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The Anti-Microbial Activity of Polyherbal Extract Using Escherichia Coli and Saccharomycescerevisiae

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ABSTRACT

One of the biggest issues in contemporary life is antimicrobial resistance. Therefore, a remedy to this issue is required. Combinations of various antimicrobial medications ought to be more effective than single medications.

The usual plants that grow in our area have antibacterial properties.One of the main plants with strong antibacterial action is Azadirachtaindica. Eryngiumfoetidum and

Mimosa pudicaboth have demonstrated antibacterial action and are readily available. The combination of these three herbal plants extracts ought to provide stronger antibacterial effects than any of the plants separate extracts.

Raw plants are initially gathered from the area.The Azadirachtaindica, Eryngiumfoetidum, and Mimosa pudica leaves and roots are employed. To get rid of dirt and undesired detritus, plants are thoroughly washed and dried in the shade. The plant leaves are then thoroughly pulverized individually.Then, using a Soxhlet device and ethanol the powdered plants are extracted separately. The extraction process is carried out until a clear solution is found. The ethanol is then removed from the obtained extracts by evaporation. DMF (dimethyl formamide) is used to dilute the extract so that 1000mcg, 2000mcg, and 3000mcg of each plant extract and plant extract mixture is also prepared in three different concentrations.The Disc Plate method, which employs Saccharomyces cerevisiae and E. coli, is used for antimicrobial assay. It is decided to use nutrient agar for bacteria and sabouraud dextrose agar for fungi. The prepared substance discs dipped in plant extracts and a combination are then added to the inoculation medium in an aseptic space with a laminar airflow system after it has been first inoculated with the organisms. The plates are then left to incubate for 24 hours.

The outcomes were as anticipated; the plant extract combo displayed a greater zone of inhibition than the plant extracts taken separately. It measures the zone of inhibition.

Keywords: Antimicrobial resistance, poly herbal extract

INTRODUCTION

Azadirachtaindica', Mimosa pudica², and Eryngium foetidum³ are commonly used in traditional medicine for their therapeutic properties. Anti- microbial activity 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 antifungals are used against fungi. The combination of extracts of these plants may possess a potent synergistic activity when compared to individual extracts.

Azadirachtaindica, Mimosa pudica, and Eryngiumfoetidum these plants have been used for centuries to treat various ailments such as fever, cough, wounds, and infections[4] Azadirachtaindica, also known as neem, is a tree commonly found in India, Bangladesh, and Pakistan. Its bark, leaves, and seeds are used in traditional medicine to treat skin conditions, fever, and digestive issues. It is also known for its antimicrobial and antifungal properties. Mimosa pudica, commonly known as touch-me-not or sensitive plant, is a herb native to South America. Its leaves and roots have been used in traditional medicine to treat urinary tract infections, digestive issues, and inflammation. It is also known for its antibacterial and antifungal properties. Eryngiumfoetidum, also known as culantro or recao, is a herb commonly found in Latin America and the Caribbean. Its leaves and roots have been used in traditional medicine to treat fever, cough, and digestive issues. It is also known for its antimicrobial and antiinflammatory properties. Recent studies have shown that these plants have strong antimicrobial activity against a range of bacteria, fungi, and viruses[5]. This has led to increased interest in exploring the potential of these plants in the development of novel antimicrobial agents. In this article, we will explore the antimicrobial potential of Azadirachtaindica, Mimosa pudica, and Eryngiumfoetidum and their potential use in modern medicine.

Azadirachta indica

Figure 1

Kingdom	Plantae
Division	Magnoliophyte
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceae
Genus	Azadirachta
Species	A.indica

Table 1

Azadirachtaindica, also known as neem, is a tree native to India and has been used for centuries in traditional medicine to treat a variety of ailments. In recent years, there has been an increasing interest in the potential antimicrobial properties of neem extracts. Studies have shown that neem can inhibit the growth of a wide range of bacteria, fungi, and viruses.

The active compounds in neem, such as azadirachtin, nimbin, and nimandial, are thought to be responsible for its antimicrobial effects. Neem extracts have been shown to be particularly effective against bacterial pathogens such as Staphylococcus aureus and Escherichia coli. They have also been found to be effective against fungal pathogens like Candida albicans and Aspergillusniger.

In addition to its antimicrobial properties, neem extracts have also been shown to have anti-inflammatory, antioxidant, anti-diabetic, and anticancer properties, which may contribute to their therapeutic potential. It is also known to boost the immune system and improve liver function. Overall, the antimicrobial properties of AzadirachtaIndica make it a promising candidate for the development of new antimicrobial agents for the treatment of infectious diseases. Further research is needed to fully understand the mechanisms of action and potential clinical applications of neem extracts.

Mimosa pudica

Figure 2

Kingdom	Plantae
division	Magnoliophyte
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Mimosa
Species	m.pudica

Table 2

Mimosa pudica, also known as the "touch-me-not" plant, is a common herb found in many parts of the world. This plant has long been used in traditional medicine to treat various ailments such as wounds, skin infections, and respiratory problems. Recent studies have shown that Mimosa pudica has powerful antimicrobial properties that can be used to combat a wide range of infectious microorganisms.

The leaves of the Mimosa pudica plant contain compounds such as tannins, alkaloids, and flavonoids that exhibit strong antimicrobial activity against bacteria, fungi, and viruses. These compounds work by disrupting the cell membranes of the microorganisms, preventing their growth and proliferation. Studies have also shown that Mimosa Pudica can be effective against drug-resistant strains of bacteria such as MRSA (Methicillin-resistant Staphylococcus aureus) and E. coli. This makes it a promising natural

alternative to antibiotics, which are becoming increasingly ineffective due to the emergence of antibiotic-resistant bacteria.

In addition to its antimicrobial properties, Mimosa pudica has also been found to have anti-inflammatory and antioxidant effects, making it a valuable herb for overall health and well-being. It is known for its wound-healing properties. The plant contains compounds that help in reducing inflammation and promoting the growth of new tissue. It is also known to have anti-anxiety and antidepressant properties, making it a potential alternative treatment for mental health conditions. Overall, the antimicrobial potential of Mimosa pudicais a promising area of research that could lead to the development of new natural treatments for infectious diseases.

Eryngiumfoetidum

Figure 3

Kingdom	Plantae
Division	Magnoliophyte
Class	Magnoliopsida
Order	Apiales
Family	Apiaceae
Genus	Eryngium
Species	E.foetidum

Table 3

Eryngiumfoetidum, also known as "culantro" or "Mexican coriander," is a popular plant used in traditional medicine across the globe. It has been recognized for its antimicrobial properties, and studies have shown that extracts from this plant have the potential to inhibit the growth of various types of bacteria and fungi. One study found that the essential oil of Eryngiumfoetidum exhibited significant antibacterial activity against Staphylococcus aureus and Escherichia coli, two types of bacteria that are commonly responsible for causing infections in humans.

The antimicrobial properties of Eryngiumfoetidum are thought to be due to the presence of various bioactive compounds, such as flavonoids, alkaloids, and terpenes. These compounds have been shown to have a range of health benefits, including antioxidant and anti-inflammatory effects. Overall, the findings of these studies suggest that Eryngiumfoetidum has the potential to be a valuable source of

natural antimicrobial agents that could be used in the development of new drugs to combat infectious diseases.

Aqueous extract of Eryngiumfoetidum had strong antifungal activity against Candida albicans, a type of fungus that can cause yeast infections. It also possesses a wide range of ethnomedicinal uses including treatment for burns, earache, fevers, hypertension, constipation, fits, asthma, stomach ache, worms, infertility complications, snake bites, diarrhea and malaria. It also uses as natural remedy for digestive issues, fever, and inflammation. It is also known to have anti-cancer properties and can help in reducing cholesterol levels.

The AzadirachtaIndica, Mimosa Pudica, and EryngiumFoetidum these plants can be used in different ways to help treat infections. They can be used in the form of teas, tinctures, or extracts. The active compounds in these plants can help to fight off the infection and boost the immune system. However, it is important to consult with a healthcare professional before using these plants as a treatment for infections. The combination of extracts of these plants may possess a potent synergistic anti-microbial activity when compared to individual extracts. It's important to also consider the potential downsides and precautions when using them. Firstly, it's important to know that these plants may cause allergic reactions in some individuals

It's always recommended to do a patch test before using any new plantbased product to ensure that you are not allergic to the plant. Secondly, some of these plants have been found to have toxic properties, especially when used in large quantities or in a concentrated form. Therefore, it's important to always follow the recommended dosage and usage instructions when using these plants. Thirdly, it's important to note that these plants may interact with certain medications. If you are taking any medication, it's always recommended to consult with your healthcare provider before using any new plant-based products. Lastly, it's important to always source these plants from a reputable supplier to ensure that the product is of high quality and free from any contaminants or additives. In conclusion, while these plants offer potential antimicrobial benefits, it's important to consider the potential downsides and precautions before using them to ensure safe and effective use.

They can be used in the development of novel antimicrobial agents to combat bacterial infections. The results of this study encourage further research to investigate the mechanism of action of these plants' extracts and identify the bioactive compounds responsible for their antimicrobial activity. Future research can also focus on the efficacy of these plants' extracts as alternative treatments for bacterial infections in animals and humans. The pharmacological properties of these plants can be explored to identify potential therapeutic benefits and side effects. Further studies can also investigate the synergistic effects of combining these plant extracts with conventional antibiotics to improve their efficacy against bacterial infections. Overall, this study provides a foundation for future research to explore the potential of these plants in the development of novel antimicrobial agents and alternative treatments for bacterial infections. Further research in this field can have significant implications for public health and contribute to the development of effective and sustainable solutions to combat bacterial infections. We hope you enjoyed our article on the antimicrobial potential of Azadirachtaindica, Mimosa pudica, andEryngiumfoetidum. These plants have been used for centuries in traditional medicine, and recent studies have shown promising results in their ability to fight against harmful microorganisms. As we continue to explore the potential of natural remedies, it is important to remember the value of traditional practices and knowledge.

E-coli

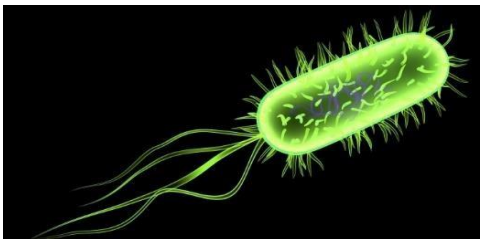


Figure 4

Domain	Bacteria
Kingdom	Monera
Phylum	Proteobacteria
Class	Gammaproteobacterial
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	Escherichia
Species	E. coli

Table 4

Escherichia colialso known as E. coli, is a Gram-negative, facultative anaerobic, rodshaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. Most E. coli strains are harmless, but some serotypes (EPEC, ETEC etc.) can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. Most strains do not cause disease in humans and are part of the normal microbiota of the gut; such strains are harmless or even beneficial to humans (although these strains tend to be less studied than the pathogenic ones)

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. E. coli is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy. E. coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. [citation needed] Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, sepsis, and Gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as hemolytic uremic syndrome; however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with E. coli.

Saccharomyces cerevisiae

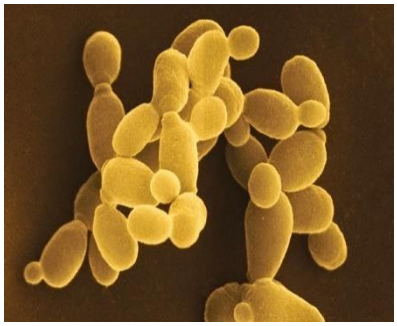


Figure 5

Domain	Eukarya
Kingdom	Fungi
Phylum	Ascomycota
Class	Saccharomycetes
Order	Saccharomycetales
Family	Saccharomycetaceae
Genus	Saccharomyces
Species	S. cerevisiae

Table 5

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. The first yeast originated hundreds of millions of years ago, and at least 1,500 species are currently recognized. They are estimated to constitute 1% of all described fungal species.

Yeasts are unicellular organisms that evolved from multicellular ancestors, with some species having the ability to develop multicellular characteristics by forming strings of connected budding cells known as pseudo hyphae or false hyphae. Yeast sizes vary greatly, depending on species and environment, typically measuring 3–4 µm in diameter, although some yeasts can grow to 40 µm in size. Most yeasts reproduce asexually by mitosis, and many do so by the asymmetric division process known as budding. With their single-celled growth habit, yeasts can be contrasted with molds, which grow hyphae. Fungal species that can take both forms (depending on temperature or other conditions) are called dimorphic fungi.

The yeast species Saccharomyces cerevisiae converts carbohydrates to carbon dioxide and alcohols through the process of fermentation. The products of this reaction have been used in baking and the production of alcoholic beverages for thousands of years. S. cerevisiae is also an important model organism in modern cell biology research, and is one of the most thoroughly studied eukaryotic microorganisms. Researchers have cultured it in order to understand the biology of the eukaryotic cell and ultimately human biology in great detail. Other species of yeasts, such as Candida albicans, are opportunistic pathogens and can cause infections in humans. Yeasts have recently been used to generate electricity in microbial fuel cells and to produce ethanol for the biofuel industry.

Yeasts do not form a single taxonomic or phylogenetic grouping. The term "yeast" is often taken as a synonym for Saccharomyces cerevisiae, but the phylogenetic diversity of yeasts is shown by their

placement in two separate phyla: the Ascomycota and the Basidiomycota. The budding yeasts or "true yeasts" are classified in the order Saccharomycetales, within the phylum Ascomycota.

METHODS

Extraction of Azadirachtaindica

Material preparation Neem seed used in this study were obtained from Bali. This raw material has water content of 7.8% and oil content of 49.58%. Prior to use, the Neem seeds were repeatedly washed to remove dirt and other impurities material, and subsequently dried in oven at 50°C until it reached constant moisture content. Then, Neem seeds were ground to get three different particle sizes (0.85-1.40 mm, 0.71-0.85 mm, and 0.425-0.71 mm).[4]

Oil extraction of Neem seeds were extracted using two solvent (n-hexane and ethanol) for 3 hours with ratio Neem seed powder weight to solvent volume of 1:5. In certain time intervals, the samples were taken and centrifuged to separate the solid fraction from solution. Filtrate was heated and evaporated to obtain solvent-free oil. Then the oil was weighed to calculate the concentration of oil in the solution. Extractions were conducted at 5 temperature level (30o, 35o, 40o, 45o and 50oC).

Extraction of Mimosa pudica

The leaves of Mimosa pudicawere procured from the Thailavaram (near SRM University) in the month of Febuary-2009. The plant was identified by Dr. D. Narashiman, Centre for Floristic Research, Department of Botany plant biology and

Plant bio-technology, Madras Christian College, Tambaram, Chennai, Tamil Nadu,

India. The voucher specimen (023/02/09) was deposited at the Department of Pharmacognosy and Phyto-chemistry M. S. A. J College of Pharmacy, India, for future reference. Care was taken to collect the healthy and young leaves ofMimosa pudica. [5]

The coarsely powdered leaves (300 g) of Mimosa pudica were extracted to exhaustion in a Soxhlet apparatus at 50 ^{o}C with 500 ml of chloroform. The extract was filtered through a cotton plug, followed by Whatman filter paper (No.1) and then concentrated by using a rotary evaporator at a low temperature (40–60 ^{o}C) and reduced pressure to provide chloroform extractive of 8.20 g.

DISC PLATE METHOD: MICROBIAL ASSAY

A stock spore suspension is prepared by cultivation of the organisms on agar or in submerged culture. When microscopic examination reveals good sporulation, the cells are separated from the medium, suspended in sterile 0.05M potassium phosphate buffer, pH7.0, and the suspension pasteurized to kill the vegetative cells. A viable spore count is made by plating. The stock spore suspension is stored at 2 to 4C and used as needed. Preparation of plates. To petri dishes (Pyrex dishes, 100 mm in diameter, selected for uniform flat bottoms 2) are added 20.0 ml of sterile assay agar, preferably with an automatic dispensing pipette. The agar is allowed to harden with the petri dish top stilted or removed. While the plates are still warm, 4.0ml of seeded agar is added and distributed evenly over the surface. The volume of spore suspension used to seed the medium depends on the concentration of viable spores. The amount to be used is best determined by actual test in the assay procedure, that quantity being selected which gives large, sharp zones. Usually, a concentration of approximately 250,000 spores per ml in the seeded agar gives the desired results. The pores are added to the liquefied agar at 60C, and. The seeded medium is maintained at that temperature until all the plates are poured. The plates are stored at 2 to 4C as soon

as they have hardened and may be kept for several days with no effect on the assay. It is essential that the plates be prepared on an absolutely level table top. A section of heavy plate glass or marble supported on adjustable legs and equipped with a level indicator serves adequately for this purpose. Preparation of the sample. All samples are diluted on the basis of their estimated potency of all on the standard curve. The initial dilution of liquid samples is made with an equal volume of 0.2 M potassium phosphate buffer, pH 7.9, and all subsequent dilutions with 0.1 M buffer. Solid samples are dissolved directly in 0.1 M buffer. Samples of submerged-culture beers are not clarified before dilution. Setting up the assay. Filter paper discs are placed, flat side down, on the agar plates. As each disc is placed, an 0.080- ml sample is immediately (within 5 seconds) pipetted on to it. Since the disc rapidly absorbs moisture from the agar it is exceedingly important that the sample be applied in the shortest possible time. As the sample is being delivered the disc is gently pressed to the agar with the tip of the pipette. Four to six discs may be placed on one plate

MATERIALS

Nutrient agar mediumcomposition

Peptone	10g
Sodium chloride	5g
Agar	20g
Distilled water	1000ml
Beef extract	10gm

Table 6

Sabouraud dextrose agar medium composition:

Peeled potatoes	200gm
Dextrose	20gm
Agar	20gm
Distilled water	1000ml

Table 7

PROPERTIES OF AGAR:

The gum is a polysaccharide derived from a seaweed gelidium species. For most bacteria it is wholly indigestible and possesses no nutritive value. It is transparent and colorless substance and melts in water at boiling temperature and solidifies at 450C. It is used in 1.5 to 3% concentration in nutrient solution and forms gel.

LITERATURE REVIEW

1. **Ashok Kumar Mandal, 5hkfidwd2ugiho1Anisha Pandey 1,2Ranjit Kumar Sah,3Adesh Baral,4and PhoolgenSah** The agar well diffusion method was used to assess the in vitro antibacterial activity of various doses of the mimosa pudica extract against four bacterial strains. By estimating the binding affinity towards the bacterial protein, the in silico molecular docking model

was utilized to determine the antibacterial effectiveness of L-mimosine against the chosen strains of bacteria employed for the in vitro study. Mimosa pudica has been discovered to have antibacterial and antioxidant properties in vitro. The agar well was used to assess the in vitro antibacterial activity of various doses of the extract against four bacterial strains.

2. **Baby Joseph, Jency George and Jeevitha Mohan** conducted the study about the Magnoliopsi da taxonomic group and Mimosasea family comprise the mimosa. It is known locally as Mimosa pudica Lin in Latin. According to Ayurveda, the root of this plant is bitter, acrid, cooling, vulnerary, and alexipharmic. Leprosy, dysentery, vaginal and uterine complaints and inflammations, burning sensations, asthma, leucoderma, tiredness, and blood disorders are among the conditions it is used to treat. Toothaches can be relieved by gargling with root decoction. It is highly helpful for urinary infections, bleeding piles, diarrhea (athisaara), and amoebic dysentery (raktaatissara). An overview of its phytochemical and pharmacological activity is provided in this review.
3. **D P Lingaraju, M S Sudarshana, C Mahendra, K Poornachandra** conducted a study and screening of of antimicrobial activity of eryngiumfoetidum This study aimed to investigate the antibacterial and antifungal activity of E. foetidum leaves. Different organic solvents were used for Soxhlet extraction, and phytochemical screening revealed the presence of glycosides, flavonoids, terpenoids, steroids, and tannins. These findings support the traditional use of E. foetidum and suggest that its leaf extract could be utilized as a potential antimicrobial agent in the development of new drugs for infectious diseases.
4. **D P Lingaraju1, M S Sudarshana, C Mahendra, K Poornachandra Rao** the Apiaceae plant, a study was created to look at the antibacterial and antifungal properties of E. foetidum leaves. Eryngiumfoetidum L. is a biennial herb. As an ethno-medicinal herb, it is used to treat a variety of illnesses. The Kodagu district of Karnataka's ethnic communities utilize leaf decoction to treat gastrointestinal issues and leaf paste to treat wounds. Petroleum ether, chloroform, ethyl acetate, and methanol were used to carry out the Soxhlet extraction. Initial phytochemical assays were performed on each extract. According to the results of the phytochemical screening, some active ingredients were found, including glycosides, flavonoids, terpenoids, steroids, and tannins. Two Gramme positive bacteria, Bacillus and Bacillus, were used to test the isolated extracts' antimicrobial efficacy.
5. **Gulzar Muhammad, Muhammad Ajaz Hussain, Ibrahim Jantan and Syed Nasir Abbas Bukhari** carried out about to mimosa pudica. Due to its thigmonastic and nyctinastic movements, Mimosa pudica Linn. (family: Mimosacee) is utilized as an attractive plant. Additionally, M. pudica is utilized to prevent or treat a number of illnesses, including cancer, diabetes, hepatitis, obesity, and urinary infections. Along with various useful secondary metabolites as tannins, steroids, flavonoids, triterpenes, and glycosyl flavones, M. pudica is well known for its anticancer alkaloid, mimosine. Different parts of M. pudica have been found to possess a wide range of pharmacological properties, including antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, antinociceptive, anticonvulsant, antidepressant, antidiarrheal, hypolipidemic activities, diuretic, antiparasitic, antimalarial, and hypoglycemic. Due to its high swelling index, the glucuronoxylan polysaccharide extruded from M. pudica seeds is employed in drug release formulations.
6. **Hassan A. Hemeg,aIhab M. Moussa,b,c, SherinIbrahim,dTurki M. Dawoud,bJwaher H. Alhaji,e Ayman S.(**2020) was carried a study and demonstrated evaluate the antimicrobial effects of ethanolic extract of five herbal plants; Guava (Psidiumguajava), Sage (Salvia officinalis), Rhamnus (Ziziphusspina Christi), Mulberry (Morusalba L.), and Olive (Oleaeuropaea L) leaves against several

microbial population representing Gram positive, Gram negative and Mollicutes; S. aureus, E. coli, Pasteurellamultocida, B. cereus, Salmonella Enteritidis and M. gallisepticum using standard agar disc diffusion technique and minimal inhibitory concentration (MIC).

7. **J.H.A. Paul, C.E. Seaforth and T. Tikasingh** conducted study of the biennial herb Eryngiumfoetidum L. is a popular therapeutic plant in the majority of tropical areas. There are a variety of other ethnomedicinal uses for it as well, including as the treatment of burns, earaches, fevers, hypertension, constipation, fits, asthma, stomachaches, worms, problems with infertility, snake bites, diarrhea, and malaria. Although no alkaloids were found, a chemical analysis of the leaves revealed the presence of flavonoids, tannins, a saponin, and many triterpenoids. E-2-dodecenal ("eryngial"), one of the main components of the plant's essential oil, is also present in smaller amounts, along with isomers of trimethylbenzaldehyde. Pharmacological studies of the aerial plant parts have shown eryngial to have anthelmintic activity, phytosterol fractions to have antiinflammatory activity, phytosterol fractions to have anti-convulsant activity in the appropriate models, and Salmonella species to have specific antibacterial activity.
8. **KorawuthPunareewattana, Glenn N. Borlacel, SupawadeeSeubsasanal, EakachalThongkham.andJareeratAiemsaard**the goal of their study was to investigate the in vitro antimicrobial activity of an ethanolic extract of Eryngiumfoetidum L. against Staphylococcus pseudintermedius, Malasseziapachydermatis, Microsporumcanis, Microsporumgypseum, and Trichophytonmentagrophytes, which are the three most prevalent dermatophytes that cause dermatitis in animals. Current antibiotic treatments for animal infectious dermatitis may have side effects, so the discovery of novel antimicrobial formulations is crucial. The E. foetidum extract had MIC, MBC, and MC values in the range of 6.25 to 25.0 mg/ml, according to a broth microdilution test, and a time-kill test revealed that this impact was dose and time dependent. The outcomes of this investigation demonstrate the efficacy of E. foetidum extract.
9. **Krishnamurthy Vijayalakshmi and RajangamUdayakumar** was carried study about Antibacterial Activity of Leaf and Root of M. pudica L. against Selected Human Pathogenic Microorganisms in 2018, The present study was aimed to investigate the antibacterial activity of leaf and root of M. pudica against selected bacteria Escherichia coli, Klebsiellapneumoniae, Proteus mirabilis, Bacillus subtilis, Pseudomonas fluorescens and Streptococcus pyogenes by disc diffusion method. The solvents acetone, aqueous, benzene, diethyl ether and ethanol were used for the preparation of extracts from leaf and root of M. pudica. In the present study, various concentrations of extracts 2.5 mg/50 µl, 3.75 mg/75 µl and 5 mg/100 µl
10. **Maria Yuliana Liauw, F. A. Natan, P. Widiyanti, D. Ikasari, N. Indraswati and F. E. Soetaredjo**, was conducted an experiment and that experiment, Neem oil extraction from Neem seeds (Azadirachta A. Juss) with n-hexane and ethanol are presented. Effects of particle size, temperature and type of solvent on the extraction kinetic and thermodynamic parameters were studied. Results showed that the maximum oil yields were 41.11% for ethanol and 44.29% for n-hexane at 50° C
11. **Mohammed a alzohairy**(Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, P.O. Box 6699, Buraidah, Saudi Arabia.) Was conducted a study about therapeutics role of azadirachtaindica (Neem) and their active constituents in diseases prevention and treatment. This literature summarizes the role of Neem (Azadirachtaindica) in disease prevention and treatment. Neem, a member of the Meliaceae family, has been extensively used in traditional

Chinese, Ayurvedic, and Unani medicines, particularly in the Indian subcontinent. Its rich antioxidant content contributes to its health-promoting effects. Animal studies have shown that neem and its constituents modulate various molecular pathways involved in cancer management, including p53, pTEN, NF-κB, PI3K/Akt, Bcl-2, and VEGF. Neem is considered safe and has been used for centuries without significant adverse effects. Further research, including clinical trials, is needed to fully understand neem's mechanisms and effectiveness in disease contexts.

12. **Mukesh C. Sharma, Smita Sharma, Devi Ahilya** conducted a study and published in 2010. This study explores the antimicrobial properties of aqueous extracts from Mimosa pudica Linn and Tridaxprocumbens Linn, two medicinal plants. The extracts were tested against various bacterial pathogens and compared to standard antibiotics. The results showed inhibitory activity against the tested organisms. Phytochemical screening revealed the presence of tannins, flavonoids, saponins, and alkaloids in the plants. These findings provide scientific validation for the traditional and ethnoveterinary use of these plants. The study highlights the potential of these natural extracts as alternatives for treating infections, considering the limitations of current drugs and the growing resistance to antimicrobial agents.
13. **Raja Ratna Reddy Y, Krishna Kumari C, Lokanatha O, Mamatha S, Damodar Reddy C** carried a study and published work on 2013, Screening medicinal plants for bioactive compounds leads to the development of affordable antimicrobial agents that are safer and more effective. AzadirachtaIndica, commonly known as neem, is a versatile tree with numerous health benefits. They utilized the agar well diffusion method and micro-broth dilution methods to determine the minimum inhibitor concentration (MIC). The results demonstrated that the leaf extract exhibited strong antimicrobial activity against both bacteria and fungi at all tested concentrations (500, 1000, and 2000 µg/ml). 14.**RekhaRajendran, R Hemachander, T Ezhilarasan, C. Keerthana, DL Saroja, KV Saichand and Mohamed Gasim Abdullah** carried out the invitro antioxidant activity against free radical scavenging by DPPH, nitric oxide, super oxide dismutase, and reducing power ability of the chloroform extract of Mimosa pudica Lin., leaves was tested. Because of its cytotoxic, antidiarrheal, and anti-hyperglycemic qualities, Mimosa pudica Lin. has been utilized for centuries. The effects were equivalent to those of the common antioxidant ascorbic acid, and the concentration affected the antioxidant characteristics. The results suggest that the chloroform extract of Mimosa pudica Lin., is a potential source of natural antioxidants and that the extract has constituents that were capable of showing antioxidant activity and the presence of phenolic compounds was further confirmed by thin layer chromatography and highperformance thin layer chromatography. Preliminary phytochemical analysis revealed the presence of phytoconstituents such as steroids, flavonoids, glycosides, alkaloids, and phenolic compounds.
15. **Y.H.LOO, P.S. SKELL, ANDH. H. THORN BERRY University of Illinois, Urbana, Illinois JOHNEHRLICH Parke, Davis** and Company, September 7, 1945 et al was demonstrated and studied as initial challenges was developing an assay method suitable for both beers and streptomycin solutions containing organic solvents and reagents. Various methods, such as agar-dilution and agardiffusion, have been used to define streptomycin potency and determine its presence in body fluids. Due to inconsistent results with the agar-diffusion method, the authors aim to share their experiences and the current procedure employed in their laboratories for streptomycin assay

AIMS AND OBJECTIVE

AIM

The purpose of the study was to extract mixture of the leaves of Azadirachtaindica, Mimosa pudica, and Eryngiumfoetidumfollowed by the evaluation of the antimicrobial activity of the polyherbal mixture on the Escherichia coli and saccharomyces cerevisiae

OBJECTIVES

- Extraction of mixture of Azadirachtaindica', Mimosa pudica, and Eryngiumfoetidum
- Antimicrobial assay of Azadirachtaindica, Mimosa pudica, and Eryngiumfoetidum

MATERIALS AND METHODS

MATERIALS

CHEMICALS	EQUIPMENTS
EXTRACTION	
•Ethanol	• Soxhlet apparatus • Round bottom flask • Measuring cylinder • Heating mantle • Cotton • Electronic balance • Rota vapour
MICROBIAL ASSAY	
• Nutrient agar medium • Sabouraud dextrose medium • Water • Ciprofloxacin • DMF	• Antibiotic disc • Discs • Petri dish • Micropipette • Autoclave • Aseptic area • Incubator

Table 8

METHOD FOR Azadirachtaindica

Extraction

- Take AzadirachtaIndica leaves which is washed in water then it is dried at 3545 °C.
- The dried leaves are then pulverized in electric grinder.
- Take 20g powder and poured into the Soxhlet apparatus which the powder is plugged in between cotton
- 125 ml of 96% ethanol is poured to soak the powder
- 50 ml of ethanol is added to the round bottom flask and the apparatus is arranged
- This is heated at 78° C for 7 hours.
- The resulting extract was dried in a rotavapour to get mass which is then stored at 4° c.

Dilution

- 1g of Azadirachtaindica resulting mass after extraction was taken
- Add it into a 100 ml conical flask
- Add dimethyl formamide and make up

- Take 1ml, 2ml and 3ml from the conical flask
- Add it to 10ml standard flask marked with 1, 2 and 3. Makeup to 10ml
- Resulting in 1000mcg/ml, 2000 mcg/ml and 3000 mcg/ml

Preparation of medium

Nutrient agar

- weigh 1.15g nutrient agar medium and add to a conical flask
- then add 25 ml of water to it and mix well
- plug it with cotton and cover with paper
- sterilize the medium and petri-dish in autoclave at 1700°C
- then transfer the medium into petri-dish in aseptic area and keep aside to set

Sabouraud dextrose agar medium

- weigh 3.25g sabouraud dextroseagar medium and add to a conical flask
- then add 25 ml of water to it and mix well
- plug it with cotton and cover with paper
- sterilize the medium and petri-dish in autoclave at 1700°C
- then transfer the medium into petri-dish in aseptic area and keep aside to set **Microbial assay**

E. coli

- Selected and labelled the culture
- Prepared nutrient agar plate Allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on bacterial cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

Saccharomyces cerevisiae

- Selected and labelled the culture
- Prepared sabouraud dextrose medium is allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on fungal cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

METHOD FOR Mimosa pudica

Extraction

- Take Whole plant of Mimosa Pudica which is washed in water then it is dried at 35-45 °C.
- The dried leaves are then pulverized in electric grinder.
- Take 20g powder and poured into the Soxhlet apparatus which the powder is plugged in between cotton
- 125 ml of 96% ethanol is poured to soak the powder

- 50 ml of ethanol is added to the round bottom flask and the apparatus is arranged
- This is heated at 78° C for 8 hours.
- The resulting extract was dried in a Rota vapour to get mass which is then stored at 4° C.

Dilution

- 1g of Mimosa Pudica resulting mass after extraction was taken
- Add it into a 100 ml conical flask
- Add dimethyl formamide and make up
- Take 1ml, 2ml and 3ml from the conical flask
- Add it to 10ml standard flask marked with 1, 2 and 3. Makeup to 10ml
- Resulting 1000mcg/ml, 2000 mcg/ml and 3000 mcg/ml

Preparation of medium

Nutrient agar

- weigh 1.15g nutrient agar medium and add to a conical flask
- then add 25 ml of water to it and mix well
- plug it with cotton and cover with paper
- sterilize the medium and petri dish in autoclave at 1700°C
- then transfer the medium into petri dish in aseptic area and keep aside to set

Sabouraud dextrose agar medium

- weigh 3.25g sabouraud dextrose agar medium and add to a conical flask
- then add 25 ml of water to it and mix well
- plug it with cotton and cover with paper
- sterilize the medium and petri dish in autoclave at 1700°C
- then transfer the medium into Petri dish in aseptic area and keep aside to set

Microbial assay

E. coli

- Selected and labelled the culture
- Prepared nutrient agar plate Allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on bacterial cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

Saccharomyces cerevisiae

- Selected and labelled the culture
- Prepared sabouraud dextrose medium is allowed to solidify completely before applying the disc. inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on fungal cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.

- Indicate whether test organism is resistant or sensitive to the antibiotic.

Figure 7

SOXHLET APPARATUS

Figure 6 EXTRACTS OF THE PLANTS

METHOD FOR Eryngiumfoetidum

Extraction

- Eryngiumfoetidumplants were collected, cut into pieces and shade dried.
- Then it is coarsely powdered
- Take 20g powder and poured into the Soxhlet apparatus which the powder is plugged in between cotton
- 125 ml of 96% ethanol is poured to soak the powder

- 50 ml of ethanol is added to the round bottom flask and the apparatus is arranged
- This is heated at 78° C for 8 hours.
- The resulting extract was dried in a rota-vapour to get mass which is then stored at 4° C.

Dilution

- 1g of Eryngiumfoetidum resulting mass after extraction was taken
- Add it into a 100 ml conical flask
- Add dimethyl formamide (DMF) and make up
- Take 1ml, 2ml and 3ml from the conical flask
- Add it to 10ml standard flask marked with 1, 2 and 3. Makeup to 10ml
- Resulting 1000mcg/ml, 2000 mcg/ml and 3000 mcg/ml

Preparation of medium

Nutrient agar

- weigh 1.15g nutrient agar (NA) medium and add to a conical flask
- then add 25 ml of water to it and mix well
- plug it with cotton and cover with paper
- sterilize the medium and Petri dish in autoclave at 1700°C
- then transfer the medium into Petri dish in aseptic area and keep aside to set

Sabouraud dextrose agar medium

- weigh 3.25g sabouraud dextrose agar medium and add to a conical flask
- then add 25 ml of water to it and mix well
- plug it with cotton and cover with paper
- sterilize the medium and petri-dish in autoclave at 1700°C
- then transfer the medium into petri-dish in aseptic area and keep aside to set

Microbial assay

E. Coli

- Selected and labelled the culture
- Prepared nutrient agar plate Allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on bacterial cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

Saccharomyces cerevisiae

- Selected and labelled the culture
- Prepared sabouraud dextrose agar (SDA) medium is allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on fungal cultures.

- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

METHOD FOR POLYHERBAL EXTRACT

Dilution

- Take 3.33gm of each extract and add it into a 100-standard flask
- Add DMF and make up to 100ml
- Then take 1ml, 2ml, 3ml from the above resulting solution into a 100 ml standard flask
- Make up to 100 ml to give 1000 mcg/ml, 2000 mcg/ml and 3000 mcg/ml respectively

Microbial assay

E. Coli

- Selected and labelled the culture
- Prepared nutrient agar plate Allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on bacterial cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

Saccharomyces cerevisiae

- Selected and labelled the culture
- Prepared sabouraud dextrose agar (SDA) medium is allowed to solidify completely before applying the disc.
- inoculated by streak plate method.
- Forceps was taken and sterilized in alcohol and often flamed and carefully take the discs and placed it on bacterial cultures.
- Incubated the plate in inverted position for 16-18 h at 37°. Observe the zone of inhibition around the antibiotic disc.
- Indicate whether test organism is resistant or sensitive to the antibiotic.

PRECAUTIONS:

1. Beef extract and peptone are hygroscopic powders, so weigh them quickly on a butter paper and recap the bottles tightly to prevent their solidification.
2. Do not spill the above two ingredients during weighing and transfer. They are foul smelling avoid inhaling their dust.
3. The conical flask for preparing the nutrient broth should be of appropriate size so that the required quantity of the medium to be prepared occupies only half of its volume. This precaution is to he taken to avoid spoilage of cotton plug during autoclaving cycle due to the boiling of the medium.
4. Validate the sterility of the nutrient broth by incubating it for 24 hours at 37°C. Any turbidity indicates that the medium is not sterile.

Nutrient Agar may be used to prepare Petri plates or Slants.

RESULTS AND DISCUSSION

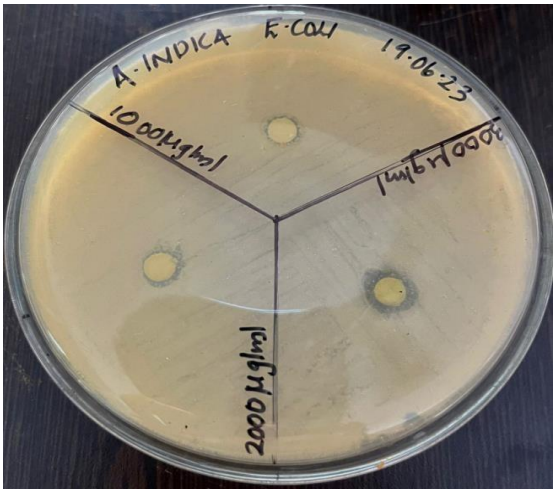


Figure 8
ZONE OF INHIBITION FOR AzadirachtaindicaIN E. coli

Figure 9 ZONE OF INHIBITION OF Mimosa pudica IN E. coli

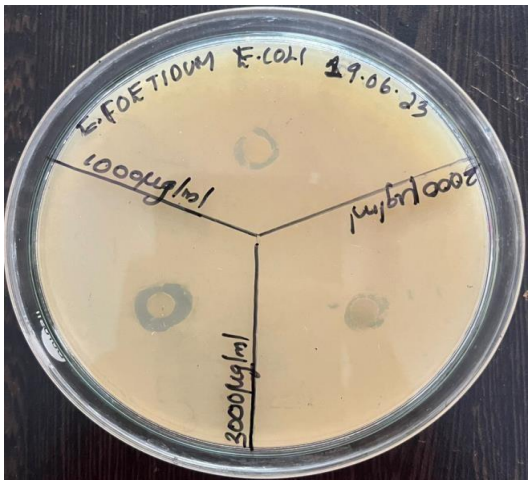


Figure 10 ZONE OF INHIBITION OF Eryngiumfoetidum IN E. coli

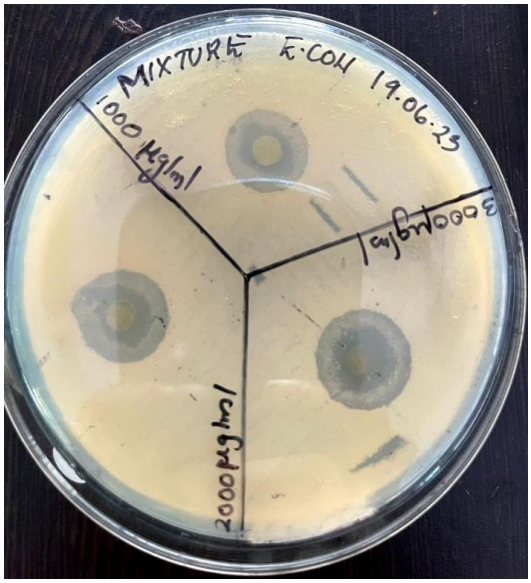


Figure 11 ZONE OF INHIBITION OF MIXTURE IN E. coli

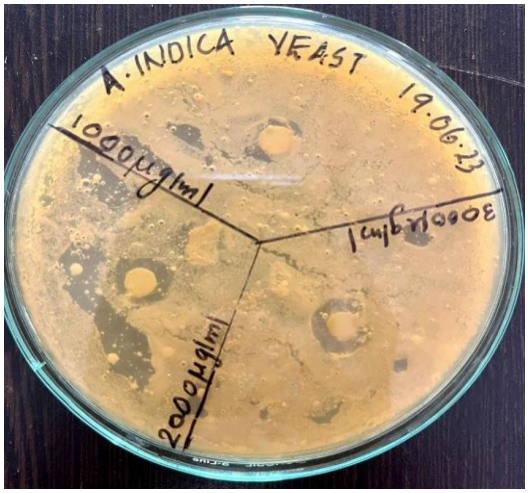


Figure 12 ZONE OF INHIBITION OF Azadirachtaindica IN YEAST

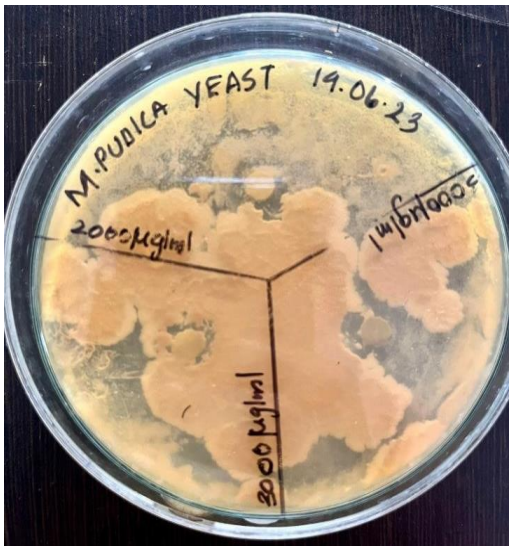


Figure 13 ZONE OF INHIBITION OF Mimosa pudica IN YEAST

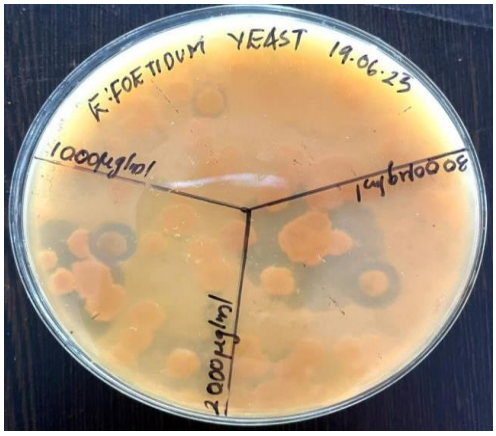


Figure 14 ZONE OF INIHIBITION OF EryngiumfoetidumIN YEAST

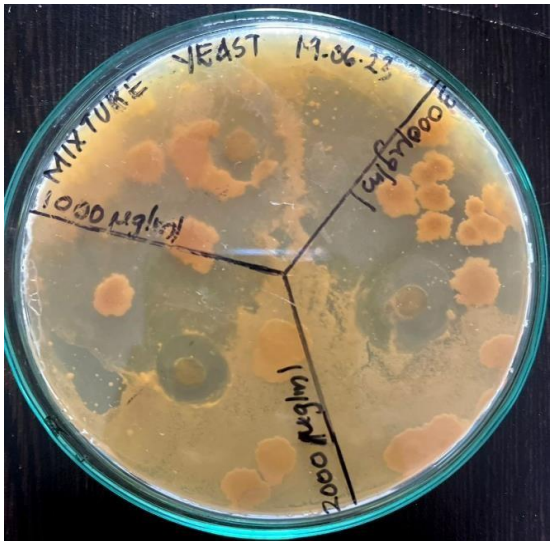


Figure 15 ZONE OF INHIBITION OF MIXTURE IN YEAST

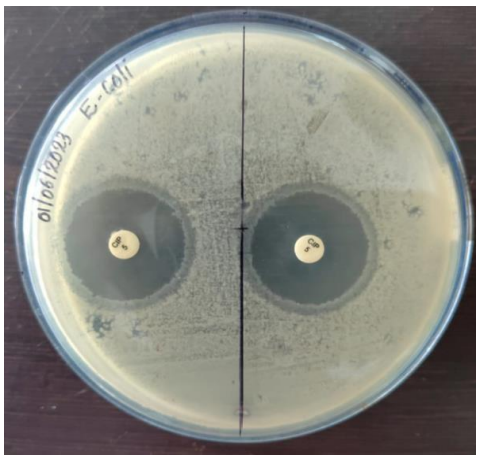


Figure 16 ZONE OF INHIBITION OF CIPROFLOXACIN IN E. COLI

Figure 17 ZONE OF INHIBITION OF CLOTRIMAZOLE IN YEAST

ZONE OF INHIBITION

PLANT	CONCENTRATION	ZONE OF INHIBITION FOR ECOLI	ZONE OF INHIBITION FOR YEAST
Azadirachtaindica	1000 mcg/ml	7 mm	7 mm
	2000 mcg/ml	9 mm	9 mm
	3000 mcg/ml	11 mm	11 mm
Mimosa pudica	1000 mcg/ml	8 mm	8 mm
	2000 mcg/ml	10 mm	10 mm
	3000 mcg/ml	12 mm	11 mm
Eryngiumfoetidum	1000 mcg/ml	9 mm	8 mm
	2000 mcg/ml	10 mm	10 mm
	3000 mcg/ml	12 mm	11 mm
MIXTURE	1000 mcg/ml	15 mm	12 mm
	2000 mcg/ml	17 mm	14 mm
	3000 mcg/ml	18 mm	17 mm
CIPROFLOXACIN	5 mcg/ml	25 mm	-
CLOTRIMAZOLE	10 mcg/ml	-	10mm

Table 9

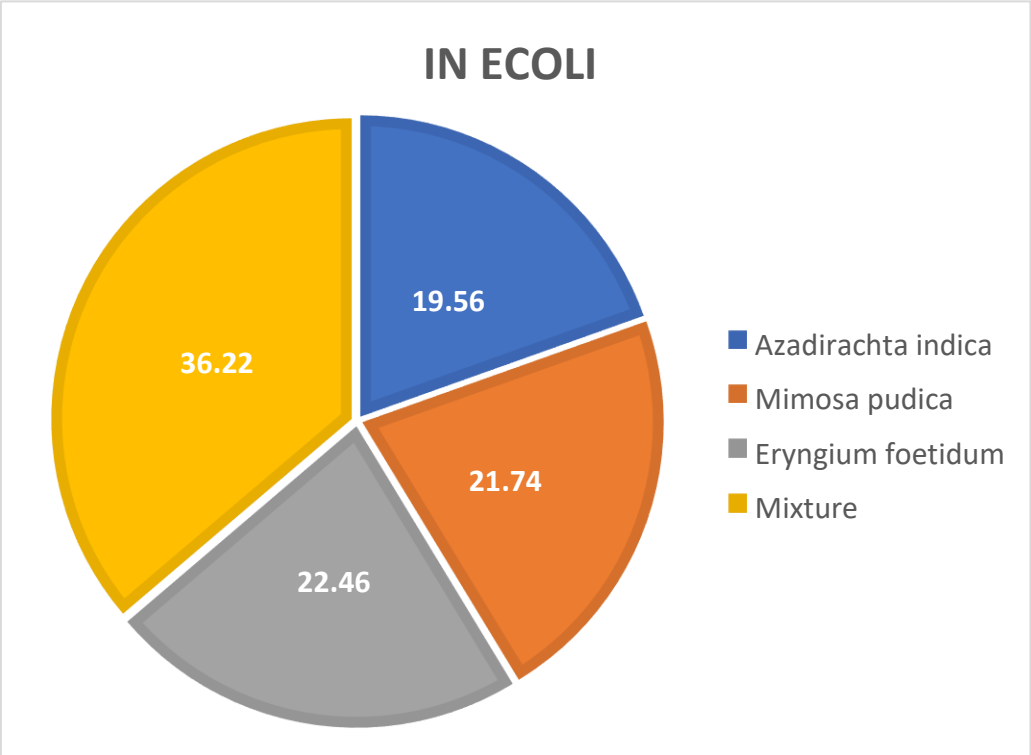


Figure 18

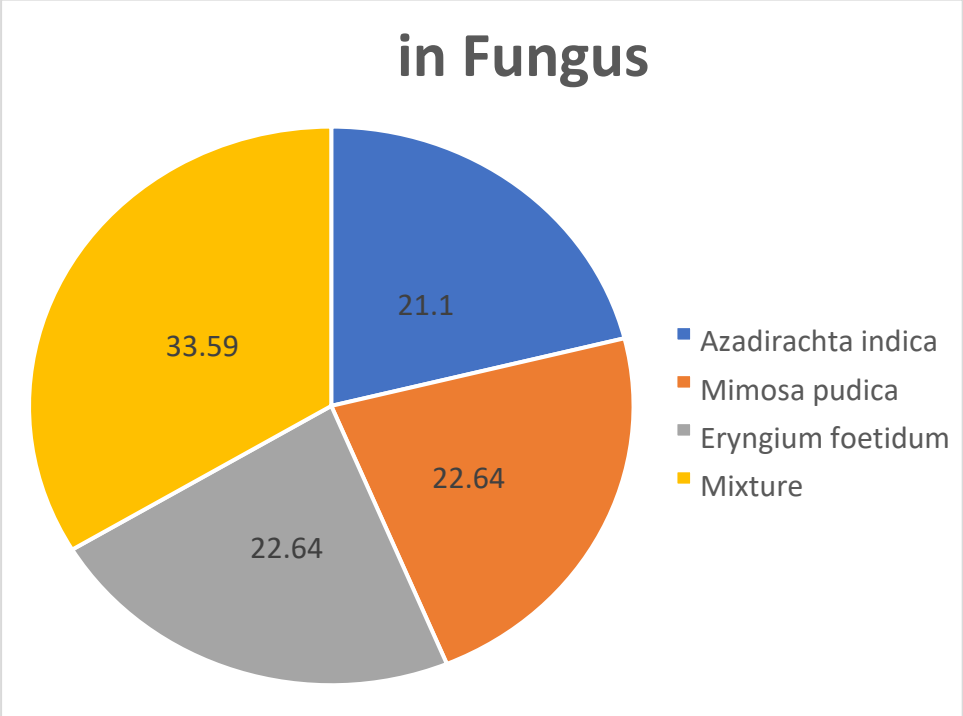


Figure 19

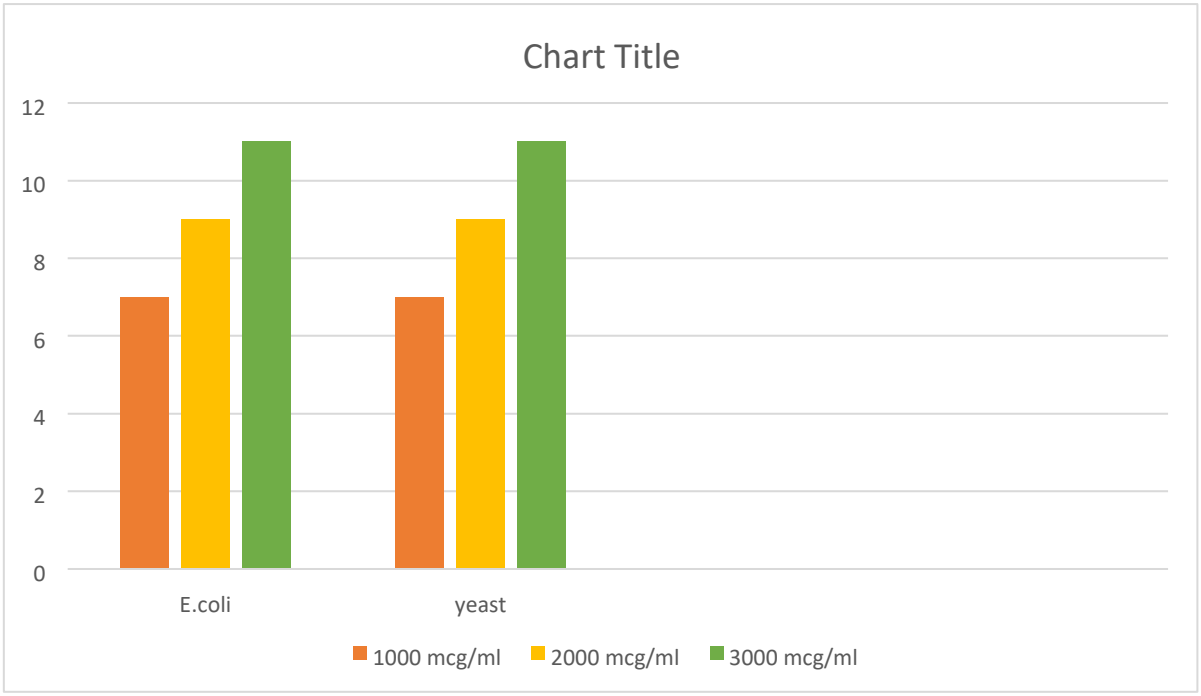


Figure 20 Azadirachtaindica

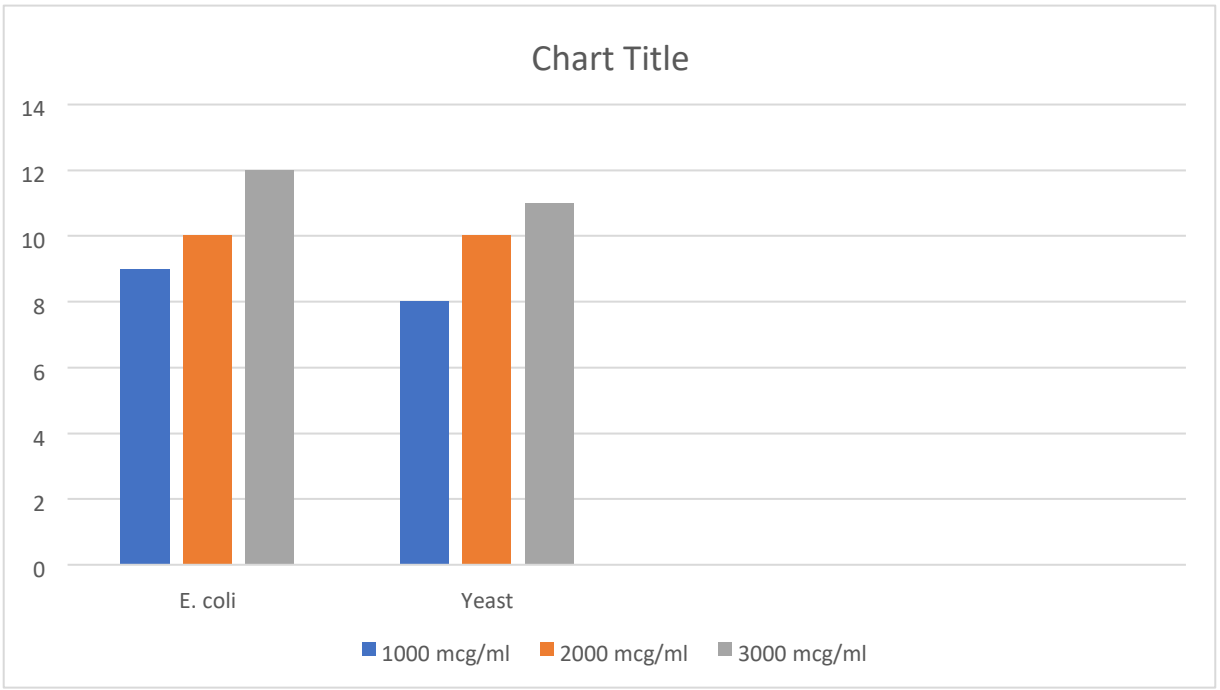


Figure 21 Eryngiumfoetidum

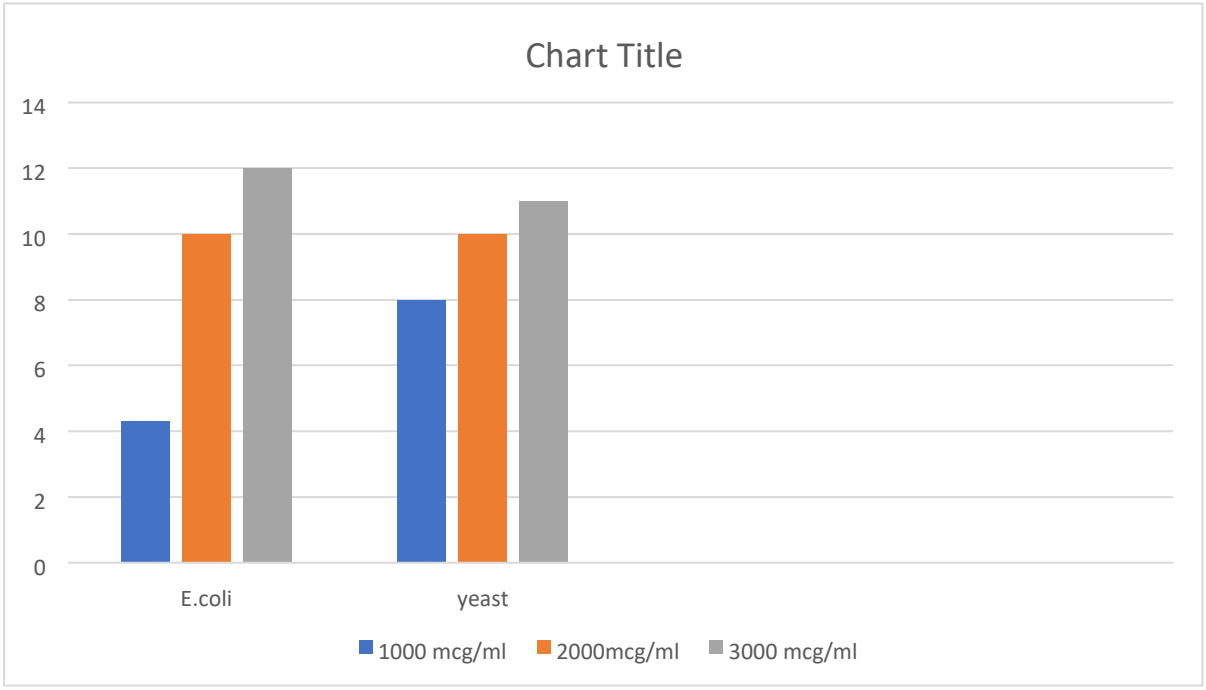


Figure 22 Mimosa pudica

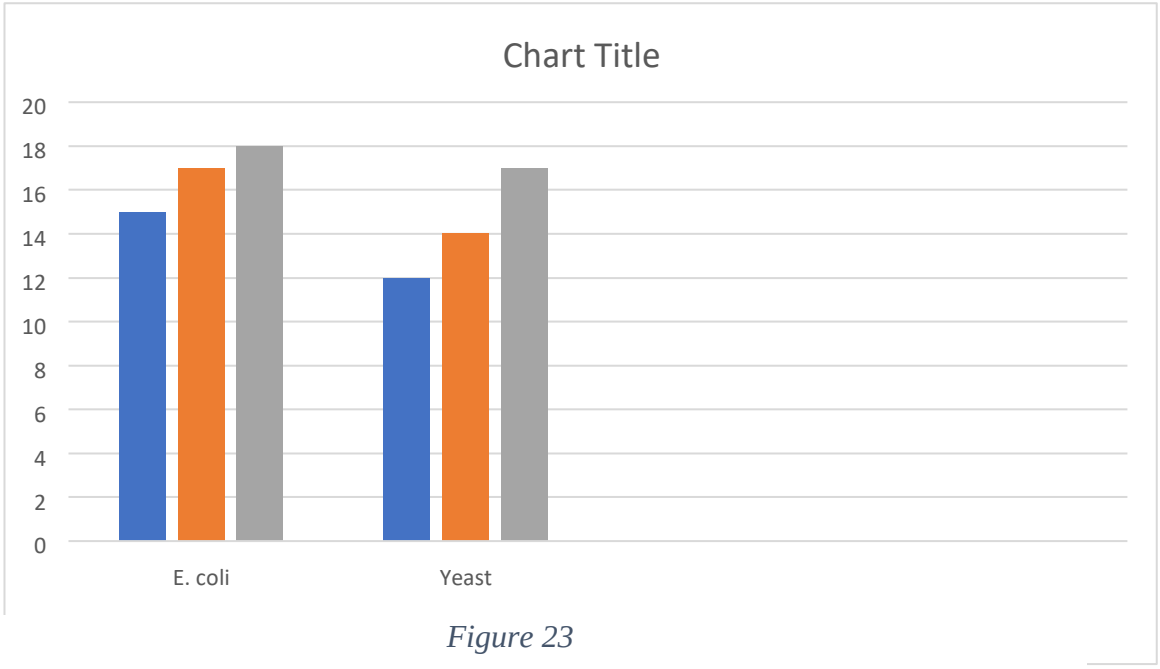


Figure 23

mixture

DISCUSSION

The data provided represents the results of an experiment evaluating the inhibitory effects of different plant concentrations on the growth of E. coli and yeast. Three plant species, namely Azadirachtaindica, Mimosa pudica, and Eryngiumfoetidum,were tested at various concentrations (1000 mcg/ml, 2000 mcg/ml, and 3000 mcg/ml). Additionally, a mixture of these plants was also included in the study. Two parameters were measured: the zone of inhibition for E. coli and the zone of inhibition for yeast, both denoted in millimeters (mm).

For Azadirachtaindica, the results showed that as the plant concentration increased from 1000 mcg/ml to 3000 mcg/ml, the zone of inhibition for both E. coli and yeast also increased. At 1000 mcg/ml, the inhibition zones were 7 mm for both E. coli and yeast, while at 2000 mcg/ml, they increased to 9 mm, and at 3000 mcg/ml, they further increased to 11 mm.

Similarly, for Mimosa pudica, increasing the plant concentration led to larger inhibition zones. At 1000 mcg/ml, the inhibition zones were 8 mm for both E. coli and yeast. At 2000 mcg/ml, they increased to 10 mm, and at 3000 mcg/ml, they further increased to 12 mm for E. coli and 11 mm for yeast.

Eryngiumfoetidum also exhibited increased inhibition zones with higher plant concentrations. At 1000 mcg/ml, the inhibition zones were 9 mm for E. coli and 8 mm for yeast. At 2000 mcg/ml, they remained relatively constant, with 10 mm for both E. coli and yeast. At 3000 mcg/ml, there was a slight increase to 12 mm for E. coli and 11 mm for yeast.

The mixture of all three plants showed the most pronounced inhibitory effects. At 1000 mcg/ml, the inhibition zones were 15 mm for E. coli and 12 mm for yeast. At 2000 mcg/ml, they increased to 17 mm for E. coli and 14 mm for yeast. Finally, at 3000 mcg/ml, the inhibition zones reached their highest values of 18 mm for E. coli and 17 mm for yeast.

Two standard drugs, ciprofloxacin and clotrimazole, were also included in the experiment. Ciprofloxacin at a concentration of 5 mcg/ml exhibited a substantial zone of inhibition of 25 mm against E. coli. Clotrimazole, at a concentration of 10 mcg/ml, resulted in a zone of inhibition of 10 mm against yeast.

SUMMARY AND CONCLUSION

SUMMARY

Antimicrobial resistance is one of the major problems faced by modern society. So, it's necessary to find a solution for this problem. combination of different antimicrobial drugs should be effective than individual drugs.

The common plants which are available at our locality exhibits antimicrobial activity.

Azadirachtaindica is one of the major plants which shows potent antimicrobial activity. Mimosa pudica, eryngiumfoetidum are also have proven antimicrobial activity and also are easily available. The mixture of the extracts of these three herbal plants should exhibit more antimicrobial activity than individual extracts of these plants.

At first raw plants are collected from the locality. Leaves of the

Azadirachtaindica, Eryngiumfoetidum and leaves and roots of the Mimosa pudica are used. Plants are washed thoroughly under tape water to remove unwanted debris and mud from the plants. then they are thoroughly dried at shade. Fleshy leaves of the eryngiumfoetidum should dried well. Then the leaves of the plants are powdered well separately.

The 20g of the powdered plants are then extracted separately using

Soxhlet apparatus, ethanol (175ml for each plant) is used as solvent. Extraction is continued till obtaining the clear solution. Then the obtained extracts are evaporated to remove the ethanol till obtaining the powder form of the crude extract. The extract is diluted using DMF (dimethyl formamide) as a solvent to get 1000mcg,2000mcg,3000mcg of each plant extract. Similarly, mixture of the plant extracts is also made in three different concentrations.

For antimicrobial assay Disc Plate method by using E. coli and saccharomyces cerevisiae is performed. Nutrient agar medium for bacteria and sabouraud dextrose agar medium for fungus is selected. The prepared medium Is first inoculated with the organisms and then discs dipped in the plant extracts and

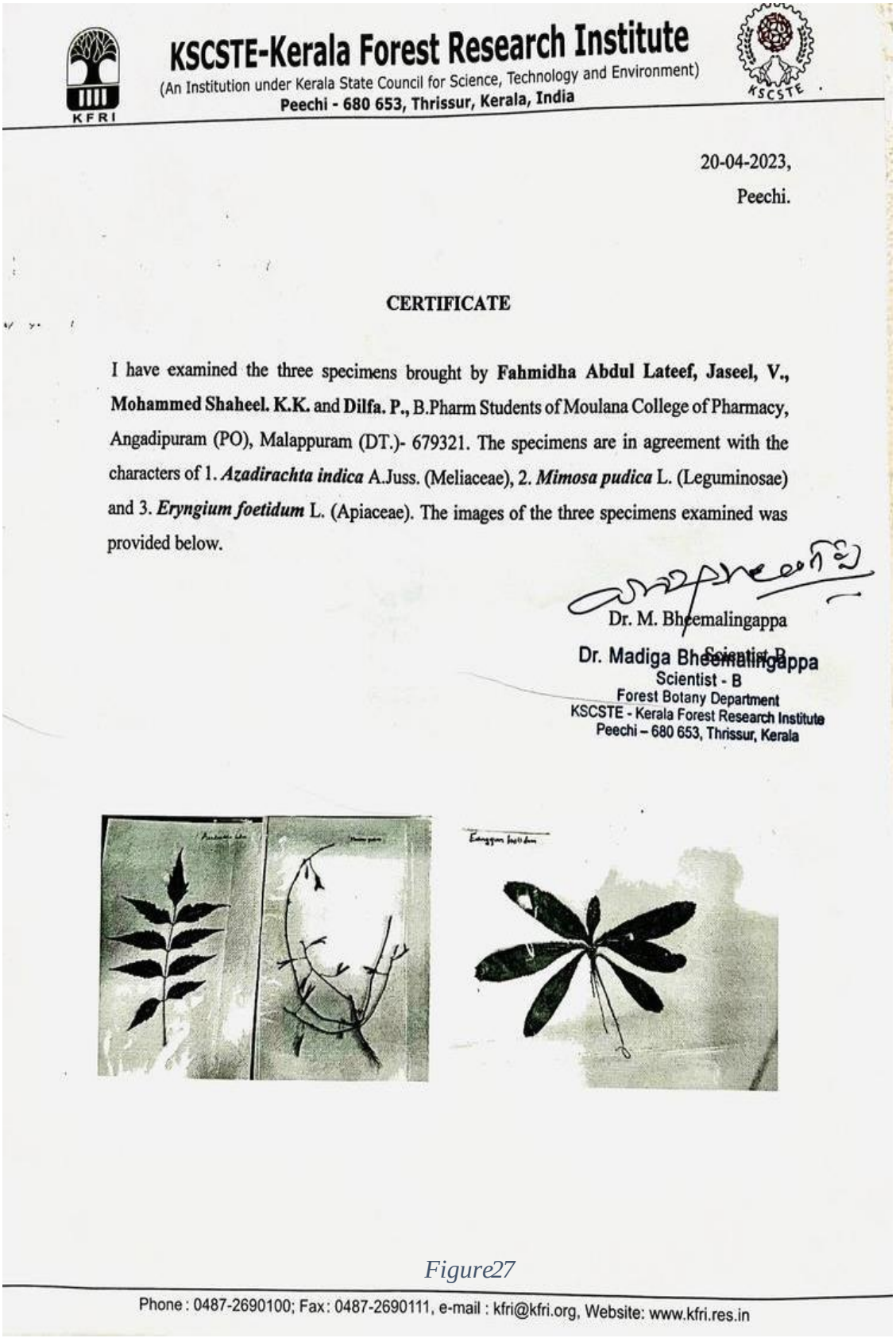
mixture are placed in the inoculated medium in an aseptic area with laminar airflow system. Then the plates are incubated for 24 hours.

The results were as expected, the mixture of the plant extract has shown more zone of inhibition than the individual plant extract. The zone of inhibition is measured.

Figure 24 HERBERIUM OF AZADIRACHTA INDICA

Figure 25 HERBERIUM OF MIMOSA PUDICA

Figure 26 HERBERIUM OF ERYNGIUM FOETIDUM



KSCSTE-Kerala Forest Research Institute
(An Institution under Kerala State Council for Science, Technology and Environment)
Peechi - 680 653, Thrissur, Kerala, India

20-04-2023,
Peechi.

CERTIFICATE

I have examined the three specimens brought by **Fahmidha Abdul Lateef, Jaseel, V., Mohammed Shaheel. K.K.** and **Dilfa. P.,** B.Pharm Students of Moulana College of Pharmacy, Angadipuram (PO), Malappuram (DT.)- 679321. The specimens are in agreement with the characters of 1. ***Azadirachta indica*** A.Juss. (Meliaceae), 2. ***Mimosa pudica*** L. (Leguminosae) and 3. ***Eryngium foetidum*** L. (Apiaceae). The images of the three specimens examined was provided below.

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Figure27

Figure 27 PLANT AUTHENTICATION CERITIFICATE

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