHANA GEETHA, S. SELSIYA, B. SHARONE SHIBI, A. SINDHUJA, K. SIVA PRIYA DHARSHINI, S.SIVA SURYA DEVI, R. SUBHA LAKSHMI, M. SUJI, (Reg.no 18SUMB35-18SUMB42)**, during the academic year 2020-2021 in St. Mary's College (Autonomous), Thoothukudi, and submitted as a partial fulfilment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by the university of Manonmaniam Sundaranar.

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Signature of the External Examiner

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DECLARATION

We hereby declare that the dissertation work entitled **"A STUDY ON ABILITY OF HUMAN URINE ODOUR REDUCTION OF BACTERIA ISOLATED FROM COW'S SHELTER SOIL"** is a bonafide record of the work completed by us during the academic year 2020-2021 in St. Mary's college (Autonomous), Thoothukudi, and submitted as a fulfilment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by the Manonmaniam Sundaranar University. We also affirm that this is a original work done by us under the supervision of Mr.C.Edward, M.Sc., M.Phil., Assistant Professor of department of Microbiology St. Mary's College (Autonomous), Thoothukudi.

Signature of the Students

Signature of the Guide

Place: Thoothukudi

Date:

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CONTENT

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ABBREVIATION

ml	millilitre
Si	Silicon
gm	gram
Hrs	hour
%	percentage
min	Minutes
GC-MS	Gas chromatography- Mass spectrometry
DF	Dilution factor
CFU	Colony forming unit
H_2S0_4	Sulfuric acid
NH_3	Ammonia
CH_4N_2O	Urea

INTRODUCTION

A study on ability of human urine Odour reduction of bacteria isolated from Cow's Shelter Soil

INTRODUCTION

Urine is a liquid waste material secreted by the renal tubules, it accumulates in the urinary bladder and excreted via urethra. While it is composed of 91 to 96 percent water, it contains many other components, both solid and liquid. Over 99 percent of urinary solutes are composed of only 68 chemicals which have a concentration of 10 mg/L or more, 42 compounds are actually in the form of such as Electrolytes, Nitrogenous compounds, Urea and Organic acids. While urea in urine is odorless, a large number of different compounds affect the smell of urine, these compounds are continuously created as urea breaks down, which explains why stale urine smells much worse than fresh urine. It can quickly break down into other nitrogen-containing compounds, such as ammonia and trimethylamine. Odors are generally generated from the decomposition of the faeces and urine.

Together, faecal and urine odours contain more than two hundred VOCs from various chemical classes such as carboxylic acid, sulfur compounds, nitrogen compounds, aldehydes, alcohols, phenols, ketones, steroids, lactones, alkanes and terpenes. Toilet malodor consists of a complex mixture of volatile compounds arising from faecal material and stale urine. Undesirable odours contribute to air quality concerns and affect human lifestyles. Odour affects human beings in a number of ways. Strong, unpleasant or offensive smells can interfere with a person's enjoyment of life especially if they are frequent and/or persistent. Though foul odour may not cause direct damage to health, toxic stimulants of odour may cause ill health or respiratory symptoms. Secondary effects, in some, may be nausea, insomnia and discomfort. Very strong odour can result in nasal irritation, trigger symptoms in individuals with breathing problems or asthma. (Sneh Gupta *et al*.,2015)

Commercial chemicals have recentlycome onto the market which are claimed by their manufacturers to control odor. The compounds fall into six broad categories: **Digestive Agents**: Contain bacterial cultures or enzymes to biologically control odor production while improving solids breakdown. **Masking Agents**: Mixtures of aromatic oils which cover the odor of manure with a stronger more "pleasant" odor. The masking agent does not reduce the smell and even if the product effectively covers the manure odor, the covering odor may still be objectional to some people**. Deodorants**: Selected chemicals that react with the odorants to inhibit their release as a gas or to neutralize their unpleasant odors. A deodorant usually does not use another masking odor. **Absorbents:** Products which have a large surface area, such as charcoal and clay, that absorb the odorous chemicals before their release to the environment. Feed Additives. Added to the feed to improve animal performance and reduce manure odors. Miscellaneous. Products which do not belong in any of the other categories include a large number of chemicals such as chemical oxidants or germicides. The digestive agents, absorbents. feed additives, and many of the compounds in the miscellaneous category are designed to prevent release of odor compounds or prevent their formation. Deodorants and masking agents affect the human response to odors. Many attempts to destroy air- borne odor molecules have been only partially successful at best. Most of the chemical reactants are specific for a special class or type of odorant or contaminant. In addition, the reagent frequently contributes a noxious species to the partially deodorized air either as a by – Product of the reaction or as unreacted reagent molecules.

Numerous studies have been carried out by different researchers on the composition of air fresheners, toiletry cleaners and their relative health effects. For instance, Fleming indicated that some compounds in such products including benzene derivatives, pinene and limonene, aldehydes,

phenol, and cresol may pose serious health effects. Other common chemicals that could be found in air fresheners include VOCs such as benzyl alcohol, toluene, myrcene, phthalates, artificial musks, lilial, and linalool have been related with adverse effects such as asthma attack, mucosal symptoms, infant illness, breathing difficulties, and migraine headaches from a health perspective (Fatima Ibrahim ALshaer *et al*., 2019).Soil microorganisms are the greatest contributors to the diversity of terrestrial ecosystems and in soils anaerobic and aerobic micro habitats co-exist. Microbes play an important role in both production and reduction of malodors (Zhu Jun., 2000). Microbial treatments have been extensively used in municipal wastewater to degrade organic matter (Low and Chase., 1999) and microbial treatments are emerging to treat livestock wastewater. Microorganisms live naturally in manure and they digest solids and breakdown various components. One recent study (Rahman and Mukhtar, 2008) suggested that microbial treatment is effective in reducing odour from waste water and swine and poultry.

Hence, considering the ill effect of chemical deodorizer and microbial nature of deodorizing ability of malodourous compound an attempt has been made in the present study to identify the microbes that inhabit the cow's shelter soil that have the ability to remove or reduce the selected odorous substances (sulfides, ammonia, and VFAs) from stale urine.

AIM AND OBJECTIVE

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 To screen and isolate the human urine odour reduction bacteria from Cow shelter's soil.

 To identify the odour reduction bacteria.

 To treat the bacterial extract with urine sample for analysing reduction ability.

 To analyze the changes in urine chemical compounds after treating with bacterial extract using GC-MS.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The isolation of certain bacteria from the soil, which can decompose hydrogen sulfide and methyl mercaptan and have potential applications in microbial deodorization. Generally, the odor of feces is attributed to fatty acids, sulfur-containing compounds, indole, skatole, and ammonia. Mass spectrometry (TCT/GC/MS) measurements were performed to identify the malodorous compounds. (Hiroshi Sato *et al*.,2002).

In general, odor can be "measured" using chemical (analytical) or sensory methods. The analytical techniques include chemical analysis and direct reading instrumental analysis. Chemical analysis is the indirect assessment involving the collection of a sample which, when analyzed, will give the concentration of the various chemical species present. This includes substance-specific wet chemistry methods, as well as sample collection followed by analysis by means of instruments such as gas chromatography (GC). Direct reading instrumental analysis provides information on the concentration of specific chemical species or their concentrations relative to each other (Elefteria Psillakis *et al.,* 2006).

The ability of microorganisms to remove selected odorous substances (sulfides, ammonia, and VFAs) from solid poultry manure and compost from green wastes. (Kim *et al.,* 2007).

Treatment of organic wastes and animal breeding are associated with odor emissions, which often impact environmental health and quality of life. Representative odors related to pig and poultry breeding as well as organic waste processing include ammonia and its derivatives, sulfuric compounds, volatile fatty acids (VFAs) and phenolic compounds (S.Borowski *et al.,* 2008).

A multipurpose sampler (Gerstel MPS), designed for liquid large volume, gaseous and

headspace samples was used for the GC-MS analysis of organic volatile in human urine. Headspace sampling with a volume, -temperature and speed- controlled-gas tight syringe was combined with a temperature-controlled cold injection system (CIS) for cold traping, enrichment and focusing of analytes. Regular 2ml GC vials filled with 1 ml acidified urine were used as headspace sampling vials. A 100-vial autosampler tray was equipped with an additional temperature and heating time controlled "preheating station" for five vials. Profiles of organic volatiles in human urine were determined and 34 components identified. Tri methyl amine (TMA) and 4 heptanone as two metabolites of medical interest were quantified (Hans Gunther Wal *et al.,* 2009).

With the development of national economy and improvement of people's living standard, odor problem becomes more and more important, as well as odor pollution treatment. In every treatment method, biological deodorization has been the research emphasis in recent 10 years, because of its many advantages, such as high deodorization efficiency, simplified device and low treatment cost. (Xiaohui WANG et al., 2011).

A number of physical, chemical and biological methods have been developed to abate the odours. Sometimes, a single method or a combination of two or more methods is used to treat waste gases. Unlike biological methods, physical and chemical methods have lower efficiency and higher installation and operation costs which restrict their implementation (Sonil Nanda *et al.,*2012).

Over the years, latrine users have developed their own strategies to reduce smell from latrines. Observations have shown that people pour wood ash, disinfectants, pesticides, oil, laundry

and soapy water, detergents, car – battery acids, and a range of substances in to the latrines to reduce the smell (Thilde Rheinlander et al.,2013).

The understanding of human reactions to odours and their importance for human well-being and health is of major concern in the control of malodours and manipulation of odours in the environment. Depending on the type and concentration of odorants, evoked responses may be positive as well as negative. Adverse environmental impact and excessive air pollution, apart from possible psychological and depressive effects, may also have a physical impact on exposed individuals. (Sven Nimmermark *et al.,*2014).

Flavors and fragrances are widely used in food, beverage, feed, cosmetic, detergent, chemical and pharmaceutical formulations. Recently due to consumer's increased interest and health awareness in natural products, there has been more stress towards the use of natural fragrances and flavors obtained from natural sources. Research has shown that microbes can also be used to produce aromas and fragrances. (Sneh Gupta *et al.,*2015).

Microorganisms are the agents that carry out the biodegradation of VOC's and odours. For the degradation of VOCs, usually mixed populations of bacteria or fungi have been extensively used. Mixed cultures often originating from wastewater treatment plants or of similar origin have been used as inoculums. Bacillus has been found effective in degrading oxidation products as many bacilli produce extracellular hydrolytic enzymes that breakdown lipids, permitting the organisms to use these products as carbon sources and electron donors. Methylotrophic microbes of Hypho microbium genus and autotrophic microbes of Thiobacillus genus has been found efficient

in degrading dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) compounds (Irfana Showq et al.,2015).

Biological deodorization technology has the ad- vantages of high treatment efficiency, no secondary pollution, simple equipment, easy operation, low cost, convenient management and maintenance, and so on. However, the growth of microorganisms is affected by temperature, pH and other environmental factors. The stability of biological deodorization needs to be further improved. Biological deodorization technology mainly includes biological filter method, biological trickling filter method, soil filter method, etc. (Jian-cheng *et al.,* 2016).

Society is suffused with fragranced consumer products: air fresheners, cleaning products, soaps, hand sanitizers, laundry supplies, and personal care products, to name a few out of hundreds.1 Fragranced products emit a range of volatile organic compounds (VOCs), such as terpenes (e.g., limonene), which often dominate pollutants found indoors, and generate secondary pollutants such as formaldehyde (Anne Steinemann *et al.,* 2016).

Odorous compounds include organic or inorganic molecules. The two major inorganic odors are hydrogen sulfide and ammonia. Organic odors are usually the result of biological activity that decomposes organic matter and forms a variety of extremely malodorous gases including indoles, skatoles, mercaptans, and amines. (Majid Rahimi Pordanjani *et al.,*2017).

Poultry production systems are associated with emissions of odorous volatile organic compounds (VOCs), ammonia (NH3), hydrogen sulfide (H2S), greenhouse gases, and particulate matter. Development of mitigation technologies for these emissions is important. Previous

laboratory-scale research on microbial mineral treatment has shown to be effective for mitigation of NH3, H2S and amines emissions from poultry manure (Kajetan Kalus et al.,2017).

Urine is a complex mixture of numerous substances. Human urine is composed primarily of water (95%). The rest is urea (2%), creatinine (0.1%), uric acid (0.03%), chloride, sodium, potassium, sulphate, ammonium, phosphate and other ions and molecules in lesser amounts. The components of urine have the potential to serve as important biomarkers and diagnose numerous diseases. Urine is a rich bodily fluid in terms of its contents. Over 3000 metabolites have been defined in urine in the past three decades. However, studying with urine has challenges. Determining the exact composition of urine is both difficult and expensive. (Neslihan Sarigul *et al.,*2019).

In recent years, bioremediation has received great attention. This is because the approach is more reliable and environmentally-friendly. Microorganisms including bacteria, fungi, yeast and algae with a high neutralizing ability have been reported in literature. Biological deodorization is becoming popular, and can be designed for virtually complete removal of odours without causing secondary pollution. Bacteria play important roles in deodorization in other biological methods such as bio cover and bio filter (Evans M.N. Chirwa *et al.,*2019).

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secondary pollution. Bacteria play important roles in deodorization in other biological methods such as bio cover and bio filter (John B.J. et al.,2019).

Urine components are affected by body metabolism but also by consumed food and drinks. Therefore, it needs to be stressed that not every odor noted in the urine should be recognized as alarming. For example, shortly after ingestion of asparagus urine might have a distinct sulfurous smell (reminiscing cooked cabbage) in some individuals. Although exact molecules that are responsible for that odor have not been unambiguously identified, several VSCs like methanethiol or dimethyl sulfide are suspected. (Izabella Mogilnicka *et al.,* 2020).

At present, most of the microbial deodorizers used in domestic market are imported from foreign countries. EM bacteria developed by Professor Bijazov of Ryukyu University in Japan are the earliest microbial agents. After many years of technological development, EM bacteria have been applied in various fields deodorized mixed domestic sewage by EM flora. It is found that XM bacteria in EM flora can remove 87% of the odor, and the deodorization effect is obvious (Hong Chengyang *et al.,*2020).

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of soil sample

The soil sample for the present study was collected from Cow's shelter of (domestic farm) and brought to the lab.

Preparation of soil extract

To isolate the culture from respective soil the given sample (10 gm soil) was dissolved with 100 ml of distilled water and then filter. Then the filtrate was used for further isolation.

Isolation of Bacteria from the soil

Isolation of bacteria from the soil was done by spread plate technique. 1gram of soil was added to 99 ml of distilled water and then sample was serially diluted up to 10-6 dilution to determine the population of bacteria. The dilutions were plated on sterile petri plates containing nutrient agar medium and plates were incubated at 37°C for 24-48 hours for isolation of bacteria.

Identification of bacteria

Simple Staining

The bacterial culture was smeared with a simple stain. A thin smear of pure isolate colony was made on a clean glass slide dried in air and fixed by passing through flame of a burner. Commonly used basic dyes were added such as methylene blue, and then examined under the compound microscope.

Gram Staining

Isolated bacterial strains were identified by performing grams staining. A thin smear of pure isolate colony was made on a clean glass slide, dried in air and fixed by passing through flame of a burner. The smear was covered with crystal violet kept for one minute. The slide was washed with water, then covered with gram iodine and stand for one minute. The slide was again washed with water. Decolorized with alcohol, was achieved by rocking the slide gently for twenty seconds till the violet colour came off the slide and then washed with water immediately. This was counterstained with safranin for twenty seconds. Washed with water, blot dried and then examined under the compound microscope.

Motility test: The isolated cultures was tested for motility by hanging drop method and the result was observed.

BIOCHEMICAL TEST

Indole test

Peptone water medium was prepared in the test tubes and the culture was inoculated into the medium, and the tubes were incubated at 37°C for 24 hours, after incubation Kovac's reagent was added and the result was observed.

Methyl Red test

MR – VP broth medium was prepared in the test tubes and the culture was inoculated in to the medium and tubes were incubated at 37°C for 24 hours, after incubation few drops of methyl red indicator was added and the result was observed.

Voges Proskauer Test

MR – VP broth medium was prepared in the test tubes and the culture was inoculated in to the medium and tubes were incubated at 37°C for 24 hours, after incubation 0.5 ml of alpha naphthol,0.2ml of KOH were added and the result was observed.

Citrate test

Simmon Citrate Agar slant was prepared and the culture was inoculated as a single streak on the agar slant surface, and the tubes were incubated at 37°C for 24 – 48 hours, after incubation the result was observed.

Starch hydrolysis

Starch agar plates were prepared and the culture was single streaked on the surface and the plates were incubated at 37°C for 24 hours and the result was observed.

Urease test

Christensen's urea agar slants were prepared culture was inoculated on the medium as zigzag streak, and the plates were incubated at 37°C for 18 hours and the result was observed.

Casein test

Skim milk medium or casein medium, was prepared and organism was inoculated into the plate as single line streak, then plates were incubated at 37°C for 24 hours and then result was observed.

Catalase test

The catalase test was performed by slide method, on a slide, few drops of hydrogen peroxide were placed to which the culture was added and the result was observed.

Growth on selective media

After biochemical confirmation the suspected colonies were plated on selective media like Bacillus Agar medium & MYP agar medium.

Crude sample preparation for reduction test

The organisms were cultivated on a 100 ml liquid medium, which contained 40 % of urine sample,4 % of glucose, and distilled water. Each medium was inoculated with a 1 ml of microorganism culture containing 1-2x108CFU/ml. Cultivations were submerged at 250 C for 21 days. After the following days of cultivation, the samples were collected for quantitative determination of ammonia. Simultaneously, the same procedure was applied for blank sample (without microorganisms). (S. BOROWSKI 2008)

Determination of Ammonia by Titration with Sulfuric Acid

Method (Practical analytical chemistry manual lab Ali Albakka) 2008

•Using a bulb pipette, quantitatively transfer a 25 mL of aliquot of the diluted cleaning solution sample to a 100 mL volumetric flask.

•Add 2 drops of methyl red indicator and mix well.

•Carefully fill a 50 mL burette with standard 0.05 M sulfuric acid solution, M (sulfuric).

•Titrate the diluted cleaning solution to the first sign of a permanent pink end point (use a white tile beneath the Erlenmeyer flask during the titration). Record the titre to the nearest 0.01 ml

•Repeat the titration (steps 2 to 5) until three titres are obtained that agree within 0.10 ml Average these readings T (sulfuric).

•Calculate the concentration of ammonia hydroxide available in the undiluted cleaner sample C (ammonia), % w/v to 2 decimal places.

$$C\ (\text{ammonia}) = 2 \times M\ (\text{sulfuric}) \times T\ (\text{sulfuric}) \times Mr\ (\text{ammonia})$$

$$\frac{}{25}$$

Where Mr (ammonia) is the formula mass of ammonium hydroxide.

Following above given method, the sample was titrated and the results was observed, H2So4 was used as titrant and methyl red was used as indicator.

Preparation of samples for GC-MS analysis

In order to analyse the changes in chemical compound of urine treated with bacterial extract and control samples were extracted with hexane. Equal volume of hexane and sample was taken in a test tube and kept for 20 minutes. Then the organic phase containing the eluted compounds were given for analysis.

Gas chromatography-Mass spectrometry (GC-MS)

Procedure

The GC-MS analyses were made in QP-2010 (Shimadzu, Japan). 10 µl of extract was injected into GC-MS on a 30 m DB-1 capillary column with a film thickness of 0.25 µm (30 m X 0.32 mm i.d. coated with DB-1 using the following temperature programme, initial oven temperature of 70ºC for 35 min. The gas chromatography was equipped with Quadrupole detector. The GC-MS was under the computer control at 70 eV. Identification of unknown compounds was made by probability-based matching using the computer library built within WILEY7, NIST05 and NIST05s.

GC-MS programme

Name of GC-MS: GC-MS QP 2010 (SCHIMADZU, JAPAN)

GC-parameters

Column Oven Temperature	:	70.00ºC
Injection Temperature	:	250.00ºC
Injection Mode	:	Splitless
Sampling Time	:	3.00 min
Flow Control Mode	:	Linear Velocity
Pressure	:	18.8 kPa
Total Flow	:	30.0 ml/min
Column Flow	:	1.47 ml/min
Linear Velocity	:	44.4 cm/sec
Purge Flow	:	3.0 ml/min
Split Ratio	:	17.4
Ion Source Temperature	:	230.00 ºC
Interface Temperature	:	270.00 ºC
Solvent Cut Time	:	3.00 min

Detector Gain Mode	:	Relative
Detector Gain	:	0.10 kV
Threshold	:	500

MS-Parameters

Start Time	:	5.00 min
End Time	:	35.00 min
ACQ Mode	:	Scan
Event Time	:	0.50 sec
Scan Speed	:	10.00
Start m/z	:	40.00
End m/z	:	500.00

RESULTS

RESULTS

Isolation by using serial dilution technique

The isolation of human urine odor reducing bacteria from soil was performed with serial dilution technique using soil filtrate and the number of colonies for each dilution was calculated and then selective organisms were maintained for further identification and the results are tabulated. (Table 1 and Fig 4, 5 and 6)

Identification of Odour reduction bacteria

The isolated colonies were identified with Microscopic observation, Cultural characteristics and Biochemical tests, the suspected colonies were streaked on the respective selective media and the results are tabulated. (Table 2 & 2.1) (Fig 7 - 14)

Growth on selective media

After biochemical confirmation by standard biochemical tests, the confirmed Bacillus sps colonies were developed on Bacillus Agar medium and MYP medium. (Fig 15, 15.1&15.2).

Crude sample preparation for reduction test

To confirm the reduction of odor compound in urine, the crude sample of broth culture along with urine was prepared for further analysis. It was confirmed by the clear turbidity. (Fig 16).

Quantitative determination of Ammonia by Titration with Sulfuric Acid

The reduction rate of ammonia in urine was calculated in control (normal urine) and bacterial treated sample by titration method with sulfuric acid as titrant and the methyl red indicator was

used and the results are tabulated (Table 3 and Fig 17 & 18).

Preparation of sample for GC-MS analysis

In order to analyse the changes in chemical compounds of urine after treatment, the samples were extracted with hexane, then the organic phase containing eluted compounds were separated. (Fig 19 & 20).

Gas chromatography-Mass spectrometry (GC-MS)

The identified and characterization of chemical compounds in hexane crude extract (both normal and bacterial broth treated) are shown according to their elution order on a VF-5 capillary column. The major changes in chemical compounds of urine in hexane crude extract were found (Shown in Table 4 and 5 & Fig 21 & 22).

RESULT TABLES

RESULTS

Table 1: SERIAL DILUTION METHOD

S.NO	DILUTION FACTOR	COLONIES
1.	**10^{-1}**	**260**
2.	**10^{-2}**	**200**
3.	**10^{-3}**	**180**
4.	**10^{-4}**	**129**
5.	**10^{-5}**	**97**
6.	**10^{-6}**	**64**

CALCULATION

For Dilution 10^{-4}:

No. of. Cells present in sample $= \frac{\text{No. of. Colonies}}{\text{Volume of sample}} \times DF$

$= \frac{129}{1} \times 10^{-4} = \frac{129}{1000}$

$= 1.29 \times 10^{-2}$ CFU/ML

For Dilution 10^{-5}:

No. of. Cells present in sample $= \frac{\text{No. of. Colonies}}{\text{Volume of sample}} \times DF$

$= \frac{97}{1} \times 10^{-5} = \frac{97}{10000}$

$= 9.7 \times 10^{-3}$ CFU/ML

MICROSCOPIC EXAMINATION

STAINING	RESULT
Simple Staining	Rod
Gram Staining	Positive
Motilily	Motile

BIOCHEMICAL TEST

TEST NAME	RESULT
Indole	Positive
Methyl Red	Positive
Voges Proskaeur	Negative
Citrate	Positive
Starch Hydrolysis	Positive
Casein Hydrolysis	Positive
Lipid Hydrolysis	Negative
Gelatin Hydrolysis	Positive
Urease	Positive
Catalase	Positive

TITRATION

CALCULATION

Normal Urine

$$\text{Ammonia\%} = \frac{(\text{reading}) + 0.5 \times 17 \times 100}{1000 \times 5}$$

$$= \frac{42 + 0.5 \times 17 \times 100}{1000 \times 5}$$

$$= 0.178\%$$

Treated urine

$$\text{Ammonia\%} = \frac{(\text{reading}) + 0.5 \times 17 \times 100}{1000 \times 5}$$

$$= \frac{30 \times 0.5 \times 17 \times 100}{1000 \times 5}$$

$$= 0.176\%$$

Table:3 TITRATION METHOD

S.NO	SAMPLE	AMMONIA%
1.	Normal Urine	0.178%
2.	Treated Urine	0.176%

GC – MS analysis

Chemical composition of Normal urine

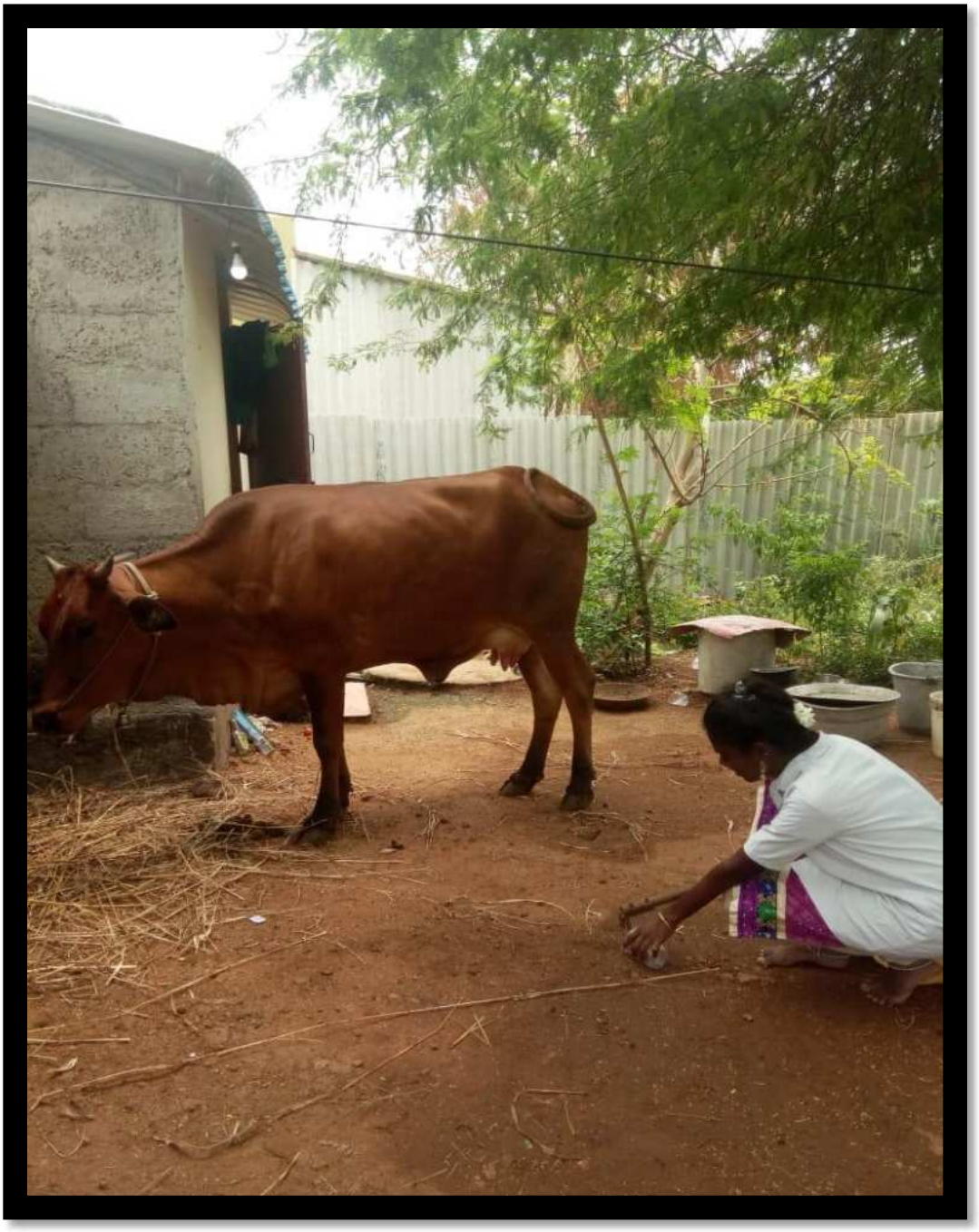
Hexane Extract	Retention time	Name of the chemical compound	Molecular formula	Range
	21.752	Hexamethyl cy clot ri siloxane	C6H18O3Si3	49
	21.752	Silane, 1,4-phenylenebis[trimethyl]	C14H26Si2	60
	21.752	2-Ethylacridine	C15H13N	41
	20.341	(2-ethylhexyl) hydrogen phthalate	C16H22O4	60
	11.986	Phenol, 2,4-di-tert-butyl	C14H22O	87
	11.486	Propanoic acid	C3H6O2	14
	11.986	Phenol, 2,5-bis(1,1-dimethylethyl)	C14H22O	83
	20.685	Benzo[h]quinoline, 2,4-dimethyl-	C15H13N	35
	21.754	De ca methyl tetra siloxane	C10H30O3Si4	38
	20.341	Phthalic acid, 2-ethoxyethyl propyl ester	C19H28O4	43

Chemical composition of Bacterial broth Treated Urine

Hexane Extract	Retention time	Name of the chemical compound	Molecular formula	Range
	21.752	Hexamethyl cy clot ri siloxane	C6H18O3Si3	47
	21.752	Silane, 1,4-phenylenebis[trimethyl]	C14H26Si2	38
	21.752	2-Ethylacridine	C15H13N	38
	20.341	(2-ethylhexyl) hydrogen phthalate	C16H22O4	59
	11.986	Phenol, 2,4-di-tert-butyl	C14H22O	64
	11.486	Propanoic acid	C3H6O2	10
	11.986	Phenol, 2,5-bis(1,1-dimethylethyl)	C14H22O	52
	20.685	Benzo[h]quinoline, 2,4-dimethyl-	C15H13N	43
	21.754	De ca methyl tetra siloxane	C10H30O3Si4	48
	20.341	Phthalic acid, 2-ethoxyethyl propyl ester	C19H28O4	43

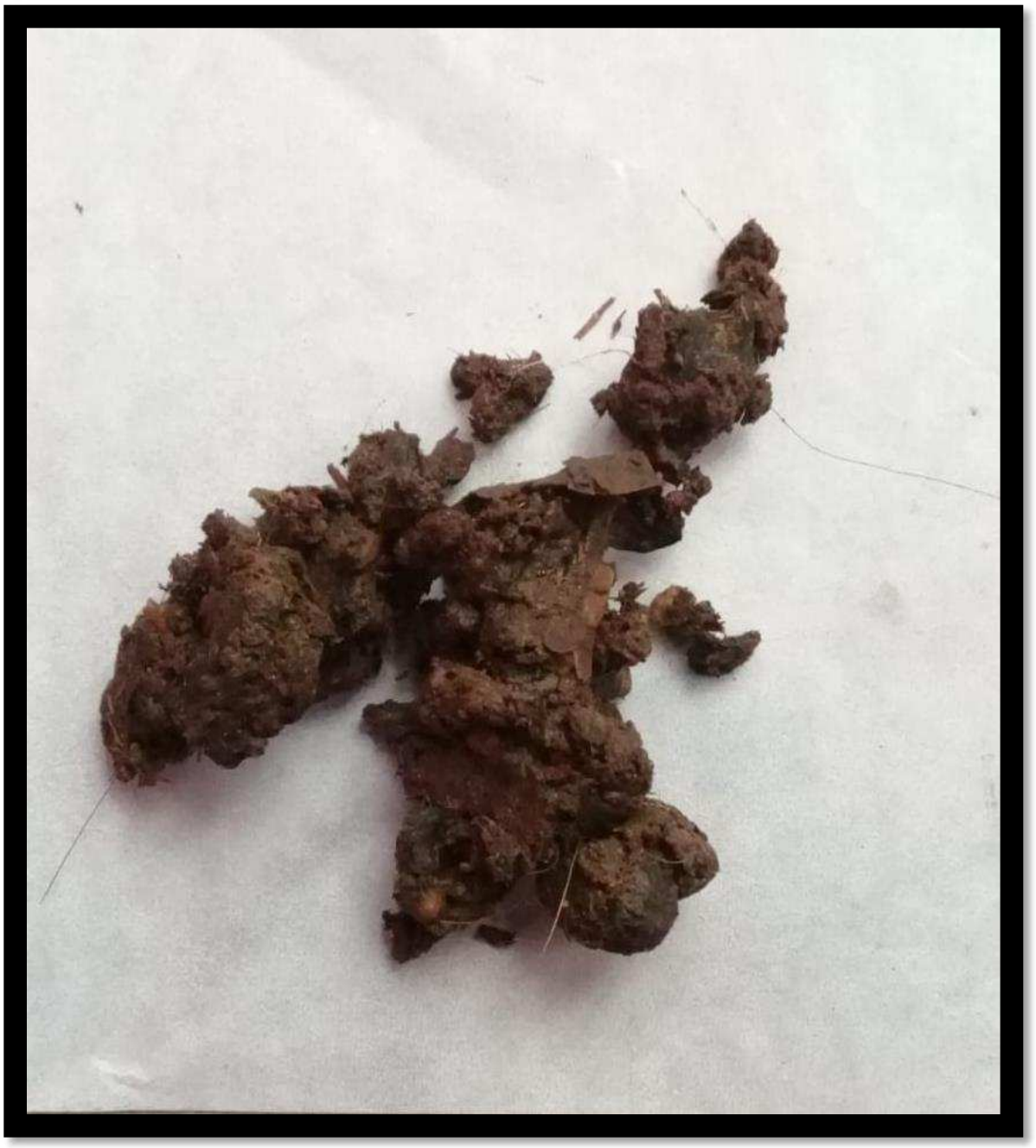
IMAGES

SOIL SAMPLE COLLECTION



(Fig: 1)

COLLECTED SOIL



(Fig: 2)

PREPARATION OF SOIL EXTRACT

(Fig: 3)

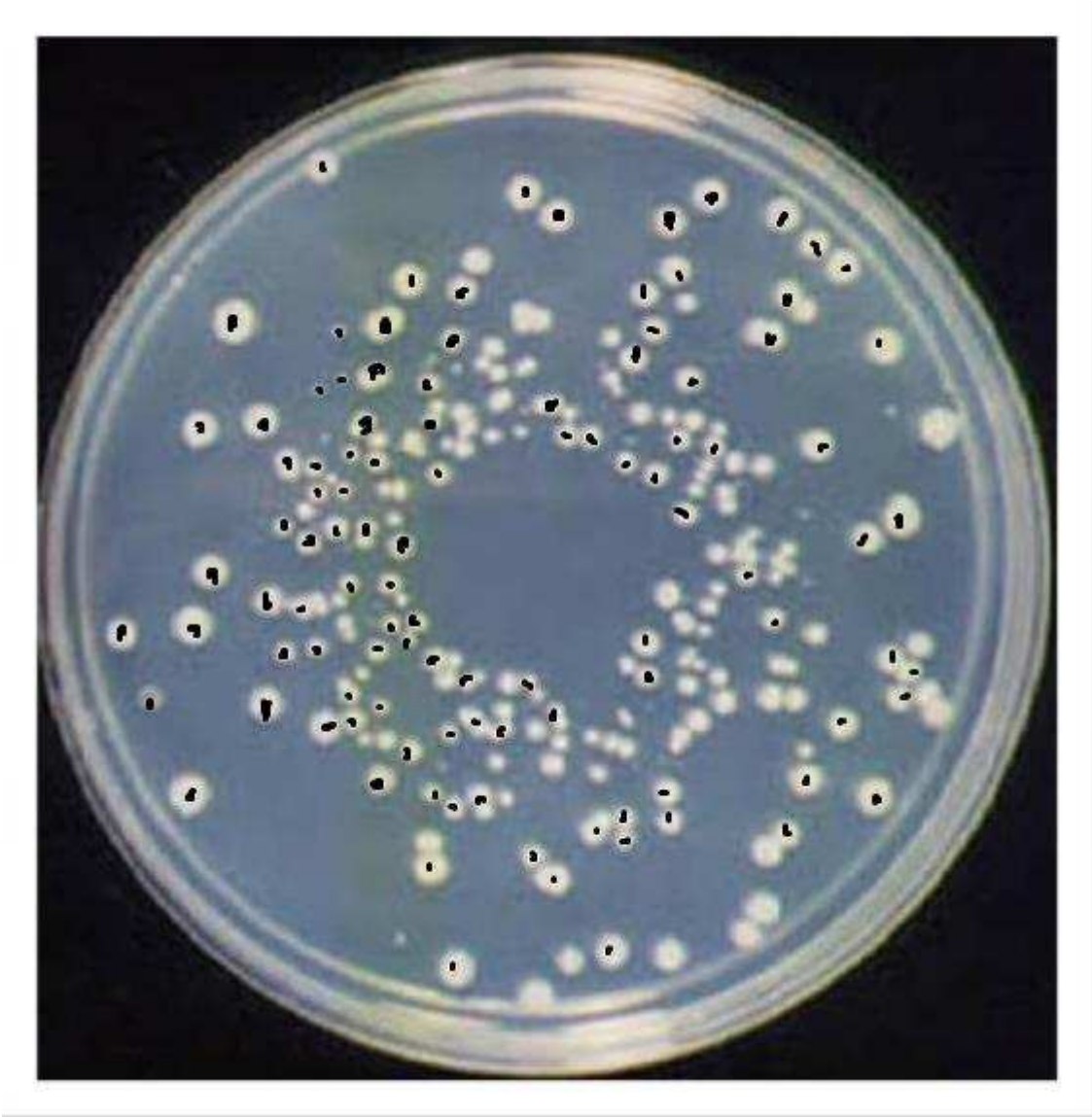
SERIAL DILUTION

(Fig: 4)

SPREAD PLATE METHOD
DILUTION 10^{-4}

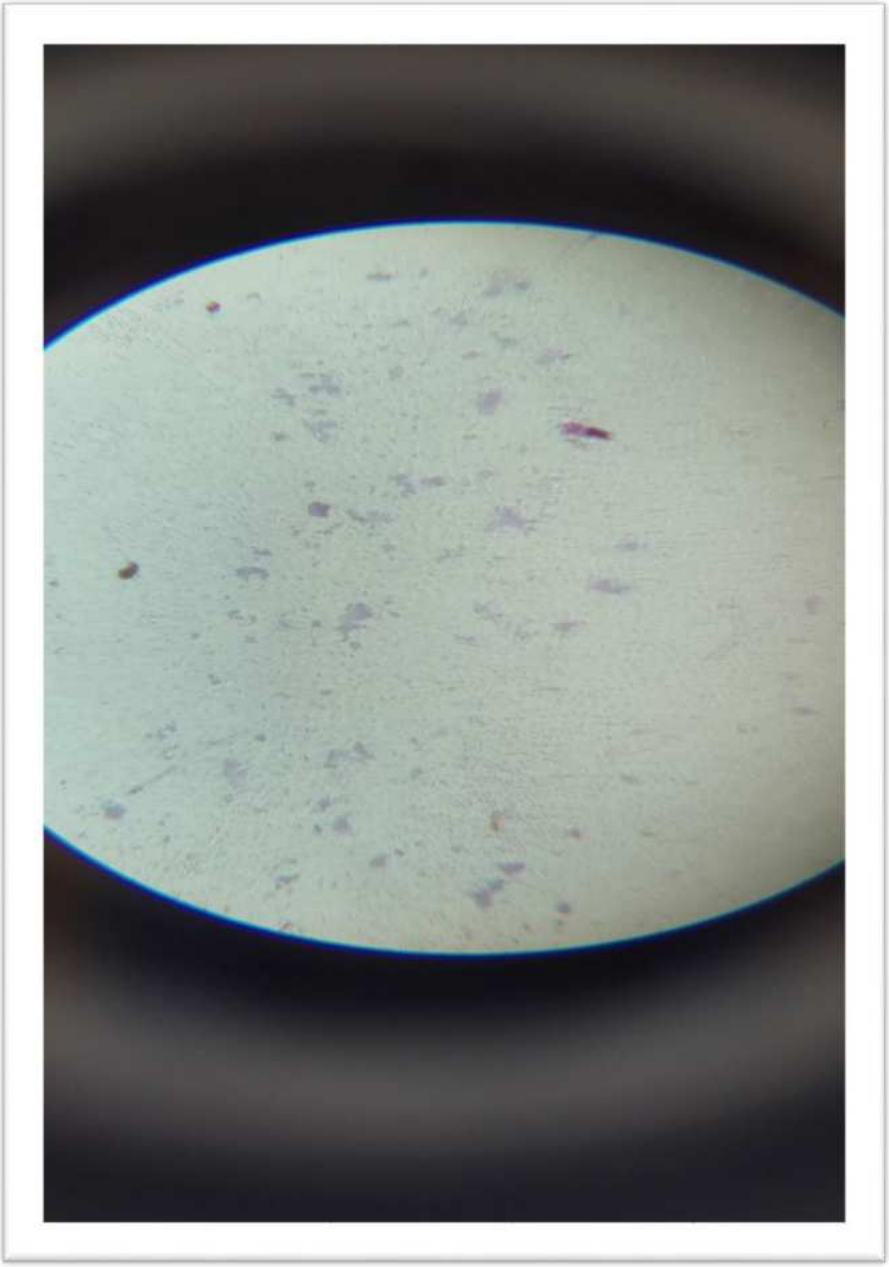
(Fig: 5)

SPREAD PLATE METHOD
DILUTION 10^{-5}



(Fig: 6)

GRAM STAINING



(Fig: 7)

IMVIC TEST

INDOLE TEST

(Fig: 8)

METHYL RED TEST

(Fig: 9)

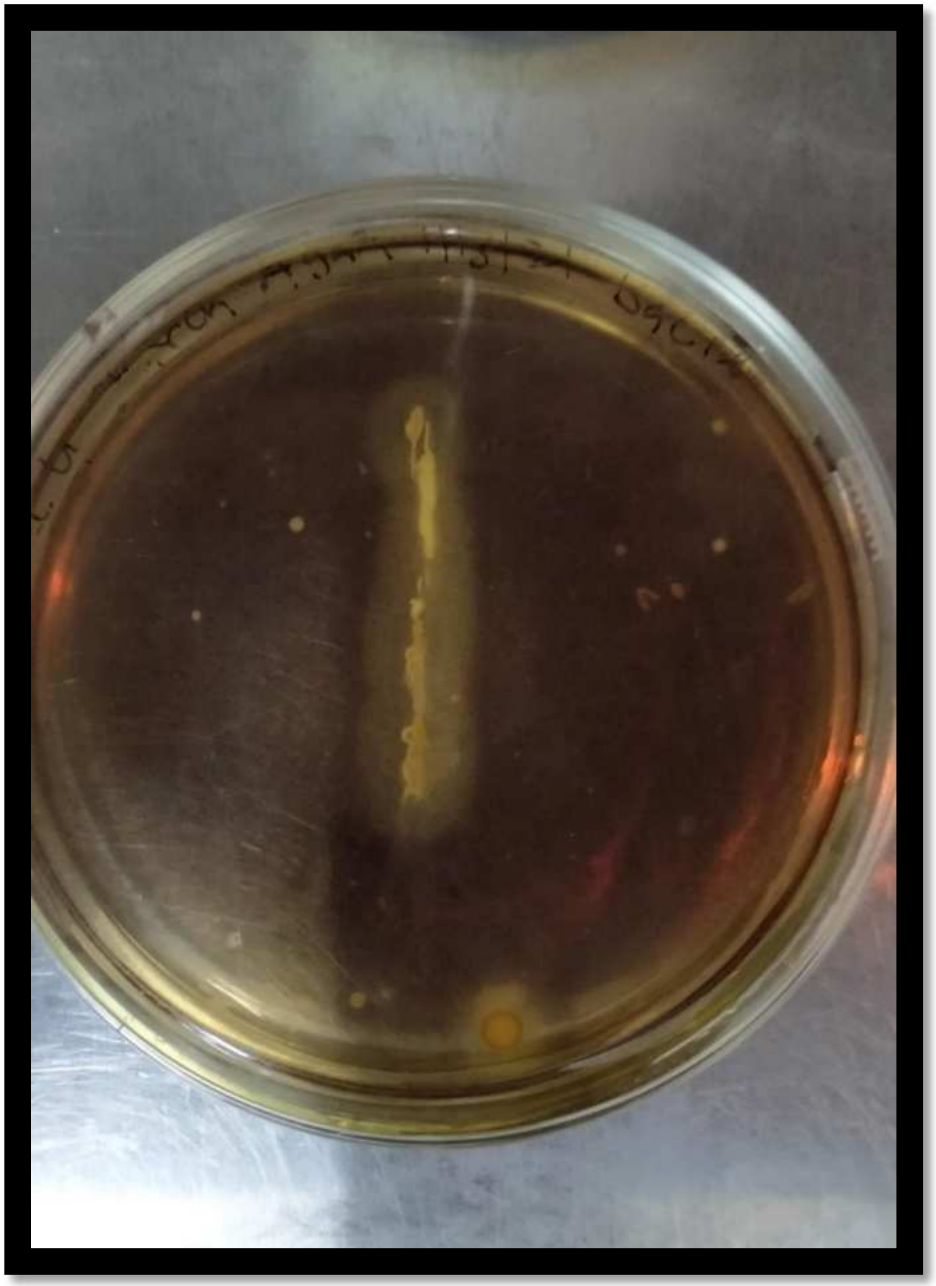
VOGES PROSKAUER TEST

(Fig: 10)

CITRATE TEST

(Fig: 11)

STARCH HYDROLYSIS



(Fig: 12)

UREASE TEST

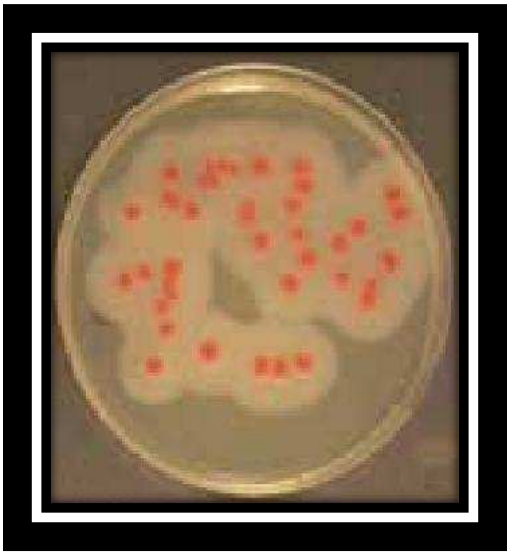
(Fig: 13)

CATALASE TEST

(Fig: 14)

GROWTH ON SELECTIVE MEDIA

(Fig: 15.1 *Bacillus* Agar Medium)



(Fig: 15.2 MYP Medium)

(Fig: 15)

CULTURE PREPARATION FOR REDUCTON TEST

(Fig: 16)

TITRATION

(Fig:17)

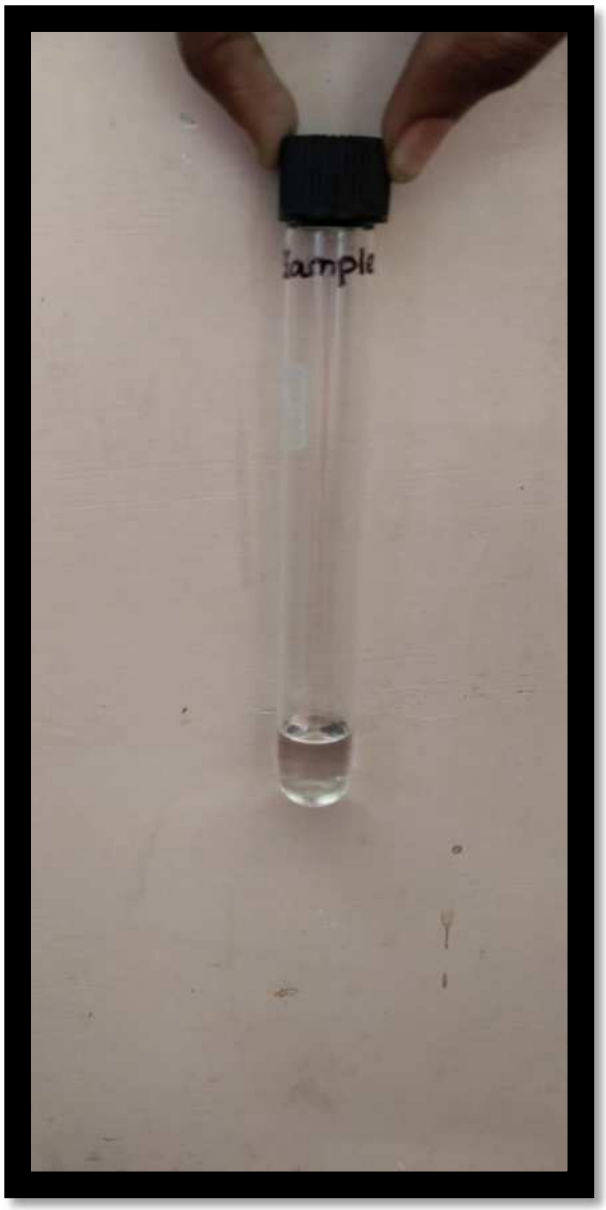
TITRATION RESULT

(Fig: 18)

PREPARATION OF SAMPLE FOR GC-MS ANALYSIS

(Fig: 19)

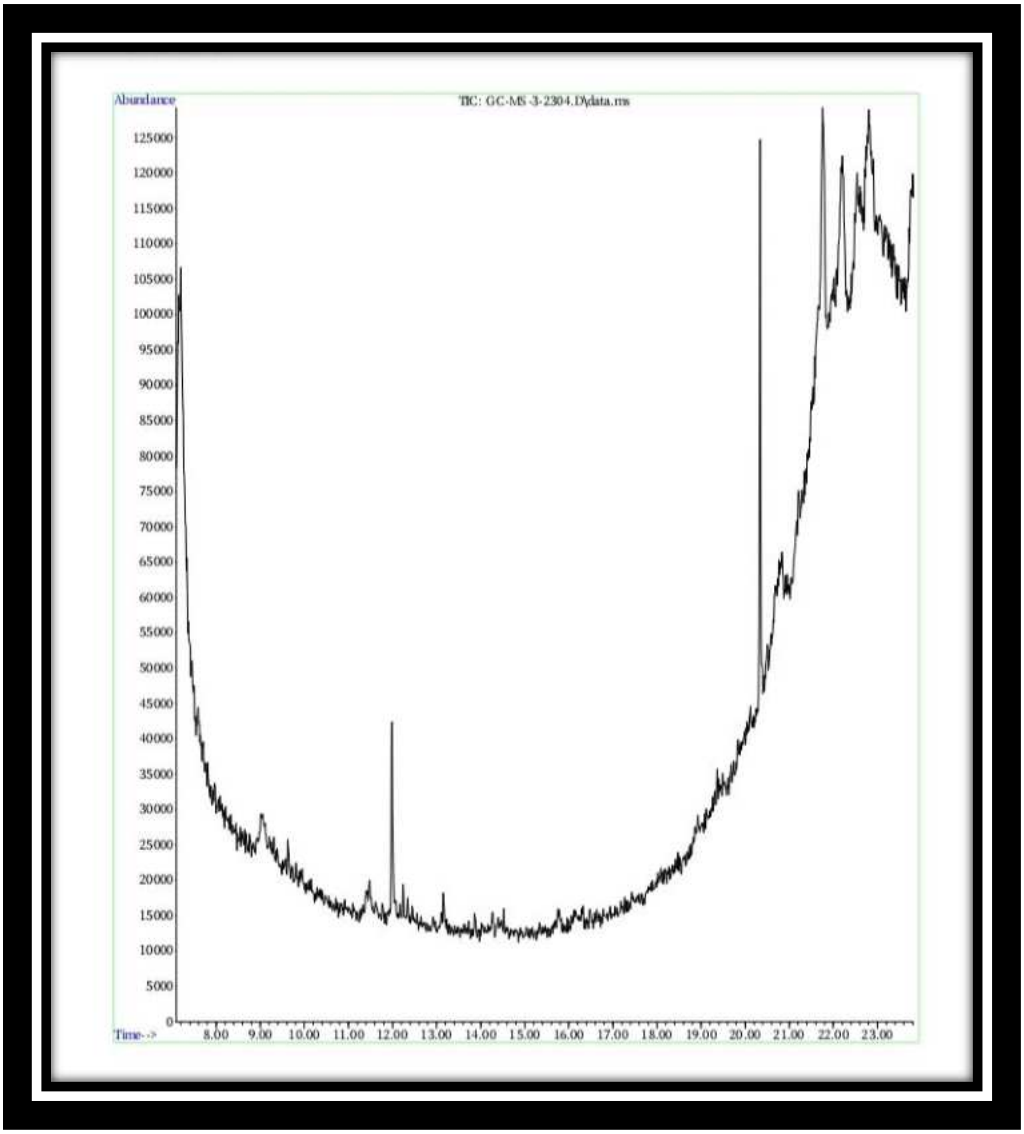
GC-MS SAMPLE



(Fig: 20)

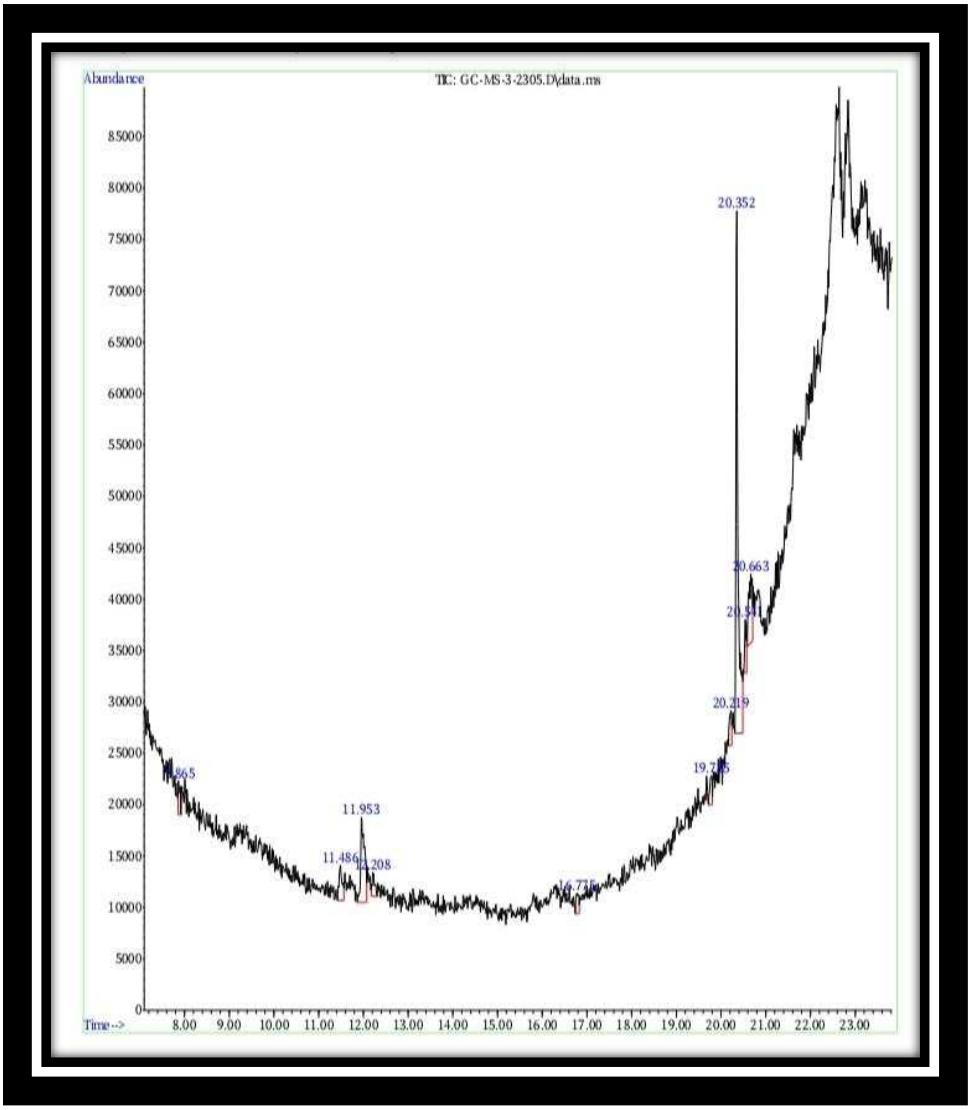
GCMS

NORMAL URINE CHROMATOGRAM



(Fig: 21)

TREATED URINE CHROMATOGRAM



(Fig: 22)

DISCUSSION

DISCUSSION

The results of experiments conducted in connection with study of ability of urine human odour reduction of bacteria isolated from the Cow's Shelter soil are discussed as follows.

The total number of colonies isolated from the serial dilution method are given in (Table 1and Fig 4,5,6) and it reveals that the number of colonies in each plate decreases as the dilution factor increases and (10^{-4} and 10^{-5}) dilution plates were selected for further study. Then microbial identification of colonies were performed based on Bergeys's manual, and from the results of microscopic examination, Biochemical characterization and cultural characteristics on growth medium, it is confirmed that the isolated colony is ***Bacillus Sps***.(Table 2 & 2.1) (Fig 7 - 16). Further more in order to determine the ability of urine odor reduction of isolated culture, the culture crude broth was treated with urine sample and after incubation the samples were titrated with sulfuric acid, after titration the amount of ammonia in control urine was found to be 0.178%, while in the bacterial broth treated urine was 0.176% (Table 3), from the result it clearly indicates that, the organism has the ability to reduce the ammonia to a certain extent (0.02%). .Boric acid titration yielded a higher recovery rate (99.2 %) of ammonia than back titration (98.6 %). Nevertheless, both titration methods fulfilled the recovery requirements of ± 2 % (98 % - 102 %) (J R Huizenga *et al.,* 1994). The effects of a variety of substances which either may normally appear in urine, or be experimentally added, were also evaluated. No significant alterations of ammonium measurements were produced by para-amino Hippurate (PAH), 2 g/l00 ml; creatinine, 1 g/l00 ml;

urea, 10 g/100 ml; uric acid (saturated); NaCl, 400 mEq/liter; KC1, 320 mEq/liter; or CaCl2, (JULIA.A. *et al*., 2014).Next to titration assay differences in the chemical components of normal urine and crude broth treated urine were analyzed using GC – MS assay. The results of GC – MS assay (Hexamethyl cy clot ri siloxane 49 and 47, Silane, 1,4-phenylenebis[trimethyl] 60 and 38, 2-Ethylacridine 41 and 38,(2-ethylhexyl) hydrogen phthalate 60 and 59, Phenol, 2,4-di-tert-butyl, 87- 64, Propanoic acid 14 and 10) clearly indicate that there is variation in the range and significant alteration in the urine chemical components after treating with bacterial extract (Table 4 and 5& Fig 17 and 18).Now in many clinical and diagnostic laboratories, automated, quantitative, and qualitative procedures for complex mixture analysis of urinary organic acids by GC/MS are used.All six quantified AAs (OTOL, 26DM,OANS, 1AMN, 2AMN and 4ABP) and corresponding structural isomers—m-toluidine (MTOL), p-toluidine (PTOL), 2,3-dimethylaniline (23DM), 2,4-dimethylaniline 24DM), 2,5-dimethylaniline (25DM), 3,4-dimethylaniline (34DM), 3,5-dimethylaniline (35DM), m-anisidine (MANS), p-anisidine (PANS), 2ABP, and 3ABP were separated using GC.(Shrila Mazumderet.al 2019). First-void urine samples were obtained from 24 elderly, asymptomatic men (median age 62.9 years). The headspace above pH adjusted urine samples were extracted using a carboxen/polydimethylsiloxane soild base micro extraction fibre and volatile organic compounds analysed by gas chromatography/mass spectrometry. A total of 147 compounds were identified in the headspace of urine. Four out of the nine bacterial strains that were capable of degrading butyric acid isolated from pit latrine faecal sludge were of *Bacillus genus*. These efficiently butyric acid degrading strains were identified as *Bacillus cereus, Lysinibacillus fusiformis, Bacillus subtilis and Bacillus methylotrophicus* with nucleotides

homology of 100%. To the best of our knowledge this is the first time that these bacterial species of *Bacillus* genus has been reported for biological deodorization application. The use of indigenous bacterial strains with butyric acid degrading capabilities as seed onto pit latrine contaminated with butyric acid could prove a more environmentally-friendly approach to bioremediation, which would have enhanced sustainable development rather than the use of alien bacterial strains. The results suggested that the bacterial strains could be potential candidates for pit latrine biological deodorization (John B.J *et al.,* 2019). The removal characteristics of dimethyl sulfide (DMS) with a peat packed tower were studied. The peat itself did not remove DMS. The peat inoculated with activated sludge as a source of microorganisms showed an efficient removal of DMS. Dominant microorganisms for degradation of DMS in the peat packed tower were some chemo lithotrophic and non-acidophilic sulfur-oxidizing microorganisms originating from sludge. A dominant DMS-oxidizing strain Au7 was isolated and identified as chemo lithotrophic *Thiobacilli.* (Xiaohui WANG *et al.,* 2011).

Based on the results of above study, it is clearly evident that *Bacillus sps* isolated from the soil of Cow's shelter and its metabolic products can be used as a substitute for chemical deodorizer, as it has the ability to reduce the ammonia in the urine through its chemical components and biological activity while ammonia is formed from urea degradation. Moreover, the production of aroma compounds from microbial culture or their enzyme preparations offers several advantages over traditional methods. Hence, it is recommended that the microbial metabolites can be produced in large quantities by the use of solid-state fermentation and can give high quality yields with better product characteristics along with low economical costs. It is also concluded that in future, further analysis of Cow shelter's soil on microbial diversity may offer better understanding and deep

insight in to microbial based deodorants, flavors and fragrance, that are used in home cleaning products and cosmetic industries. Furthermore, exploring knowledge on microbial diversity of this specific soil region, based on 16s r^{RNA} sequencing and other molecular approaches, may promise them to be used as cell factories for the production of wide range of bio products, used in Household cleaning product industries.

SUMMARY

SUMMARY

In this present research, an attempt was made to study the ability of bacteria isolated from the Cow shelter's soil to reduce the human urine odor as a substitute for chemical deodorizer. Initially the bacteria from the Cow Shelter's soil was isolated by serial dilution technique and identified with microscopic examination, biochemical tests and cultural characterization. The organism involved in reduction activity was identified as *Bacillus sps* based on the above tests results. The urine odour reduction ability of organism was tested with crude bacterial broth extract along normal urine by incubating at 35^{o}C for 21 days. After incubation period the samples were titrated against sulfuric acid. The titrated values were calculated as the broth turns in to pink from its methyl red mixed red color. The titrating rate clearly indicated that of two important organisms tested for the reduction of ammonia the *Bacillus sps* shown reduction up to 0.2% compared to control. (0.178 and 0.176). To understand the variations in chemical compounds and reduction rate of ammonia both normal and bacterial extract treated urine GC – MS analysis was performed and the results indicate that is variation in the range and significant alteration in the normal urine chemical components (10) after treating with crude bacterial extracts. Hence it is finally concluded that *Bacillus* sps isolated from the cow shelter's soil and its microbial components could be used as a substitute for chemical deodorizer.

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Extraction and Categorization of Pigment Generating Bacteria from Vegetable and Its Application

A DISSERTATION SUBMITTED TO

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI.

Affiliated to Manonmaniam Sundaranar University,

In partial fulfilment of the requirements for award of the degree of

BACHELOR OF SCIENCE IN MICROBIOLOGY

SUBMITTED BY

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APRIL - 2021.

BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled "Extraction and Categorization of Pigment Generating Bacteria from Vegetable and Its Application" is a bonafide record of the original work completed by M. Syma Siddika (Reg. No: 18SUMB44), S.Tamilarasi (Reg. No: 18SUMB45), Teenu Thomas (Reg. No: 18SUMB46), S.Vanaja (Reg. No: 18SUMB47), N.Vincy (Reg. No: 18SUMB48) during the academic year 2020 - 2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by the Manonmaniam Sundaranar University.

Signature of the Guide

Signature of the H.O.D

Signature of the Director
Director
Self Supporting Courses
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

Signature of the Principal
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

15/04/21

Signature of the External Examiner

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Signature of the Guide **Signature of the H.O.D**

Signature of the Director **Signature of the Principal**

Signature of the External Examiner

DECLARATION

We hereby declare that the dissertation work entitled **" Extraction and Categorization Of Pigment Generating Bacteria From Vegetable and Its Application"** is a bonafide record of the original work completed by us during the academic year 2020-2021 in St. Mary's College (Autonomous), Thoothukudi and submitted as a partial fulfilment of requirements for the award of the degree of Bachelor of Science in Microbiology prescribed by the Manonmaniam Sundaranar University. We also affirm that this is an original work done by us under the supervision of P. Raja Rajeswari M.Sc., (Ph.D.) Assistant professor of department of Microbiology, St. Mary's College (Autonomous), Thoothukudi.

Signature of the Students **Signature of the Guide**

Place: Thoothukudi

Date:

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Abstract

Bacterial pigments are naturally produced by pigment producing bacteria. Presently it has vital role in food, textile and various industries as a source of natural dyes. In this study it was done to isolate different colored pigments from pigment producing bacteria using carrot, capsicum and decade coconut collected from the local market of Thoothukudi, Tamil Nadu. Yellow and orange colored bacterial colonies where isolated from carrot and Capsicum and developed into nutrient media. A pink colored bacteria was isolated from decayed coconut and developed into nutrient media. The morphological characters were studied and observed rod and cocci shaped organisms in Gram staining. Biochemical characteristics were also performed. Maximum production of pigment was observed at 35 degrees Celsius and pH 7. The pigments were extracted from the isolated microorganisms and it was freeze, dried and powdered. Extracted pigment was applied for dyeing fabric. It showed that fabric can observe the dye after 24 hours of soaking.

INTRODUCTION

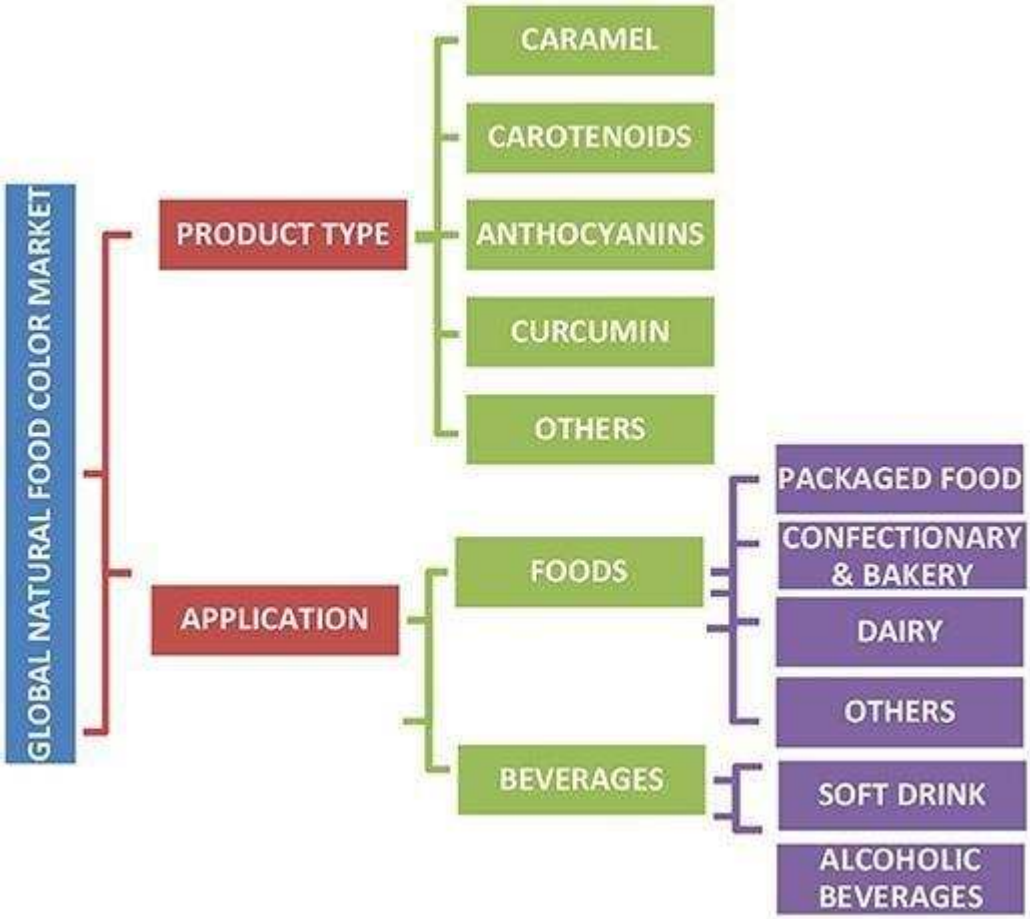
Color plays a significant role in the food production and processing sector, contributing to the sensory attribute of food. It signifies freshness, nutritional value, safety, and aesthetic value of a food, directly affecting the market value of the colored food product coloring is presumed to have originated back in 1500 BC. A Roman and Egyptians writings show activities such as the coloring of drugs and wine. In earlier times, most of the food coloring agents were derived from natural sources such as paprika, berries, turmeric, indigo, saffron, and various flowers. In the 1800's there was a shift toward development of synthetic colors due to their chemical stability, low production cost, and larger ranges of hue and shade. The first synthetic dye, Perkin's Mauve pigment, appeared in 1856 which also lead to the discovery of other synthetic dyes. However, possible side effects of synthetic colors like hyper-activity in children, allergenicity, toxicological, and carcinogenicity problems, has led to the banning of many synthetic food colorants further leading to a transition from the use of synthetic food colors, to natural ones An increase in the desire to label food as natural has also contributed food colorants exempt from certification generally include natural pigments, but no legal definition for the term ‘natural' has been adopted yet, leading to consumer, and industrial confusion. Colorants exempted from certification include a variety of pigments obtained from microbial, plant, mineral and animal sources but also include synthesized compounds that are identical to natural products, despite the common belief that colorants exempt from certification are all natural of a decline in the use of synthetic food colorants.

Natural colors are assumed safe if they are non-allergic, non-toxic, non-carcinogenic, and biodegradable, thereby rendering no risk to the environment Due to the lower risk advantage of natural colors and changing perceptions of consumers to consume natural products, there is an increasing interest in the discovery of new natural colors. The consumer demand for natural colors

and their growth as a category is predicted to increase by 7% annually in recent times, natural food colors have varied applications in the food industry, with almost all major natural pigment classes being used in at least one sector of the food industry

Fig 1: Global natural food color market

Natural colors are primarily derived from plants, insects, mineral ores or microbial sources. Microbial colorants are preferable because of scalability ease as well as a potentially lower cost of production .Microbial fermentation for the production of natural pigments have several



benefits such as cheaper production, higher yields, easier extraction, lower-cost raw materials, no seasonal variations, and strain improvement techniques to increase natural pigment .These can also have health benefits like anticancer activity, antimicrobial activity and antioxidant activity Microbes produce a variety of pigments that can be used as food colors such as carotenoids,

Flavin's, melanin's, quinines, monascins, violacein, amongst others. They can also be used as additives, antioxidants, color intensifiers, and functional food ingredients. Advances in organic chemistry and metabolic engineering have enabled the mass production of microbes of interest. Studying the biosynthetic pathway for pigment production can help in understanding the roadblocks in the production of pigments and to counter that, genes can be cloned, and recombinant DNA technology can be used to increase pigment production. Using the appropriate fermentation strategies and modifying conditions to be more suitable for the production of pigments, developing low cost processes and extraction processes, co-pigmentation strategies, have all been applied for efficient microbial pigment production. Newly emerging tools such as nanotechnology has also been effectively used in the food industry, including in pigment formulation. Natural food colorants derived from microbial sources can increase stability, shelf life, or solubility, leading to better delivery systems for food, and feed. The present review focuses on the potential of microbial pigments used as food colorants, their benefits and challenges; explores possible strategies for simplifying the process for overproduction of pigments in microbial systems, as well as the methods to improve pigment stability and formulation.

HISTORY OF COLORANTS

Until the mid-19th century all dyes were obtained from animal or plant extract. The textile industry used natural pigment, such as turmeric cochineal, wood madder, or henna. In 1856 H.Perkijn established the first industrial unit of organic synthetic dyes to produce mauve. A few years later the discovery of diazotization and a coupling reaction by Peter Griess was the next major advance for development of the color industry. In the 19th century, synthetic organic dyes were developed, creating a more inexpensive research and developed. The economic significance of the color industry is the clearly reflected in the large number of synthesized compound as many as 700 colorants are currently available.

Toward the end of the 19th century, when synthetic colors were first adopted for use on a large scale, they were hailed as a considerable technological breakthrough. The term synthetic was associated with the idea of progress and synthetic colorants were actually considered safer in food than the naturals, as they were pictorially much stronger and consequently a smaller quantity was needed to achieve a specific colored effect. Synthetic colorants were used in foods, medicines and cosmetics, but through the years their important reduced. This cut back of synthetic colorants started about five decades ago. All synthetic food compounds suffered severe criticism including synthetic additives and mostly pigments. Color additives were one of first man-made (synthetic) products regulated by law. Today, all food color additives are continuously regulated by federal authorities to ensure that foods are safe to eat and accurately labeled.

PIGMENTS

Biological pigments, also known as pigments or Bio chromes, which are the substances that produced by living organisms. Biological pigments include plant pigment and flower pigment. The various microorganism such as Micrococcus, Bacillus, Rhodotorula, Monascus, Phaffia, Sarcina and Achromobacter have the capability to produce different pigment. These colors have number of beneficial properties like anti-immunosuppressive, anti- proliferative, bio degradability etc.

NATURAL PIGMENTS

The natural pigment extracted from the microorganism are termed as ‘’microbial pigment’’ shows the naturally derived pigments from microorganism. Microorganism’s bacteria, algae, and fungi produce variety of pigments and therefore, are the promising source of food colorants. These pigments from biological or microbial sources have desirable properties like stability to heat, light and pH.

Plant pigment plays an important role in plant metabolism, and they are also important for human. Each pigment category has a family of compounds within them, each with a unique name, chemical structure, chemical properties and unique color.

Plants pigments are classified into chlorophyll, anthocyanin, carotenoids, and betalins. Vegetables contain several classes of pigments like green chlorophyll, the yellow orange, red carotenoids, red-blue, violet anthocyanin, and red- violet betalins.

Pigment that has synthetic origin creates a toxicity problem therefore it creates a mounting in interest on nature pigment that are originated from sources are good alternative one to synthetic pigment. Normally natural pigment is obtained from mainly two sources, they are plant and microorganisms. The pigment obtained from plant have many draw backs like instability against light, or adverse pH, low water solubility and are often non availability throughout the year. The microbial pigment known for its good stability and the availability of cultivation technology. They can be easily produced and fast growth in cheap culture medium. They are independent from weather conditions and colors of different shades. Genes *Micrococcus* produce yellowish pigment carotenoids. It's the most prominent pigment present in the microbial world.

MICROBIAL PIGMENT

Microbial pigments are characteristics property of some bacteria, which are useful in the identification. Bacterial pigments offer various applications due to their increased biodegradability and increased compatibility with the environment.

MICROBIAL PIGMENTS THAT CAN BE USED AS A FOOD GRADE COLORS

Some of the major pigments found in micro-organisms which are used as food colorants are canthaxanthin, astaxanthin, Prodigiosin, phycocyanin, violacein, riboflavin, beta-carotene, melanin, and lycopene. Microbial pigments can be either inorganic or organic, although organic pigments tend to be more useful as food colorants.

1. Canthaxanthin- is an orange to deep pink colored carotenoid that is lipid soluble and a potent antioxidant. It is isolated from *Brady rhizobium Sp*, is a trans-carotenoid pigment, and is approved as a food colorant and used in a range of foods as well as salmon and poultry feed.
2. Astaxanthin- is a red-orange pigment, naturally found in basidiomycetes yeast, microalgae, salmon and crustaceans, red shrimp, cray fish, feathers of some birds and is lipid soluble. It's an approved coloring agent used in fish and animal foods.
3. Prodigiosin- Many strains of *Serratia marcescens*, produce a red pigment, which shows antibacterial, antimalarial, antibiotic and antineoplastic activity. It has been successfully applied as coloring agents in yogurt, milk and carbonated drinks.
4. Phycocyanin- is a blue pigment produced by chlorophyll A containing cyanobacteria. *Aphanizomenon flos-aquae* and *Spirulina* produces phycocyanin which is being used in the food and beverage industry as the natural coloring agent 'Lina Blue' and is also found in sweets and ice cream.
5. *Violacein- Chromobacterium violaceum* is one of the most prominent producers of this purple pigment, other bacterial species also produces the pigment and mostly have a purple hue. It exhibits antifungal, antibiotic, antitumor and antibacterial properties. Violacein has shown potential use in food, cosmetic and textile industries.
6. Riboflavin- Water soluble vitamin B2, is a yellow colored pigment and produced by various microorganisms. It is used in diary items, breakfast cereals, baby foods, sauces, fruit drinks, and energy drinks.
7. Beta-carotene- A red-orange colored organic pigment, mostly extracted from the beta-carotene rich algae, Dunaliella salina. Production of β-carotene through fermentation of *Blakeslea trispora* produces a pigment equivalent to pigments produced through a

chemical process and is an acceptable coloring agent. It is used in a variety of food items ranging from red to yellow in color.

8. Melanin- Melanin's are natural pigments present in animals, plants and in many micro-organisms. They are widely used in eye glasses, cosmetic, food items, sunscreen protection creams, pharmaceuticals and food items.
9. Lycopene- widely present and consumed in tomatoes, a brilliant red pigment consisting of carotenoid. It has been isolated from microbes like Fusarium, Sporotrichioides, and Blakeslea trispora, and has the potential to attenuate persistent diseases such as some types of cancers and coronary heart disease. It is used in meat coloring in countries like the USA, Australia and New Zealand

Table 1. Microbial pigments that are being used or with high potential to be used as natural food colorants.

Serial no	**Micro- organisms**	**Pigments**	**Color/appearance**
1.	*Staphylococcus aureus*	Zeaxanthin	Golden yellow
2.	*Serratia marcescens*	Prodigiosin	Red
3.	*Phaffia rohodozyma*	Astaxanthin	Red
4.	*Blakesela trispora*	Lycopenebeta carotene	Red yellow- orange
5.	*Flavobacterium spp.*	Zeaxanthin	Yellow
6.	*Pseudomonas aeruginosa*	Pyocyanin blue	Green
7.	*Dunaliella salina*	Monoscorubramin, Rubropunctatin	Cream

Now a days the use of bacteria or natural pigments is increased in food, cosmetics, pharmaceuticals, textiles, and in printing dyes industries by the increased awareness of toxicity of

synthetics colors.

Colors plays an important role in food. The word bio color stands for something natural of coloring purpose. These colors are taken from the fruits, seeds, roots, vegetables and also from microorganisms and they are also known as ‘’ bicolor’’. The natural pigment extracted from the microorganisms is called ‘’Microbial Pigments’’. The colored pigments are produced by Microorganisms bacteria, Algae, and Fungi. Pigmented Microorganisms shows anti-cancer properties and it has a provitamin-A source. These colors are used for artificial coloring in food. In food industry, a large amount of food wastes like peel, sags, kernel, seeds are used for the production of substrate of which bicolor producing microbes.

Plants pigments are classified into chlorophyll, anthocyanin, carotenoids, and betalins. Vegetables contain several classes of pigments like green chlorophyll, the yellow orange, red carotenoids, red-blue, violet anthocyanin, and red- violet betalins.

Microbial pigmented cells are widely used in industrial application. In traditionally peoples are more desired to prefer in natural sources to add color in clothes, medicines, cosmetics, food etc.

THE BENEFITS OF USING MICROBIAL PIGMENTS AS FOOD GRADE COLORING AGENTS

Micro-organisms are found in almost every environmental niche and have various roles in nature. They are also affiliated with food and are accountable for the fermentation of food products. Microbial pigments are a better alternative to synthetic food colors compared to plants because of their availability, non-seasonality, scalability, higher yield per hectare, and straight forward down streaming processing. Microbial pigments like that of Monascus, Arpink Red (natural red- industrial name) from Penicillium oxalicum, and β-carotene from Blakeslea trispora

and Astaxanthin from various microbes are already used in the food industry to color foods. A lot of research has been done to lower production and processing costs for natural colors, to increase stability and shelf life, so that it can compete with the use of synthetic colors. Many of these pigments not only work as coloring agents but also impart health benefits (Bioactivity of various microbial pigments mentioned in table. Micro-organisms produce a large quantities of pharmacologically and biologically active compounds that can have a diverse range of activities, including antioxidants, antimicrobial, anticancer, immuno-regulatory, and anti- inflammatory compounds.

ANTIOXIDANT ACTIVITY

Microbial pigments like violacein, carotenoids, anthocyanins, and naphthoquinone have been shown to be potent antioxidants agents. Violacein which is a purple pigment largely produced by Pseudoalteromonas and Chromobacter violaceum is a powerful antioxidant which stimulates mucosal defense mechanisms to protect against oxidative damage in gastric ulcers. Staphylococcus aureus produces a yellow pigment called staphyloxanthin, that prevents carbon tetrachloride induced oxidative stress in swiss albino mice. There are many other pigments that can act as antioxidants such as Astaxanthin, Granadaene, Canthaxanthin, Lycopene, Riboflavin, β- carotene, Torularhodin, etc.

ANTICANCER PROPERTY

Anticancer activities in microbial pigments have been reported in a number of studies. These pigments can induce apoptosis, which lead to the destruction of cancerous cells. Scytonemin which is a green-yellow pigment, produced by the aquatic cyanobacteria, inhibits the action of the cell cycle regulatory protein kinase, thereby showing an antiproliferative effect. Prodigiosin is red

pigment `which is a potent anticancer compound, produced by *Serratia marcescens* and *Pseudomoalteromonas rubra*. It shows an apoptotic effect against human cervical carcinoma. Anticancer activity is shown by synthetic indole derivatives and analogs of Prodigiosin in-vitro. Violacein showed cytotoxic effects on HL60 leukemia cells through a TNF signaling cascade and the activation of Caspase-8 and p38 MAPK. There are various pigments that can act as anticancer agents such as Astaxanthin, Canthaxanthin, Lycopene, Monoscorubramin, Riboflavin, Rubropunctatin, β-carotene, Torularhodin, and others.

ANTIMICROBIAL ACTIVITY

Many micro-organisms produce antimicrobial compounds, some of which are presently used as antibiotics. A pigment obtained from an endophytic fungus was shown to be more potent than the commercially available antibiotic Streptomycin. It was effective against bacteria like Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi and Vibrio cholera. It is known that violacein causes growth inhibition, additionally also killing the bacteria. It also exhibits antifungal, antiprotozoal and antiviral activities. The recent emergence of antibiotic and multi drug resistant microbial strains has led to a search for new and novel compounds that can be used as antibiotics. Finding novel microbial pigments that have both pigment producing and antimicrobial properties is highly advantageous.

CHALLENGES FACED IN NATURAL FOOD COLOURS

Natural pigments have many product challenges with respect to cost, application, process, and quality. Microbial pigments have a weaker tinctorial strength and may react on different food matrices, causing undesirable flavors and odors. Synthetic food colorants that the food industry came to rely on over the past 50–60 years are relatively well- behaved and consistent in their performance. Replacement of synthetic colors with natural colors in the food industry is

challenging, particularly with regard to the relatively low range of natural colors approved for food use.

Deodorization is another issue that arises in natural pigment products as many of the available natural pigments have an odor that is undesired in the food products. Furthermore, natural colors are generally more sensitive to light, pH, UV, temperature, oxygen, and heat, leading to color loss caused by fading and a decreased shelf life. Some natural pigments are sensitive to other ambient conditions like metal ions, proteins or organic compounds. It is well-known that vitamin C will enhance the stability of beverage products, which are colored with carotenoids like beta- carotene and paprika oleoresin, but the same vitamin will cause the degradation of anthocyanins.

Major microbial pigments like carotenoids, chlorophyll, anthocyanins, and others also face such limitations. Carotenoids, which are strongly colored isoprenoid plant compounds and highly conjugated, are unstable when exposed to oxygen or light. Chlorophyll undergoes rapid degradation due to enzymatic reactions or factors like light, oxygen, heat or acid, leading to the formation of chlorophyll derivatives.

Formulation of these natural colors is challenging and methods such as micro-encapsulation can be applied to improve stability and in some cases solubility.

Many fungal pigments are prohibited as natural colorants due to the presence of mycotoxins. It is therefore important to use non-toxic and non- pathogenic strains for natural pigment extraction. When a promising pigment-producing microbe is discovered, metabolic engineering can be used for controlled biosynthesis of the pigment and toxin production.

Bicolor are those coloring agents which are obtained from the living sources. They are generally extracted from fruits, vegetables, roots, and microorganism .example Annatto, Anthocyanin,

Beetroot, carrot, tomato, onion, capsicum, organic colorants that are derived from natural edible sources using recognized food preparation methods.

Production of bio coloring is done in many methods by biotechnology approach by microorganisms by using certain enzymes. Biotechnology natural production is based on the genetic modification for pigment production, improving the traditional methods for extraction of pigments, Microbial production of pigments in vivo pigment production by plant tissue culture.

Microorganisms aided extraction the natural coloring extraction, especially those obtained through aqueous extraction or direct processing of plant materials. It also contains high amount of unwanted dissolved solids like salts, organic acids, phenolics, sugar etc.

Review of literature

The first synthetic color, mauvine was developed by a Sin William Henry Perkin in 1856 and this development started a history of synthetics colorants ‘’-Welford, 1980

Use of natural pigments in food is known from Japan in the Shoshoni text of the Nara period (8^{th} century) which contains reference to colored soybean and adzuki- bean cakes, so it appears that colored produced food had been taken at least by people of some section. Thus, studies on natural pigment are greatly impulse by their multiple function 7. Newsome RL. Food colors. Food Technology 1986.

Since color is an important attribute that determine the consumer acceptance of foods, color additives are important in food industry. As a result, various synthetic food has been manufactured but many of them comprise various hazardous effects – (Fabre *et.al* 1993)

In nature, color rich and pigment producing microorganisms (fungi, yeasts and bacteria) are quite common. Babu S Shenolikar IS. ‘’Health and nutritional implications of food color’. India journal of medical research 102(1995) 245-249

Due to toxicity of several artificial colorants uses of natural additives are of increasing interest. Increasing consumer awareness put string emphasis on the production of bio colors or natural color extracted from fruits, vegetables, roots, and microorganism. (Pattanaik *et.al* 1997)

Natural colors are generally extracted from the fruits, vegetables, roots and Microorganisms and are often called ‘’Bio colors’’ because of their biological origin (Pattanaik *et al* …1997).

Pigments produced from natural sources are of worldwide interest and is gaining significance. Natural pigments are obtained from ores, insects, plant and Microbes. (Pattanaik P. Roy U I 1997)

Pigments produced by bacteria are one of the traditional uses in oriental countries and have been a subject of intense research in the present decades of its potential for application (Henry ….*et*

al in 1997)

There is growing interest in the food industry in the use of natural ingredients. Ingredients, such as colors, are considered natural when derived from biological sources like plants or microorganisms. Pattanaik P. *et al* ‘’bio colors’’; New generation additives of food ‘’ Indian food industry 16.5 (1997); 21-32.

Nowadays there is rapid emergences of antibiotic resistance pathogens causing life threatening infections so it creates demand for new antibiotic in spite of considerable progress in the fields of chemical synthesis and engineered biosynthesis if antimicrobial compounds. (Carbonell GV, *et al*.’’clinical reference and virulence factors of pigmented Serratia marcescens’’. FEMS immunology and medical microbiology (2000) 143-149

Henna was used even before 2500 BC, while saffron is mentioned in the Bible Gulrajani Ml. Introduction to natural dyes. Indian J fiber texture Res 2001

The art of coloring spread widely with the advancement of civilization 8. Krishnamurthyku, Siva R, Senthil TK. Natural dye yielding plants shervaroy Hills Eastern Ghats 2002

Natural pigments posse’s anticancer activity, contain pro vitamin- A and have some desirable properties like stability to light, heat and Ph (Joshi…*et al* in 2003)

Pigment is the important group of organic constituents of bacterial protoplasm. some of these, like Prodigiosin, pyocyanin, violacein, phenazine, iodine and melanin are metabolic by products formed under special circumstances – (Giri AV, et al ‘’the novel medium for the enhanced cell growth and production of digiosin from Serratia marcescens isolated, BMC microbiology 4(2004);11

Microbial colorants are in use in the fish industry already, for example to improve the pink of the farmed salmon. In nature, color rich and pigment producing microorganism (fungi, yeast, and bacteria) are fairly common. (A, Motensen, 2006) (C.K. Venil *et al* 2013).

Among natural pigments, pigments from microbial sources are potentially good alternative to synthetic pigments (Dufossel….2006)

Natural pigments and synthetic dyes are extensively used in various fields of everyday life such as food production, textile industries, paper production and agriculture practices (Cserhati *et al* 2006)

Natural color or dyes derived from flora or fauna are believed to be safe because of non-toxic, non-carcinogenic and biodegradable in nature 1. Critead vilarem.G. G improving light fatneos of natural dyes on cotton yarn. Dyes pigment 2006

Traditional sources of colorants include natural product such as flavonoids and anthraquinones product by plant and animals. For example, carminic acid, a deep red anthra quinones produced color d by scale insects is now used a pigment in paints, crimson, ink, cosmetic, and food color 2. Dapson Rw. the history chemistry and modes of action of carmine and related dyes. Biotech Histochem 2007

Microbial pigments are of industrial interest because they are often more stable and soluble than those from plants or animal sources (Gunasekaran *et al* 2008).

Microorganisms produce various pigments like carotenoids, melanin's, flavins, quinones, Prodigiosin and more specifically manascins, violacein or indigo- Pufose L. ''pigments, Microbial' 'Encyclopedia microbiology 4(2009);457-471

Many artificial synthetic colorants are widely used in food stuff, dye, cosmetic, and pharmaceuticals manufacturing processes. The synthetic pigments which are used produce harmful effect to human and pollute water and soil. In textile industry during manufacturing and usage approximately 10-15%of the dye is lost directly to wastewater and pollute the environment (Palanivel Velmurugan *et al* 2009)

Prodigiosin a red color pigment from Serratia marcescens was also shown as an

antibacterial agent against gram positive and gram-negative bacteria (Mikael and Yousif 2009).

Bacteria pigment production is now one of the emerging fields of research to demonstrate its potential for various industrial application 3. Venil Ck Lakshmana perumalsamy p. An insightful overview on microbial pigment; Prodigiosin Ele JBiol 2009

Pigment producing Microbes are Bacteria, fungi, yeast, Microalgae and are quite common in nature. Microbes can produce a large a large amount of stable pigment such as carotenoids, Canthaxanthin, flavonoids,

Prodigiosin and some xanthophylls as astaxanthin. Hence, Microbial pigments production is now one of the emerging fields of research to demonstrate its potential for various industrial application (Goswami *et al*…. 2010)

Prodigiosin, from Serratia marcescens showed a potent apoptotic effect against human cervix carcinoma cells in a close dependent manner with a mean IC50 of 700 (Kavitha *et al* ...2010)

Most of the bacterial pigment production is still at the R&D stage. Hence, crook on the bacterial pigment should be intensified especially in finding cheap and suitable growth medium which can reduce the cost and increase its applicability for industrial production Ahmad As, `Ahmad. Application of Bacterial pigments as colorants. The Malaysian perspective. New York; springer briefs in molecular science; 2012

The pigment producing bacteria were identified as follow; yellow-orange as not gelatinous, regular and violets, although irregular variants and non-pigmented colonies can also be procedure for the isolation of Prodigiosin, a known red pigment produced. (Ahmad *et al*….2012)

Another important class of pigments comprises of carotenoids produced by bacteria, fungus, and algae which are known to enhance immune response (Lo *et al*...2013).

The industry is now able to some microbial pigments for applications in food, cosmetics or

textiles. Bello E Kizito and Nwankwo to ‘’ Antibiotic activity of Streptomyces isolates collected from soil of Kogi central, Nigeria ‘’. Journal of pharmacy and biological sciences 8.4 (2013);79-84

Pigment production from microorganism is easy, lesser requirements of expensive culture medium and it is independent of weather conditions (Bhat *et al* 2013).

Microorganisms such as fungi and bacteria provide a reality available alternate source of naturally derived pigments ‘’- (Arul Selvi *et al* 2014)

Pigments of extremophiles are very colorful and are required for respiratory and photosynthetic functions (Rokade and Pethe.,2016)

Colonies producing pigments were selected for further studies. Coagulase test. This test was done by inoculating the pigmented bacteria in tube containing 0.5% Pl.

MATERIALS AND METHODS

Collection of sample:

Vegetable such as carrot, capsicum and decayed coconut is collected from the V.O.C market, Thoothukudi district. All the fruits and vegetable sample were collected in a clean, try and sterile container. And the sample is immediately transferred to the laboratory in a sterile conditions from these samples, pigment producing bacteria were isolated and the bacterial culture is used for the present study.

Fig 1: Sample Collection

Media used:

Nutrient agar media is used for the screening of pigment producing bacteria the collected sample such as carrot, capsicum and decayed coconut.

Isolation of pigment producing organisms:

Carrot:

Collected carrot sample is peel off and crushed using a mortar and pestle. Then the extraction of carrot is collected in a beaker. Collected extracted carrot sample were used for serial dilution up to 10^{-7}. 10^{-2} to 10^{-4} dilution were plated and kept for incubation at 37^{0} c for 48 hours. The nutrient agar

plate were observed on 10^{-2} dilution of carrot sample. The pigment producing organism was observed on 10^{-2} of carrot sample. The isolated pigment producing organism from 10^{-2} dilution of collected sample was orange in color. Which was further streaked on sterile nutrient agar plate to obtain pure culture. The orange color pigment producing bacteria was further characterized for identification.

Capsicum:

Collected capsicum sample is peel off and crushed using a mortar and pestle. Then the extraction of capsicum is collected in a beaker. Collected extracted capsicum sample were used for serial dilution up to 10^{-7}. 10^{-2} to 10^{-4} dilution were plated and kept for incubation at 37^0 c for 48 hours. The nutrient agar plate were observed on 10^{-2} dilution of capsicum sample. The pigment producing organism was observed on 10^{-2} of capsicum sample. The isolated pigment producing organism from 10^{-2} dilution of collected sample was yellow in color. Which was further streaked on sterile nutrient agar plate to obtain pure culture. The yellow color pigment producing bacteria was further characterized for identification.

Isolation of bacteria from pinkish red discolored spoiled coconut

The pinkish red discolored spoiled coconut was swabbed using sterile swab and inoculated in 50ml of sterile nutrient broth in 250ml conical flask and incubated in rotatory shaker at 28^0 c for 48hours. After 48hours 10µl of reddish nutrient agar Petri plates and swabbed was done and incubated in incubator at 28^0c for 48 hour. The red pigment colony was selected and detailed morphological, biochemical characterization was done.

Purification of culture:

Pigmented bacterial isolates from carrot, capsicum and decayed coconut sample by streaking onto nutrient agar plate and it was incubated for 24 hours at 30^0c.

Maintenance of culture:

Pigmented bacterial cultures from carrot, capsicum, and decayed coconut sample were grown

on nutrient agar and it was maintained at 2-4^{0}c temperature in refrigerator and sub cultured into respective medium.

Characterization and identification of pure culture of pigment producing bacteria:

Colony characterization of pigment based on its shape, color, gram staining. The biochemical tests performed were indole, methyl red test, vogues-proskauer, urease, Simon, citrate, TSI, catalase, nitrate reductase test. Identification of isolates obtained in pure culture were characteristics, on media, microscopically by gram staining and various biochemical test recommended in the life science manual.

Gram staining:

The stain makes use of the differing membrane structures between gram positive (single cell membrane with a tough outer cell wall of peptidoglycan), and gram negative organisms (have two layers of membranes, with a thin layer of peptidoglycan sandwiched between them).

Prepare a bacterial smear and heat fixed on a slide, pour a few drops of crystal violet on a smear wait for 1 minute and wash with water. Now fixed the smear with gram's iodine for 1 minute and wash again with water and decolorize the stain with 95% ethyl alcohol drop wise, wash with water and counter stain with safranin (45 sec) and again wash with water. After dying examine under oil immersion.

Indole:

This is mainly used to perform whether the given organism has the ability to utilize tryptophan or not. Red layer formed on surface of the media showed negative result.

Methyl red test:

Methyl red test is used to differentiate the gram negative intestinal bacteria on the basis of end product formed by the fermentation of glucose. Some organisms produce large amounts of acids lower the pH to lower than 5.0. These organisms produce great amount of gas due to the presence of

the enzyme formic hydrogenase. Red color developed indicates positive result and yellow color developed indicates. Negative result

Voges proskauer test:

This test is mainly performed to find whether the given organism producers non-acid end product as alcohols. VP test detects the presence of acetoin, which is a precursor to 2, 3 butane diol.

Urease test:

The rapid urease test involves incubating a gastric biopsy in a urea broth that contains the pH indicator phenol red. If gastric helicobacter are present, helicobacter urease breaks down the urea, with the release of an ammonia, arise in pH and a color change occur.

Citrate test:

The citrate test detects the ability of an organism to use citrate as the sole source of carbon and energy test**.**

Triple sugar iron:

The Triple sugar iron test is a microbiological test roughly named for its ability to test a microorganism's ability to ferment sugars and to produce hydrogen sulphide.

Catalase test:

It is used to test the presence of enzyme catalase. (H_2O_2) accumulates, it becomes toxic to the organism. Catalase decomposes (H_2O_2) and permits the organism to survive solely obligate anaerobes lack this protein. Bubbling (O_2 gas is liberated from the H_2O_2) shows positive result and no bubbling denoted negative result.

Nitrate Reductase test:

It is used to differentiate between bacteria based on their ability or inability to reduce nitrate (NO_2) to nitrite (NO_2) using anaerobic respiration or nitrogen gas (N_2) by the enzyme nitrates. The production of nitrate to nitrate or nitrogen gas takes places under anaerobic condition in which an

Organism derives its oxygen from nitrate.

Nitrate broth were inoculated with bacterial suspension. Incubate the tubes at optimum temperature 30^0c or 37^0c for 24 hours. After incubation look for nitrogen gas before adding the reagents. Add 6-8 drop of nitrate reagent. Observe the reaction within a minutes or less.

Appearance of red color denotes that nitrate is reduced to nitrite and it's a positive result. If there is no color change the confirmation test was done adding a small pinch of zinc powder.

Starch Hydrolysis test:

Starch hydrolysis is used to determine the ability of an organism. Also to differentiate organisms based on their α-amylase enzyme activity. In the starch hydrolysis test, the test bacteria are grown pm agar plates containing starch. If the bacteria have the ability to hydrolyses starch, it does so in the medium, particular in the medium, particularly in the areas surrounding their growth while the rest the area of the plate still contain non-hydrolyses starch. Since no color change occurs in the medium when organisms hydrolyses starch, iodine solution is added as an indicator to the plate after incubation while the non-hydrolyses starch forms dark blue color with iodine, its hydrolyses end products do not acquire such dark blue color with iodine. Consequently, transparent clear zones are formed around the colonies that hydrolyses starch while the rest of the plate show a dark blue coloration as iodine for the colored complex with starch. Using a sterile technique make a single streak inoculation of organism to the center of labelled plate. Incubate the bacterial inoculated plates for 4-8 hours at 37^0c. Following incubation flood the surface of the plates with iodine. Examine for the clear Zone around the line of bacterial growth.

Lipid hydrolysis test:

Lipid hydrolysis is used to determine the ability of the organism. Also to identify bacteria capable of producing the exoenzyme lipase. In this experimental procedure, tributary agar is used to demonstrate the exoenzyme lipase. The medium is composed of nutrient agar supplemented with the

Triglyceride tributary as the lipid substrate. Tributary from an emulsion when dispersed in the agar, producing an opaque medium that is necessary for observing exo-enzymatic activity following inoculation and incubation of agar plate cultures, organisms excreting lipase will show a zone of lipolysis, which is demonstrated by a clean area surrounding the bacterial growth. This loss of opacity is the result of the hydrolytic reaction for lipid hydrolysis. In the absence of lipolytic enzymes, the medium retain its opacity. This is a negative reaction. Inoculated the tributyrin agar medium with single line streaking of organism. Incubate anaerobically in a gas pak jar immediately after streaking and transfer into the incubator maintained at 35-37^{0}c for 24-48 hours for anaerobes and for aerobes incubate the plate at 35^0-37^{0}c for 24-48hours. Examine the tributyrin agar plate culture for the presence or absence of a clear area or zone of lipolysis, surrounding the growth of each of the organism

Gelatin hydrolysis:

This test is used to the ability of an organism to produce gelatinases. Gelatin hydrolysis tests helps in the identification of *serratia, pseudomonas, flavobacterium*, and *clostridium.* The test distinguishes the gelatinase positive, non-pathogenic staphylococcus epidermis. The test can be used to differentiate genera of gelatinase producing bacteria such as serratia and proteus from other members of the family *Enterobacteriaceae.*

Gelatin is a protein derived from animal connective tissue, collagen, which forms a solid structure at lower temperatures. Protein is metabolized or degraded by a group of enzymes called gelatinase. Gelatin into polypeptides and individual amino acids.

The degradation of gelatin, like most proteins, occurs in two steps; the first step involve the degradation of gelatin into polypeptides into amino acids. Gelatinase is important in most bacteria as the gelatin is a large polymer and thus cannot be transported into the cell. The enzyme thus, breaks down gelatin into smaller units, which can be transported into the cell and utilized by the bacteria. In

The hydrolysis test media with gelatin is used, and its hydrolysis is observed either by the liquefaction of the media or by flooding with mercuric chloride. The mercuric chloride added to the medium precipitates gelatin is hydrolyses appear clear.

The gelatin medium in the tube is inoculated with 4-5 drops of a 24-hours incubated at 37^0c in air for 24-48 hours. If the organisms grow well 25^0c. After the first incubation, the tubes are to be placed at 4^0c for another 24hours.

Screening of pigment producing Bacteria

Isolated colonies of identified cultures were suspended in 2% glycerol containing Nutrient broth in a flask and the flasks were incubated for 48hrs. Extensive growth of pigment producing bacteria was seen in the flasks after 48hrs. Extraction of pigments from pigment producing bacteria was harvested by centrifuging at 6000rpm for 20min. Then supernatants were discarded and the pellets were re-suspended in ethanol. Then the mixture was vortexed and the suspension was centrifuged at 6000rpm for10 min and supernatant was collected. Centrifugation was repeated till the pellet changes to colorless. After centrifugation, supernatants containing diffused pigments were filtered through filter paper. Then the filtrates were kept in water bath for ethanol evaporation. After evaporation of ethanol, dry pigment was collected.

EXTRACTION AND DRYING OF PIGMENT:

Orange, yellow and pink pigments were extracted from the isolated from microorganisms *Enterobacteria, Micrococcus and serretia* respectively. The collected extracted pigments were dried and it was powdered.

Pigments that are isolated from the microorganisms were checked for the microbial action. Extracted pigments were streaked on nutrient agar plates. And the plates were incubated at 30 degree celcius for 24 hrs. After incubation there was no microbial growth.

Extracted extra cellular pigments were used for dyeing the fabrics. These microbial pigments

can be used for dyeing cloth, paper, pencil, soap etc., and also it can be used in food industries.

Dyeing and characteristics of the pigments

Method of Dyeing

Dyeing was attempted using an organic solvent solution of the pigment. After extraction of the pigment and air-drying of the extract, a fixed amount of dry pigments was dissolved in a various amount of organic solvents. Cotton fabrics were immersed in the various solution for half a day and the extent of dyeing. The cotton were dyed very well in the ethanol and methanol solutions.

Chromatography analysis

Thin-Layer Chromatography Qualitative analysis of pigments was carried out by Thin Layer Chromatography (TLC). The samples were spotted on the baseline of the TLC plates with the help of a capillary tube and then allowed to dry at room temperature; this step was repeated three to four times. The TLC plates were then placed in a pre-saturated TLC chamber containing the mobile phase (chloroform: methanol in the ratio of 9:1) (Priya *et al*., 2017) after 45 minutes, the TLC plates were carefully removed and the Retention factor (Rf) value was calculated according to the following equation from the chromatogram.

$$Rf = d / D$$

Where, d= Distance travelled by solute front

D= Distance travelled by solvent front.

FTIR ANAYSIS

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterize some inorganic. Examples include paints, adhesives, resins, polymers, coatings and drugs. It is a particularly useful tool in isolating and characterizing organic contamination.

PRINCIPLE

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range are measured as wave numbers typically over the range 4000 – 600 cm-1.

The background emission spectrum of the IR source is first recorded, followed by the emission spectrum of the IR source with the sample in place. The ratio of the sample spectrum to the background spectrum is directly related to the sample's absorption spectrum. The resultant absorption spectrum from the bond natural vibration frequencies indicates the presence of various chemical bonds and functional groups present in the sample. FTIR is particularly useful for identification of organic molecular groups and compounds due to the range of functional groups, side chains and cross-links involved, all of which will have characteristic vibrational frequencies in the infra-red range.

APPLICATION

ENVIRONMENT

Infrared spectroscopy is a valuable technique for monitoring air quality, testing water quality, and analyzing soil to address environmental and health concerns caused by increasing pollution levels. The technique offers a “green” method of testing and fast, accurate results with the added benefit of saving money on the cost of consumables.

FTIR can be used in all applications where a dispersive spectrometer was used in the past (see external links). In addition, the improved sensitivity and speed have opened up new areas of application. Spectra can be measured in situations where very little energy reaches the detector and scan rates can exceed 50 spectra a second. Fourier transform infrared spectroscopy is used in geology, chemistry, materials and biology research fields.

BIOLOGICAL MATERIALS

FTIR is used to investigate proteins in hydrophobic membrane environments. Studies show the ability of FTIR to directly determine the polarity at a given site along the backbone of a transmembrane protein.

MICROSCOPY AND IMAGING

An infrared microscope allows samples to be observed and spectra measured from regions as small as 5 microns across. Images can be generated by combining a microscope with linear or 2-D array detectors. The spatial resolution can approach 5 microns with tens of thousands of pixels. The images contain a spectrum for each pixel and can be viewed as maps showing the intensity at any wavelength or combination of wavelengths. This allows the distribution of different chemical species within the sample to be seen. Typical studies include analysing tissue sections as an alternative to conventional histopathology and examining the homogeneity of pharmaceutical tablets.

ADVANTAGE

The multiplex or Fellgett's advantage. This arises from the fact that information from all wavelengths is collected simultaneously. It results in a higher signal-to-noise ratio for a given scan-time for observations limited by a fixed detector noise contribution (typically in the thermal infrared spectral region where a photo detector is limited by generation-recombination noise). For spectrum with resolution elements, this increase is equal to the square root of alternatively, it allows a shorter scan-time for a given resolution. In practice multiple scans are often averaged, increasing the signal-to-noise ratio by the square root of the number of scans.

The throughput or Jacqui not’s advantage. This results from the fact that in a dispersive instrument, the mono chromator has entrance and exit slits which restrict the amount of light that passes through it. The interferometer throughput is determined only by the diameter of the collimated beam coming from the source. Although no slits are needed, FTIR spectrometers do require an

aperture to restrict the convergence of the collimated beam in the interferometer. This is because convergent rays are modulated at different frequencies as the path difference is varied. Such an aperture is called a Jacqui not stop. For a given resolution and wavelength this circular aperture allows more light through than a slit, resulting in a higher signal-to-noise ratio.

The wavelength accuracy or Connes' advantage. The wavelength scale is calibrated by a laser beam of known wavelength that passes through the interferometer. This is much more stable and accurate than in dispersive instruments where the scale depends on the mechanical movement of diffraction gratings. In practice, the accuracy is limited by the divergence of the beam in the interferometer which depends on the resolution.

Another minor advantage is less sensitivity to stray light that is radiation of one wavelength appearing at another wavelength in the spectrum. In dispersive instruments, this is the result of imperfections in the diffraction gratings and accidental reflections. In FT instruments there is no direct equivalent as the apparent wavelength is determined by the modulation frequency in the interferometer. (P. de Hasseth, J. A. 2007).

Result and Discussion

Identification and characterization of the pigment producing bacteria

The vegetable samples collected were used for isolation of pigment producing bacteria. Three color pigment producing bacteria were identified and characterized which were yellow, Orange and pink color in (Fig 2)

Fig 2: Morphological characteristics of pigments producing Bacteria:

Table 1: Morphological characteristics

S.No	Color	Shape	Gram Characters	Motility
1)	Orange	Rod Shape	Negative	Motile
2)	Yellow	Cocci	Negative	Non Motile
3)	Pink	Rod Shape	Negative	Motile

These bacteria were then identified and characterized with the help of morphological characteristics and biochemical tests (Table 2)

Table 2: Biochemical tests

S.NO	Biochemical Test	*Enterobacter*	*Micrococcus*	*Serratia*
1)	Gram Staining	-ve	-ve	-ve
2)	Indole	-ve	+ve	+ve
3)	Methyl Red	-ve	-ve	-ve
4)	Voges Proskauer	-ve	+ve	+ve
5)	Urease	-ve	+ve	+ve
6)	Citrate	+ve	-ve	-ve
7)	Triple sugar ion	-ve	-ve	-ve
8)	Catalase	+ve	+ve	+ve
9)	Nitrate reduction	+ve	-ve	-ve
10)	Starch hydrolysis	-ve	+ve	+ve
11)	Gelatin hydrolysis	+ve	-ve	-ve

+ve Sign strands for positive and –ve sign strands for Negative

Their identification at genus level was done. In the present study, the three pigment producing bacteria that produced intracellular pigments (Fig.2) with the help of Bergey's Manual of Determinative Bacteriology, which is presented in (Table 3).

Table 3: Genus Level Identification

Colour of Pigment	Microorganism
Orange	***Enterobacter***
Yellow	***Micrococcus***
Pink	***Serratia***

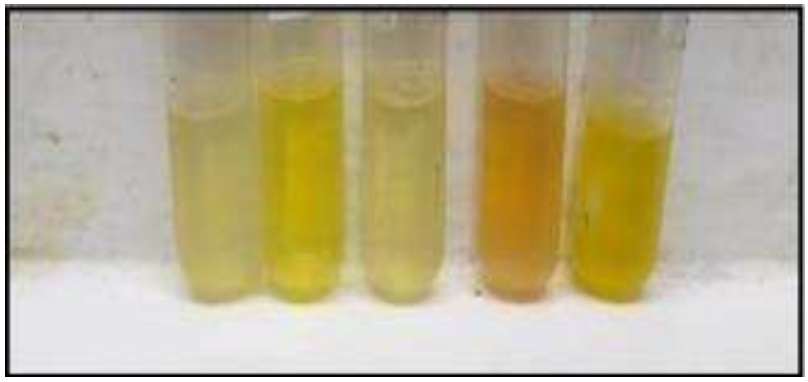
Fig 3: Nutrient agar showing pigment producing organism

Pigment producing organism from carrot, capsicum and coconut

Production of pigment

The production of pigments was carried out by growing the cultures in plain nutrient broth and 2% glycerol in nutrient broth for seven days. Both of the enlisted methods gave good results with visible pigments settled at the bottom of the flask as shown in Fig respectively. Nutrient broth contains peptone and meat extract which act as an organic carbon and nitrogen source respectively, providing the organism with the essential growth factors and vitamins that are necessary for their propagation (John and Aruna, 2019). Besides, the addition of glycerol also being a carbon source helps further in their growth.

Fig 4: Production of pigment



Extraction of pigment for the extraction of pigment-producing bacteria

Extraction of pigment for the extraction of pigment-producing bacteria, methods like centrifugation, filtration were used with ethanol as an organic solvent. The chemical composition of the extracted pigment influences the choice of organic solvent and yield directly. Carotenoids are lipophilic in nature and are soluble in organic solvents, like chloroform, acetone, methanol, ethanol etc. (Priya et al., 2017) Different solvents like chloroform, ethanol, petroleum ether, etc were screened for extraction of pigments from bacterial species, in various studies (John and Aruna, 2019). Acetone and methanol are the solvents, can extract the pigment from the cell. But the highest extraction of pigment was shown in methanol (Vora *et al.*, 2014).

Extraction of pigments from pigment producing bacteria For the extraction of pigment producing bacteria, various methods were used like centrifugation, filtration, and addition of ethanol so that cell gets lysed and intracellular pigment can be extracted. The pigments extracted were orange, yellow, and pink color.

Fig 5: Extraction of orange color pigments from pigment producing bacteria

Fig 6: Extraction of yellow pigments from pigment producing bacteria

Yellow pigment

Fig 7: Extraction of pink pigments from pigment producing bacteria

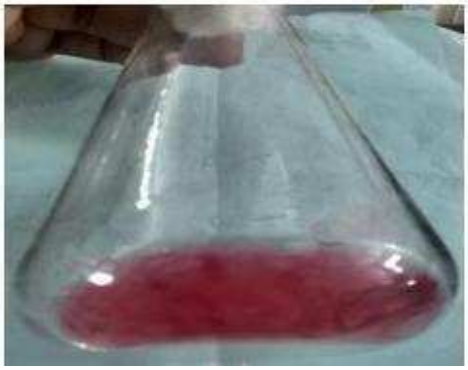


Fig 7: Extraction and drying of pigment

Pigments were extracted from the isolated microorganisms. Methanol was used for the pigment extraction and the extracted pigment was dried and it was powdered.

Chromatography analysis Thin-Layer Chromatography

Carotenoids are a group of bioactive compounds that are widely distributed in nature, which are responsible for yellow, orange, and pink pigments in various plants, microorganisms, and animals. The TLC profile of PR 3 showed Rf value of 0.73 and PR 6 with two bands showed Rf values of 0.78 and 0.9. The orange-yellow colored pigments were confirmed to be carotenoids because the Rf value of carotenoids lies within the range of 0.92 to 0.34 (Reddy *et al.,* 2003)

Identification of pigment by FTIR analysis

Identification of pigment by FTIR analysis one of the main identification tests for pigment is IR spectrum.

Fig 8: Chromatography analysis Thin-Layer Chromatography

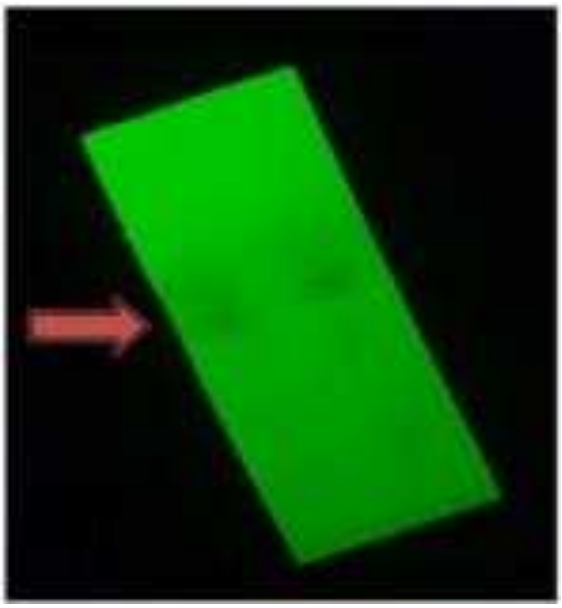
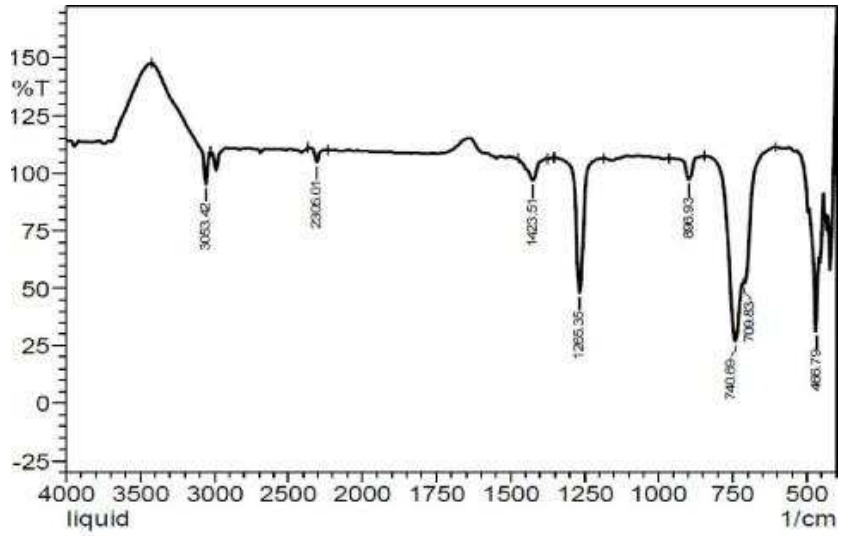


Fig 9: Identification of pigment by FTIR analysis



Pigment exhibited bright spectral absorb tionlines,466.79 cm-1 peak related to the hydroxyl group (OH), 709.83-740.69 cm-1 attribute to the O-H bending peaks, peak at 1265-1435 cm-1 related to the amino second group (NH), 2305-3053.42 cm-1 peaks related to amino group (NH) in pigment extracted from pigments producing bacteria. The IR spectral analysis of the methanol extracted pigment reveals the presence of carotenoids.

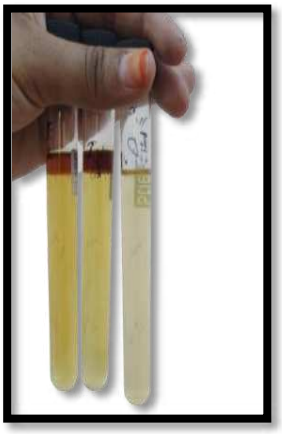
Conclusion

This present study suggests local microbial isolates which is able to produce pigment based upon morphological, physiological and biochemical studies, the orange, yellow, and pink colonies were produced. So it can be used as a potential source for pharmaceutical and other cosmetic industries. Hence it was concluded that the organisms carried out for the maximal production of the pigment so that the above pigment could be exploited in future for various applications like pharmaceuticals and cosmetics. Pigment can be looked for so that it could of great use to the mankind.

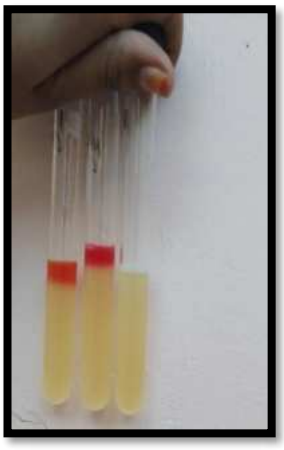
Summary

Isolated colonies of identified cultures were suspended in 2% glycerol containing Nutrient broth in a flask and the flasks were incubated for 48hrs. Extensive growth of pigment producing bacteria was seen in the flasks after 48hrs. Extraction of pigments from pigment producing bacteria was harvested by centrifuging at 6000rpm for 20min. Then supernatants were discarded and the pellets were re-suspended in ethanol. Then the mixture was vortexed and the suspension was centrifuged at 6000rpm for10 min and supernatant was collected. Centrifugation was repeated till the pellet changes to colorless. After centrifugation, supernatants containing diffused pigments were filtered through filter paper. Then the filtrates were kept in water bath for ethanol evaporation. After evaporation of ethanol, dry pigment was collected.

INDOLE TEST `

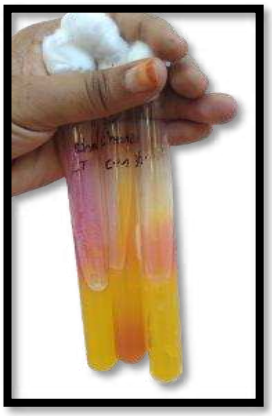


METHYL RED TEST



VOGES PROSKAUER TEST

UREASE TEST



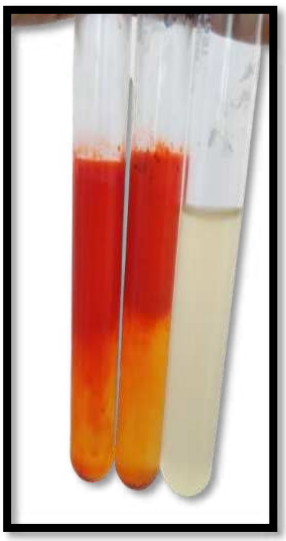
CITRATE TEST

TRIPLE SUGAR IRON

CATALASE TEST CAPSIUM

CARROT

NITRATE REDUCTASE TEST



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