Antibacterial and Antifungal Activities of Plant Extract

Shivika Chadha, Research Scholar, CT University, Ludhiana, Punjab

Abstract

The emergence of multidrug-resistant pathogens has raised serious concerns in the biomedical and clinical domains regarding the widespread and repetitive use of chemical antibiotics. Because they produce secondary metabolites, plants are attractive options for safe, natural antimicrobial agents. Extracts from four native plants—Nerium sp., Mentha sp., Aloe vera, and Eucalyptus sp.—are evaluated for their antibacterial and antifungal properties in comparison. Using a microwave, the entire phenolic and flavonoid content was extracted., and this content was then used for phytochemical assays. Antimicrobial experiments using the microdilution technique were carried out. A one-way ANOVA test and post hoc analysis were used to synthesise antibiotic sensitivity data and percentage inhibition. Nerium sp. showed a lower total phenolic content, while Mentha sp. showed a significantly higher content. Significantly, the extracts at higher concentrations showed strong antifungal and antibacterial properties that were on par with those of control antibiotics. Even at lower concentrations, eucalyptus sp. extracts demonstrated significant growth reduction. Finally, these plant extracts show great promise in the management of microbial infections, exhibiting antibiotic-like efficacy with reduced susceptibility to resistance development.

Keywords: microbial, antibiotic, antifungal, Eucalyptus sp., Aloe vera, Mentha sp., Nerium sp.

1. INTRODUCTION

Microbial illnesses are very common and make a substantial contribution to the total burden of disease, especially those that are bacterial in nature. A significant number of people contract microbiological infections each year, which puts a significant burden on a nation's social and economic stability. The widespread and recurrent use of antibiotics, which is indicative of the empiric approach to treatment, has created serious threat to the biomedical and biomedical clinical sectors. The advent of pathogens that are resistant to multiple drugs presents a formidable obstacle, giving organisms a chance to evolve diverse kinds of β-lactamases. Consequently, this calls into question the effectiveness of β-lactam antibiotics and adds to the concerning growth of drug-resistant organisms, leading to significant harm to human health. The growing danger of antimicrobial resistance in the face of traditional antibiotic treatments emphasises the urgent need for alternative tactics and therapeutic approaches[1-4]. When antibiotics are used to treat viral infections, the body's immune system is weakened, and sometimes the natural bacteria present in the body become opportunistic and cause chronic problems [5,6]. The plant extracts have great potential to treat infections caused by bacteria, fungi, viruses, and protozoa, and they have no known side effects. They are a good candidate for an antimicrobial agent due to the presence of secondary metabolites, such as flavonoids, terpenoids, tannins, and alkaloids [7]. They are antimicrobial against a range of bacteria, both Gram-positive and Gram-negative [8,9]. Elizabeth et al. [4] looked into the phytochemical screening and antibacterial activity of Dialium guineense seed extract against enteric bacteria. According to Yaraksa [3], five different medicinal herbs were used: Tristaniopsis burmanica, Capparis zeylanica Linn., Markhamia stipulata, Caryota maxima, and Amphineurion marginatum. They are beneficial not only for their antibacterial properties but also for their anti-inflammatory ones.

To extract the bioactive compounds from various plant parts, various chemicals have been employed. Alam et al. conducted a study to investigate the antibacterial qualities of methanol extract, petroleum ether, chloroform, and ethyl acetate from the root bark of Akanda (Calotropis gigantea) [10]. The outcomes showed a noteworthy antibacterial activity against a variety of the tested bacteria. To ascertain Tribulus terrestris L. saponins' antifungal properties, comparable tests were carried out on Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis, and Cryptococcus neoformans. The findings showed that saponins have a strong antifungal effect, which is explained by their capacity to damage the fungal cell membrane and impede the growth of the fungus. This demonstrates the various ways that chemical extraction techniques can be applied to investigate the medicinal potential of compounds derived from plants against infections caused by bacteria and fungi [11]. It was discovered that the clinical isolates examined in the assessment of Mentha spicata and Mentha piperita essential oils' antibacterial activity were successfully inhibited [12]. Many essential oils have been researched, eucalyptus among them, for their potential medical benefits and have been utilised in traditional medicine across the globe. Essential oils derived from Eucalyptus sp. possess analgesic, antimicrobial, antifungal, and anti-inflammatory characteristics, making them extensively employed in cosmetic, food, and pharmaceutical items [13]. There is still much to learn despite numerous researchers reporting notable successes using plant extracts against various microorganisms.

2. LITERATURE REVIEW

Budniak et al. (2021) [19] described in “The antibacterial and antifungal activities of the extract of Gentiana cruciata L. herb." L. is a well-known plant that is a member of the Gentianaceae family and has been utilised for a very long time in traditional medicine across many nations. Many ailments can be treated with Gentiana cruciata L.'s herb and roots. Researchers looked into the dried extract of the herb Gentiana cruciata L.'s antibacterial and antifungal qualities. The dry extract's phytochemical analysis showed the presence of tannins and polyphenols, both of which have antibacterial and antifungal qualities. The total content of tannins and polyphenols, recalculated at pyrogallol, was 1.84% and 8.52%, respectively. The antibacterial and antifungal activity of the dry extract of Gentiana cruciata L. herb was determined by measuring the inhibition zone diameters (mm) using the "wells" method (agar diffusion). The native extract demonstrated impressive activity against Staphylococcus aureus (25.2 mm), Bacillus subtilis (22 mm), Escherichia coli (20.1 mm), and Candida albicans (17.9 mm). Furthermore, very high activity against Staphylococcus aureus (19 mm), Bacillus subtilis (15 mm), Escherichia coli (13 mm), and Candida albicans (11.6 mm) was demonstrated by the herb Gentiana cruciata L. extract at a N:2 dilution. One potential use for the extract of Gentiana cruciata L. herb is as an active pharmaceutical ingredient in novel drug formulations.

Danish et al. (2020) [15] Described in "Antifungal and antibacterial activity of aloe vera plant extract. A well-known medicinal plant with numerous therapeutic uses is aloe vera. It is naturally composed of numerous advantageous compounds that have a broad range of therapeutic applications. This plant contains a variety of active compounds, including lignin, salicylic acid, naftoquinones, sterols, minerals, aloesin, aloeemodin, aloin, flavonoids, and amino acids. Other substances include vitamins that are soluble in fat and water, minerals, enzymes, simple and complex sugars, organic acids, and phenolic compounds. Aloe vera is used for its antifungal and antibacterial qualities against a variety of pathogenic fungal and bacterial strains. Various concentrations of Aloe vera leaf and root ethanol extract are used to treat these bacterial and fungal strains. Agrobacterium tumefacins and Escherichia coli exhibit an 18 mm zone of inhibition, which is regarded as a positive result. Around 16mm, Bacillus subtitis and Bacillus megaterium also exhibit good results. Pseudomonas aeruginosa and Proteus mirabilis exhibit a minimum zone of inhibition of approximately 11 mm. Aspergillus fumigatus, Aspergillus niger, Candida albicans, and fuserium oxysporum are the four fungal strains that were used; of these, Aspergillus niger and fuserium oxysporum exhibit the best results, measuring about 19 mm against both root extract and leaf extract.

Oluwajobi et al., (2019) [16] proposed in "Antibacterial and Antifungal activities of aqueous leaves extract of some medicinal plants. The phytochemical compositions and antibacterial and antifungal activities of the aqueous leaf extracts of Psidium guajava, Vernonia amygdalina, and Azadiracta indica were assessed in relation to microbial isolates. The standards for the antibacterial and antifungal assays were ampicillin and vastatin, respectively. The plant aqueous extract contained all of the phytochemicals that had been tested. The methanol extract of P. guajava and A. indica did not contain any glycoside or anthraquinone, respectively. Azadirachta indica recoded the lowest, while Psidium guajava recorded the highest levels of phenol (18.63±0.29 mg/g) and alkaloids (6.40±0.35 mg/g). Saponins were highest in Vernonia amygdalina (6.63±0.34 mg/g) and lowest in Azadirachta indica (2.40±0.34 mg/g). The highest tannin content, however, was found in Azadirachta indica (23.52±0.25 mg/100g). The Azadirachta indica aqueous extract exhibited a larger zone of inhibition range against fungi and bacteria, respectively, measuring between 13.00±0.05 and 14.00±0.05 mm and 10.00±0.10 to 14.00±0.05 mm. Psidium guajava exhibits greater antifungal activities and lower activity against bacteria. Conversely, Vernonia amygdalina exhibited an inhibition zone of 13.00±1.45 mm against Pseudomonas aeruginosa and Streptococcus faecalis, as well as antifungal activities ranging from 9.00±0.35 mm to 15.00±0.45 mm. In summary, compared to the other plant extracts, Azadiracta indica extract showed greater antibacterial activity and Psidium guajava showed greater antifungal activity. In order to extract the special biopotential compounds from these plants and turn them into antimicrobial agents that can be used in the food industry, more research must be done.

Hassan et al., (2019) [18] described in "Antibacterial and antifungal activities of the medicinal plant veronica biloba." Plants are inherently endowed by God with the ability to synthesise medicinal compounds, and because they are not readily available as pure compounds or as standardised extracts, they greatly aid in new discoveries in the field of chemical diversity. After extracting the medicinal plant Veronica biloba using the Soxhlet and maceration methods, a preliminary antimicrobial screening against pathogenic microorganisms was carried out. Fractionation was done with liquid-liquid extracts, including ethyl acetate, water, dichloromethane, and plant extract in hexane. The fractions were then tested using the well-diffusion method at sample concentrations of 10–30 µL for antifungal and antibacterial activity. The outcome showed that every extract had antimicrobial activity against every pathogen tested. The ethyl acetate extract demonstrated greater activity in comparison to other extracts of a similar kind. Out of all the extracts, only the ethyl acetate extract shows promise against test strains of fungi that are larger than the standard Nystatin test control and against both gram-positive and gram-negative bacteria. To treat infections brought on by microorganisms, Veronica biloba extract may be utilised.

Dahham et al. (2010) [17] discussed in "Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.)." The Natural products have recently been assessed for their potential as sources of antimicrobial agents that are effective against a range of microorganisms. The antibacterial and antifungal properties of pomegranate peel extract (rind), seed extract, juice, and whole fruit were investigated in relation to a set of chosen bacteria and fungi. Of all the extracts, the peel extract has demonstrated the strongest antimicrobial activity. The highest antibacterial activity against Staphylococcus aureus and the highest antifungal activity against Aspergillus niger were found in the selected bacterial and fungal cultures.

Erturk and Omer. (2006) [14] described in "Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants." Using agar dilution methods, the in vitro antibacterial activity of eleven ethanolic extracts derived from various spices, namely Melissa officinalis, Mentha piperita, Laurus nobilis, Rhus coriaria, Dianthus coryophyllum, Erica arborea, Colutea arborescens, and Cuminum cyminum, was assessed. These spices were gathered from various parts of Turkey as well as neighbourhood markets, and ethanol was used in the extraction procedure. The extracts were then examined for their ability to inhibit the growth of different bacterial strains. Staphylococcus aureus, S. epidermidis, Bacillus subtilis, and other Gram-positive and Gram-negative bacteria were among the strains of bacteria that were present. In addition, these extracts' potential toxicity to Aspergillus niger and Candida albicans was evaluated using the disc-diffusion and agar dilution methods. The minimum inhibition concentration (MIC) of 5 mg/mL was observed for each tested microorganism in the ethanolic extracts of M. piperita, L. nobilis, and J. oxycedrus. With a minimum inhibitory concentration (MIC) of 5 mg/mL, P. aeruginosa was the strain of bacteria most susceptible to P. nigrum and E. arborea extracts among both Gram-positive and Gram-negative bacteria tested. The extracts of L. nobilis, D. coryophyllum, J. oxycedrus, and C. arborescens showed higher inhibitory activity against the fungus A. niger and the yeast C. albicans than the common antifungal nystatin.

Table:1 Comparative table summarizing the information from the mentioned studies:

Author and Year	Title of the Study	Main Findings
Budniak et al. (2021)	"The Antibacterial and Antifungal Activities of the Extract of Gentiana cruciata L. Herb"	A herb extract from Gentiana cruciata L. has been investigated for its antibacterial and antifungal qualities. - Polyphenols and tannins were detected by phytochemical analysis. - The agar diffusion method was used to determine

		the antibacterial and antifungal activity. extremely effective against Candida albicans, E. coli, and S. aureus.
Danish et al. (2020)	"Antifungal and Antibacterial Activity of Aloe Vera Plant Extract"	Aloe vera extracts were evaluated against various bacterial and fungal strains. - Ethanol extract was applied in different concentrations (15, 20, 25, and 30µl). - Success against bacteria like E. Coli and A. tumefacins was observed. - The fungus strains A. niger and F. oxysporum outperformed a root and leaf extract by a significant margin.
Oluwajobi et al. (2019)	"Antibacterial and Antifungal Activities of Aqueous Leaves Extract of Some Medicinal Plants"	We evaluated the phytochemical compositions of P. guajava, V. amygdalina, and A. indica aqueous extracts. - examined against bacterial and fungal isolates. - The plant with the strongest antimicrobial activity was Azadirachta indica. - Psidium guajava showed reduced antibacterial qualities but greater antifungal ones.
Hassan et al. (2019)	"Antibacterial and Antifungal Activities of the Medicinal Plant Veronica Biloba"	The Soxhlet and maceration methods used to prepare Veronica biloba extracts were evaluated for their antimicrobial activity. - Water, hexane, dichloromethane, and ethyl acetate are fractionated liquid-liquid extracts. Compared to the other extracts, the ethyl acetate extract exhibited a greater level of antimicrobial activity. - Has the potential to be more potent against gram-positive and gram-negative bacterial and fungal strains than standard Nystatin. - It has been suggested to use Veronica biloba extract to treat microbial infections.
Dahham et al. (2010)	"Studies on Antibacterial and Antifungal Activity of Pomegranate (Punica granatum L.)"	Investigated the antifungal and antimicrobial qualities of pomegranate seed extract, peel extract, and juice. The antimicrobial agent found in peel extract proved to be the most effective. - The most resistant bacteria to antibacterial activity is Staphylococcus aureus. - Aspergillus niger has a strong antifungal effect.
Erturk and Omer (2006)	"Antibacterial and Antifungal Activity of Ethanolic Extracts from Eleven Spice Plants"	Examined the antibacterial activity of eleven spice plants in vitro against bacteria that are both Gram-positive and Gram-negative. - evaluated toxicity against Candida albicans and Aspergillus niger. - The MIC was 5 mg/mL for the extracts of J. oxycedrus, L. nobilis, and M. piperita. - P. aeruginosa reacted best to P. nigrum and E. arborea extracts.

3. RESEARCH METHODOLOGY

3.1 Plant collection and secondary metabolite extraction:

Aloe barbadensis, Nerium oleander, Eucalyptus spp., Mentha spp., and the natural habitat of the Guru Ghasidas Vishwavidyalaya campus in Bilaspur, Chhattisgarh, India were collected. Using a microwave-assisted extractor (MAE), the phenolic and flavonoid content of the chosen plant parts was extracted. The extracted material was then used for further phytochemical tests. By combining conventional solvent extraction techniques with microwave technology, MAE is applied to produce improved extraction kinetics. Notably, MAE is more cost-effective overall and has a number of benefits over traditional extraction methods, including shorter extraction times, fewer solvent requirements, and higher extraction efficiency [20].

- **Phytochemical Assay:** Total phenolic content (TPC) and total flavonoid content (TFC): The total phenolic content was determined using the foiln ciocalteau assay [21], which is explained below. One millilitre of the oil extract, four millilitres of the sodium carbonate (7.5%) solution, and five millilitres of the 10% folinciocalten reagent were mixed together. Following a 20-minute inoculation period at room temperature, the absorbance at 760 nm was determined. Gallic acid solution was used to create the standard curve, and the sample's phenolic content was expressed as mg of GAE (gallic acid equivalent) /gm of extract.
  The total flavonoid content was ascertained by means of the colorimetric assay with aluminium chloride. [22]. 4 ml of deionized water were used to dilute 1 ml of oil. After adding 300 µl of a 5% solution of sodium nitrate for five minutes, 300 µl of a 10% solution of aluminium chloride was added for six minutes. The final volume was increased to 10 ml with distilled water after adding 2 ml of 1M NaOH. After thoroughly mixing the solution, the absorbance at 510 nm was determined. The whole number of flavonoids was reported in milligrams of quercetin equivalents.
- **Antimicrobial Assay**
  - ✓ **Microbial media and chemicals:** Hi-media provided ciprofloxacin and nutrient growth medium. The growth of bacteria was tested using nutrient agar and nutrient broth media, while the growth of fungal species was tested using yeast peptone broth.
  - ✓ **Microbial strains:** MTCC 227 and MTCC 96 of Staphylococcus aureus were utilised for the minimum inhibitory concentration (MIC) calculation and antimicrobial investigations. While fungal inoculums were prepared in accordance with Al-Hatmi et al.[24], bacterial inoculums were prepared in accordance with Wiegand et al. [23].
  - ✓ **Antimicrobial activity as well as MIC determination:** Oils extracted from various plant leaves that contain flavonoids and phenolics are used to calculate the minimum inhibitory concentration and measure the antimicrobial activity. A 96-well ELISA plate was pipetted-grown with 50 µl test isolates. Each plant's final extract was regarded as its stock (D1: (µg/well). Nerium, Mentha, Aloe vera, and Eucalyptus have D1 values of 58, 55, 59, and 58, in that order. By diluting each stock, two additional dilutions were made: D2 and D3 . The MIC was ascertained using these three distinct oil sample dilutions (D1 to D3). The experimental plates were incubated for 24 hours at 35°C and 72 hours at 25°C for fungi and bacteria, respectively. When there were bacteria in an ELISA plate reader, the observations were made after 24 and 48 hours. In contrast, fungal growth was observed following a 72-hour incubation period. First, growth control was employed, followed by positive control (antibiotics at a concentration of 50µg/ml). Growth control was used to track the impact of

the antibiotic activity process, and positive control was used to compare the efficacy of the drug.

3.2 Analytical statistics:

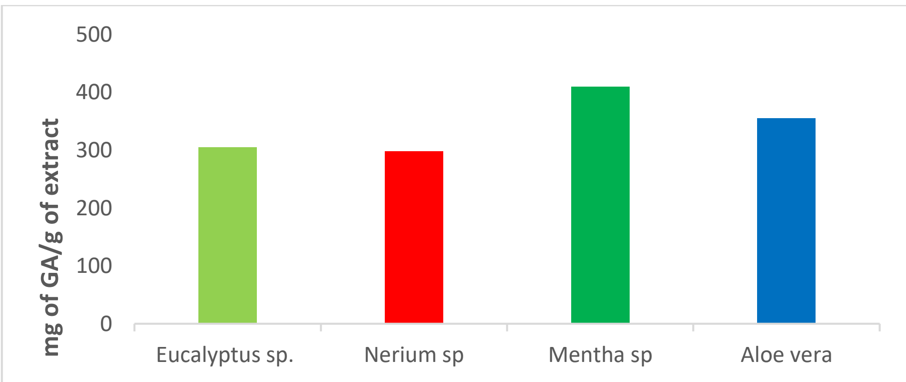
The normalcy of the continuous data was evaluated using the Kolmogorov-Smirnov test. The numerical data was displayed using the mean and SD. An integrated one-way ANOVA test and post hoc analysis were used to compile data on antibiotic sensitivity and percent inhibition. Using SPSS-created graphs, the p-value, or level of significance, was determined to be less than 0.05.

4. RESULTS AND DISCUSSION

Four native plant species were employed to compare the effectiveness of each species' antimicrobial defences. These plants are native to the area and are members of various families. Crude extracts from these plants are used by the local ethnic community to treat illnesses.

4.1 Total Phenolic and Flavonoid content:

Two significant phytochemicals that have been identified from several therapeutic plants are phenolics and flavonoids. These substances primarily aid in the plant's reaction to various stressors. The total phenolic contents of the extracted essential oils were measured and expressed in terms of gallic acid equivalent using the Folin-Ciocalteu method. The results of the findings are shown in (Figure 1A). Nerium sp. had the lowest total phenolic content and Mentha sp. the highest (all $p < 0.05$). The amounts of flavonoids in essential oil extracts were quantified and expressed in terms of quercetin equivalent using an aluminium chloride (AlCl3) spectrophotometric method. The amounts of flavonoids found in different plant extracts are shown in (Figure 1B).The concentration of flavonoids is low in comparison to the total amount of phenolic contents. It was to be expected, since plants typically contain very small amounts of flavonoids. Four experimental plants' flavonoid concentration trends are comparable to those of total phenolics. Mentha sp. had the highest value, while Nerium sp. had the lowest (all $p < 0.05$). The flavonoid concentrations of Eucalyptus sp. and Aloe vera are comparable ($p > 0.05$) and fall between those of Mentha and Nerium sp (p 0.05).



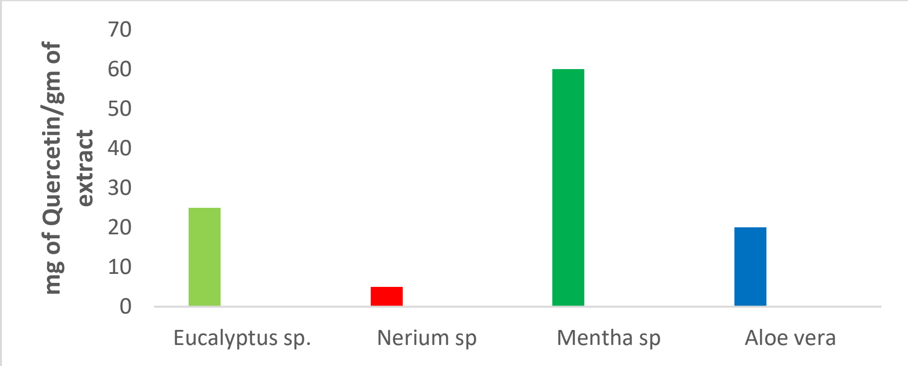
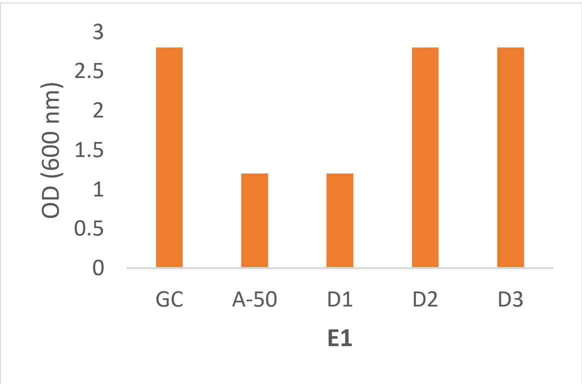


Figure 1: Four distinct plants' total phenolic (A) and total flavonoid (B) contents. There was a noticeable difference between the two small alphabets in section A and section B. A post hoc LSD one-way ANOVA was used to determine significance at $p < 0.05$.

4.2 Antimicrobial activity:

Essential oils containing phenolics and flavonoids were extracted from Nerium sp. (E1), Mentha asp. (E2), Aloe vera (E3), and Eucalyptus sp. (E4). The antimicrobial activities of the oils against bacterial and fungal species were assessed. In 96-well plates, the experiment was conducted using the micro dilution method. Different wells underwent the appropriate controls. Following incubation, absorbance was measured, and the average outcomes are shown in (Figures 2 and 3). The outcome of treatment against a bacterium is displayed in (Figure 2). Staphylococcus aureus was the bacterial strain selected for this investigation because it is a common human bacterial pathogen with a wide range of clinical manifestations and a commensal bacterium. Gram-positive S. aureusis bacteria have a coccus shape. Antibiotics, which served as the experiment's positive control, can cause it to become sensitive. Higher concentrations of plant extracts demonstrated a strong anti-pathogenic effect against Nerium sp. (E1). The activity matched that of antibiotics used at 50µg/ml at higher concentrations. Growth is unaffected and there is very little activity against the pathogen at lower concentrations (Figure 2). The extracts of Mentha Sp. (E2) and Aloe vera (E3) showed a similar outcome. Higher concentrations killed the bacterial pathogen more effectively in each of the three cases than did lower concentrations. At times, it appears as though the organism's growth is being aided by lower concentrations (Figure 2). This might be explained by changes in the biological characteristics of the active ingredients in essential oils, such as their altered antimicrobial capability, which causes them to become growth-promoting substances rather than growth-inhibiting ones. The essential oil that was extracted from the eucalyptus sample (E4) produced different results. It demonstrated a notable ability to reduce growth even at lower concentrations. Both higher and lower concentrations of eucalyptus oils effectively killed the bacteria and exhibited behaviour comparable to that of the antibiotics used as a control. Thus, it is reasonable to draw the conclusion that eucalyptus essential oil has the ability to successfully stop gram-positive bacteria from growing. Interestingly, though, when all four essential oils were compared at higher active concentrations, eucalyptus was not determined to be the best (Figure 2). In this comparison, Mentha sp. showed a much greater ability to inhibit growth, even surpassing the antibiotics that were utilised as a positive control. A similar experiment was carried out to find out how well these essential oils worked against a fungal infection.; the findings are shown in (Figure 3). Given that Candida albicans coexists with most humans in a harmless commensal state, it was the perfect choice for this study. But in the right circumstances, Candida albicans can cause anything from minor skin infections to potentially fatal systemic infections. The findings displayed in Figure 3 demonstrated comparable antifungal and antibacterial activity. When compared to lower concentrations, all four plant extracts demonstrated excellent response at higher concentrations. At higher concentrations, the growth inhibition matched or exceeded that of control antibiotics. In three of the four instances, there was no discernible difference between the growth control and lower concentrations of the various essential oils. Only in the case of eucalyptus oil was growth inhibition seen, even at lower concentrations. In our comparison of the four plant extracts at higher concentrations, eucalyptus oil was found to be the most efficacious antifungal agent. Compared to the positive control, which consisted of antibiotics, eucalyptus oil demonstrated significantly greater activity against Candida albicans. Many teams from various nations have completed related work with comparable encouraging outcomes. Gulluce et al. [25] also looked into the antioxidant and antimicrobial properties of menthalongifolia essential oil, and they found that the essential oil had strong antibacterial activity against 30 infections. And research on the antimicrobial properties of Nerium oleander leaf material. Using the well diffusion method, they extracted the leaves using the cold maceration extraction method in methanol and chloroform to combat a variety of bacterial and fungal isolates, such as E. Coli, B. subtilis, A. brasiliensis, and C. albicans. eucalyptus essential oil's antimicrobial activity and found that, depending on the size of the inoculum and the concentration of the essential oil, there was antibacterial activity of varying intensities. The antimicrobial, cytotoxic, and antidiarrheal properties of ethanol-extracted yellow oleander (Thevetia peruviana) leaves were investigated by Hassan et al. [26]. The plant extract's antimicrobial activity was found to be low. Bachir and Benali [27] investigated the essential oil of Eucalyptus globulus leaves' in-vitro antimicrobial propertiesThe results showed that the essential oil extracted from E. globules leaves exhibited antimicrobial activity against gram-positive and gram-negative bacteria, including S. aureus (E. coli).



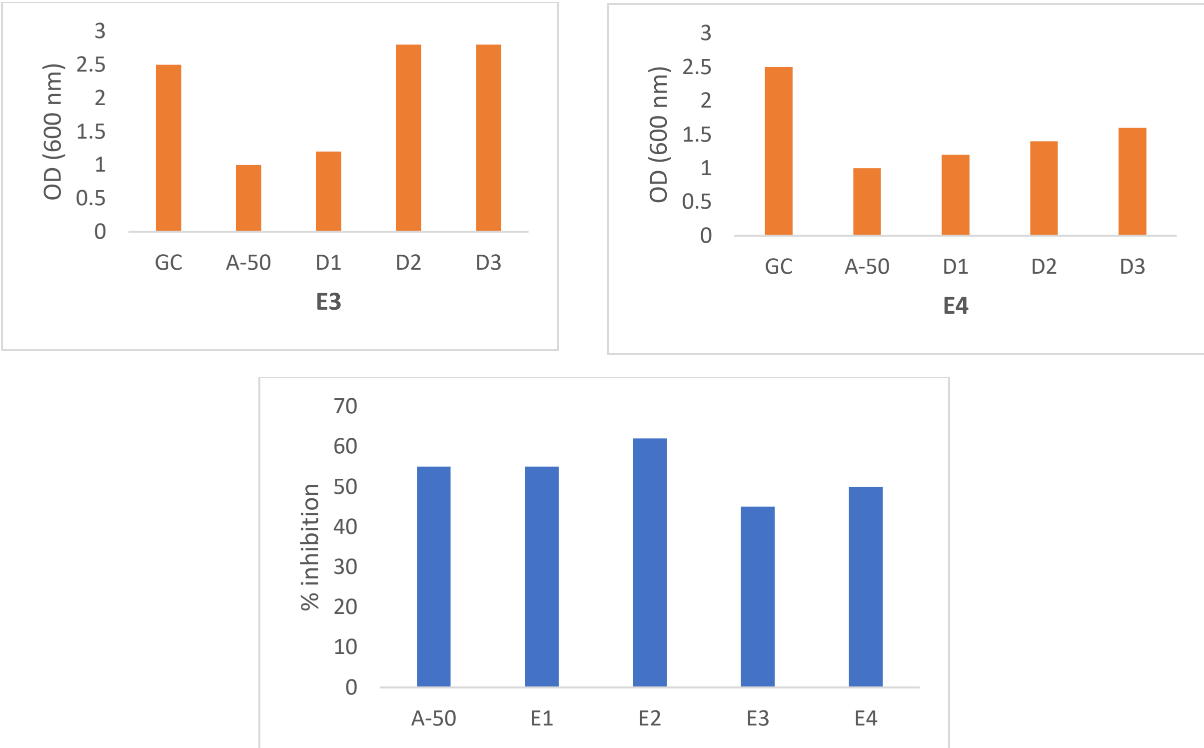
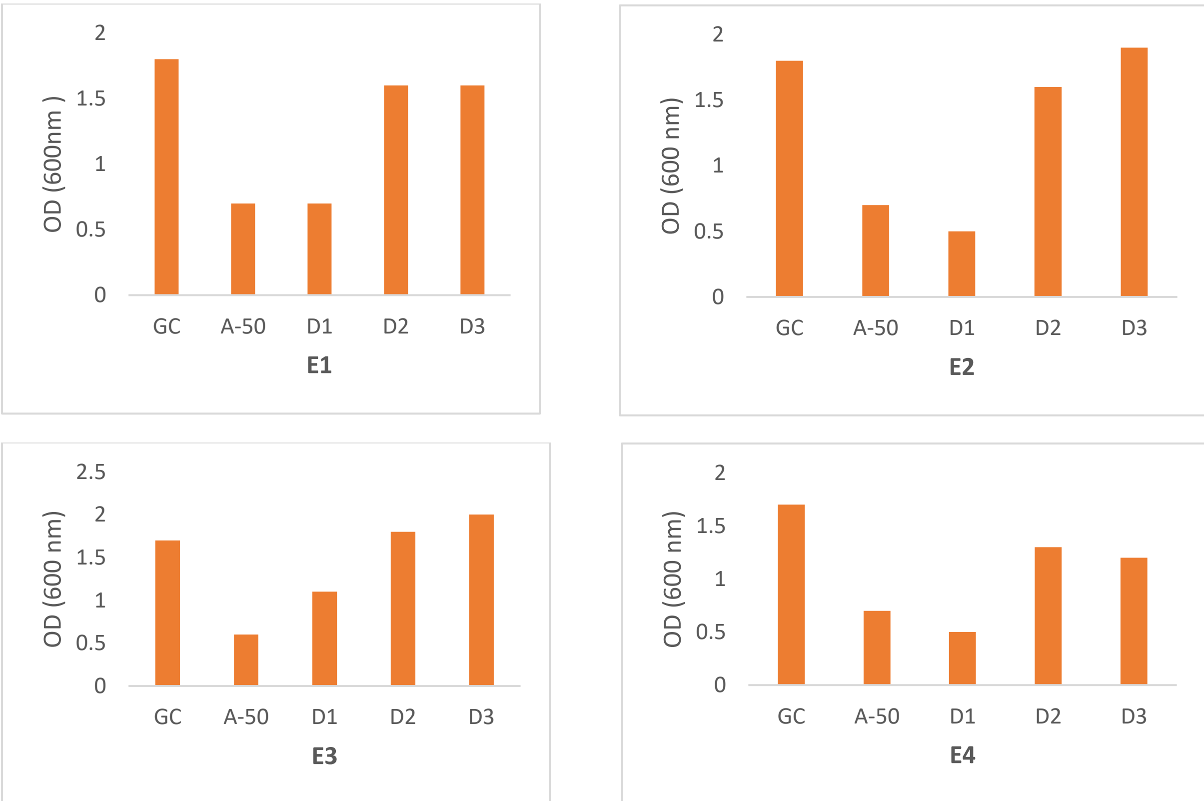


Figure 2: Plant extracts (E1: Nerium sp., E2: Mentha sp., E3: Aloe-vera, E4: Eucalyptus) were tested for their antibacterial effect and their percentage inhibition (GC: growth control; A-50: Ciprofloxacin 50 µg/ml). Significant variation was observed between different alphabets in distinct plants (E1-E4). A post hoc Tukey one-way ANOVA was used to determine significance at $p < 0.05$.



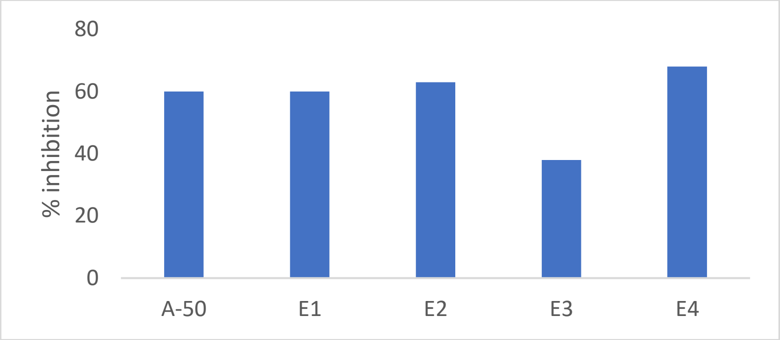


Figure 3 Plant extracts were tested for their anti-fungal effect and their percentage inhibition. Significant variation was observed between different alphabets in distinct plants (E1-E4). A post hoc Tukey one-way ANOVA was used to determine significance at $p < 0.05$.

Plant extracts with antibacterial and antifungal properties are used in a wide range of industries, including research and development, pharmaceuticals, agriculture, traditional medicine, food preservation, and environmental health. Let's explore each of these topics in more detail:

1. **Traditional Medicine:**

Wound Healing: To stop bacterial infections and encourage healing, plant extracts with antibacterial qualities are applied topically to wounds.

Infectious Diseases: In traditional medicine, plant extracts are often used to treat fungal and bacterial infections.

2. Pharmaceuticals:

Plant extracts are a source of bioactive compounds for drug discovery. These compounds are isolated and studied by scientists in hopes that they may serve as the building blocks for novel antifungal and antibacterial medications.

Synergistic Effects: When combined with other medications, certain plant compounds may increase their efficacy.

3. Agriculture:

Crop protection: In order to shield crops from bacterial and fungal diseases, plant extracts are being investigated as natural substitutes for synthetic pesticides and fungicides.

Eco-friendly and sustainable farming methods are compatible with the application of plant extracts.

4. Food Preservation:

Natural Preservatives: By preventing the growth of bacteria and fungi, plant extracts with antimicrobial qualities may be able to prolong the shelf life of food items.

Clean Labelling: Plant extracts satisfy consumer demand for clean-label food additives, as they prefer natural preservatives.

5. Research and Development:

Researchers use a technique known as "bioprospecting" to look for and identify compounds with antibacterial and antifungal qualities by studying a variety of plant species.

Mechanism Studies: Improving therapeutic applications and extraction techniques requires an understanding of the mechanisms of action.

6. Environmental Health:

Water Treatment: In areas where access to conventional medications is limited, plant extracts are taken into consideration for water treatment in order to control microbial contamination. This represents a sustainable solution.

Eco-Friendly Solutions: When addressing public health issues, plant-based antimicrobials play a part in eco-friendly strategies.

7. Challenges and Considerations:

Safety and Toxicity: In order to establish the right concentrations for use, researchers assess the safety and toxicity of plant extracts.

Standardisation: Ensuring uniformity in the antibacterial and antifungal properties of plant extracts is achieved through standardising their production.

Regulatory Compliance: Safety and efficacy requirements for products made from plant extracts must be met.

Conclusions:

The results demonstrate the efficacy of essential oils derived from four distinct plant species and corroborate past findings. The fact that these plants originated in the same region and have been used for many years to treat a range of illnesses by the local ethnic communities supports the local folklore regarding their therapeutic properties. Because of their intricate chemical makeup, essential oils are very effective because they pose a major barrier to pathogenic resistance. The results confirm the traditional use of these plants for treating infections and highlight a strategy for improving drug efficacy in the future. Finding and utilising the active ingredients in these essential oils could lead to better treatment options. This not only offers empirical support for the efficacy of native remedies but also demonstrates how modern medical advancements can be preserved alongside traditional knowledge. All things considered, it draws attention to the historical significance of essential oils derived from plants and presents prospects for future development in tackling the evolving problems associated with microbial infections. It also clarifies how effective these oils are right now. For future by using plant extracts as a starting point can help develop countermeasures for the growing threat of antimicrobial resistance. And interdisciplinary Approaches for Cooperation between pharmacology, conventional knowledge, and other fields guarantees a thorough comprehension and responsible application of plant extracts.

References:

1. Farina, V., Hauck, S. I., & Firestone, R. A. (1996). Synthesis of cephems bearing olefinic sulfoxide side chains as potential β-lactamase inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *6*(13), 1613-1618.
2. Liu, Y. C., Huang, W. K., Huang, T. S., & Kunin, C. M. (1999). Detection of antimicrobial activity in urine for epidemiologic studies of antibiotic use. *Journal of clinical epidemiology*, *52*(6), 539-545.
3. Yaraksa, N. (2019). Antibacterial activity against some human pathogenic bacteria and anti-inflammatory activity of five Thai medicinal plant extracts. *Creative Science*, *11*(1), 1-10.
4. Mehta, R., Dhruv, S., Kaushik, V., Sen, K. K., Khan, N. S., Abhishek, A., ... & Tripathi, V. N. (2020). A comparative study of antibacterial and antifungal activities of extracts from four indigenous plants. *Bioinformation*, *16*(3), 267.
5. Garrity, J. D., Bennett, B., & Crowder, M. W. (2005). Direct evidence that the reaction intermediate of metallo-β-lactamase L1 is metal bound. *Biochemistry*, *44*(3), 1078-1087.
6. Francino, M. P. (2016). Antibiotics and the human gut microbiome: dysbioses and accumulation of resistances. *Frontiers in microbiology*, *6*, 1543.
7. Talib, W. H., & Mahasneh, A. M. (2010). Antiproliferative activity of plant extracts used against cancer in traditional medicine. *Scientia pharmaceutica*, *78*(1), 33-46.
8. Neu, H. C. (1992). The crisis in antibiotic resistance. *Science*, *257*(5073), 1064-1073.
9. Alzoreky, N. S., & Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International journal of food microbiology*, *80*(3), 223-230.
10. Azim, T., Rahman, M., Alam, M. S., Chowdhury, I. A., Khan, R., Reza, M., ... & Rahman, A. S. M. M. (2008). Bangladesh moves from being a low-prevalence nation for HIV to one with a concentrated epidemic in injecting drug users. *International journal of STD & AIDS*, *19*(5), 327-331.
11. Zhang, J. D., Xu, Z., Cao, Y. B., Chen, H. S., Yan, L., An, M. M., ... & Jiang, Y. Y. (2006). Antifungal activities and action mechanisms of compounds from Tribulus terrestris L. *Journal of ethnopharmacology*, *103*(1), 76-84.
12. Zaidi, S., & Dahiya, P. (2015). In vitro antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from Mentha spicata and Mentha piperita. *International Food Research Journal*, *22*(6), 2440.
13. Sartorelli, P., Marquioreto, A. D., Amaral-Baroli, A., Lima, M. E. L., & Moreno, P. R. H. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. *Phytotherapy Research*, *21*(3), 231-233.
14. Ertürk, Ö. (2006). Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. *Biologia*, *61*, 275-278.
15. Danish, P., Ali, Q., Hafeez, M. M., & Malik, A. (2020). Antifungal and antibacterial activity of aloe vera plant extract. *Biological and Clinical Sciences Research Journal*, *2020*(1).

16. Oluwajobi, I., Kabiru, Y. A., & Jigam, A. A. (2019). Antibacterial and Antifungal activities of aqueous leaves extract of some medicinal plants. *GSC Biological and Pharmaceutical Sciences*, *9*(1), 062-069.
17. Dahham, S. S., Ali, M. N., Tabassum, H., & Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.). *Am. Eurasian J. Agric. Environ. Sci*, *9*(3), 273-281.
18. Hassan, A., & Ullah, H. (2019). Antibacterial and antifungal activities of the medicinal plant veronica biloba. *Journal of chemistry*, *2019*, 1-7.
19. Budniak, L., Slobodianiuk, L., Marchyshyn, S., Basaraba, R., & Banadyga, A. (2021). The antibacterial and antifungal activities of the extract of Gentiana cruciata L. herb. *PharmacologyOnLine*, *2*, 188-197.
20. Eskilsson, C. S., & Björklund, E. (2000). Analytical-scale microwave-assisted extraction. *Journal of chromatography A*, *902*(1), 227-250.
21. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.
22. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, *10*(3).
23. Wiegand, I., Hilpert, K., & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature protocols*, *3*(2), 163-175.
24. Al-Hatmi, A. M. S., Al-Shuhoumi, M. A., & Denning, D. W. (2020). Estimated Burden of Fungal Infections in Oman. J. Fungi 2021, 7, 5.
25. Gulluce, M., Sahin, F., Sokmen, M. Ü. N. E. V. V. E. R., Ozer, H., Daferera, D., Sokmen, A. T. A. L. A. Y., ... & Ozkan, H. İ. C. A. B. İ. (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. *Food chemistry*, *103*(4), 1449-1456.
26. Hassan, M. M., Saha, A. K., Khan, S. A., Islam, A., Mahabub-Uz-Zaman, M., & Ahmed, S. S. U. (2011). Studies on the antidiarrhoeal, antimicrobial and cytotoxic activities of ethanol-extracted leaves of yellow oleander (Thevetia peruviana). *Open veterinary journal*, *1*(1), 28-31.
27. Bachir, R. G., & Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pacific journal of tropical biomedicine*, *2*(9), 739-742.