Phytochemical Investigation and Evaluation of Anti-microbial Activity of *Leucas aspera* extract

Kamini Verma*[1], Niharika Dewangan[1], Trilochan Satapathy[2]

[1]Department of Life Science, Shri Shankaracharya Professional University, Junwani - 490020, Bhilai, Chhattisgarh, India
[2]Department of Pharmacology, Columbia Institute of Pharmacy, Tekari - 493111, Raipur, Chhattisgarh, India

***Corresponding author**

Kamini Verma

Department of Life Science, Shri Shankaracharya Professional University, Junwani – 490020, Bhilai, Chhattisgarh, India

Abstract

Traditional medicine practitioners have relied on the antibacterial and anti-inflammatory effects of the herb Leucas aspera for generations. *Leucas aspera's* phytochemical profile and antibacterial activity was determined by in vitro bioassays in this current investigation. Alkaloids, glycosides, tannins, phenolics, saponins, and flavonoids were among the bioactive metabolites identified by phytochemical screening of a 90% v/v ethanol extract with the test material. Oleanolic acid and another bioactive component were identified using Thin Layer Chromatography (TLC) with varied Rf values, confirming the plant's chemical richness. Functional groups, such as hydroxyl, alkyl, and carbonyl groups, were also found for the main bioactive compounds, and FTIR spectroscopy was also developed. *Staphylococcus aureus, Pseudomonas aeruginosa, Candida tropicalis, and Aspergillus niger* were tested for antimicrobial activity using the disc diffusion method. When tested at 6.25% concentration, the *Leucas aspera* extract had an average zone of inhibition of 9.3 mm against *Staphylococcus aureus* and 7.5 mm against *Pseudomonas aeruginosa*, compared to 28.1 mm and 24.3 mm, respectively, for the conventional antibiotic ciprofloxacin. While the control antifungal Amphotericin B produced zones of 19.5 mm and 16.2 mm, respectively, against *Aspergillus niger* and *Candida tropicalis,* the extract produced zones of 6.1 mm and 7.3 mm, respectively. According to the results, *Leucas aspera* has a modest antibacterial potential and may be used as an alternative drug. Further research is needed to isolate the active ingredients and testing for preclinical and clinical studies.

Keywords: Fungus; Bacteria; Free radical; Medicinal value; Antimicrobial resistance

Introduction

Infectious diseases are becoming more difficult to control because of microorganisms' drug resistance, which is a major public health concern known as antimicrobial resistance (AMR) [1]. However, researchers are testing for new antimicrobial drugs that may have inhibitory action against drug-resistant microbes. In this, medicinal plants and natural products are rich in lead compounds. The field of natural products chemistry has provided a wealth of medicinally useful chemicals having medicinal properties [2]. *Leucas aspera* stands out among the plants tested for bioactivity due to its history of medicinal usage and the promising array of biological activities, it belongs to the Lamiaceae family [3]. In addition to its antioxidant, antibacterial, and anti-inflammatory properties, this plant is reputed to have a vast distribution in tropical and subtropical areas. The plant’s rising profile in phytochemistry and pharmacology is largely attributable to its bioactivities [4].

Leucas aspera has a complex pharmacological profile due to its diverse array of bioactive compounds, including alkaloids, flavonoids, terpenoids, glycosides, and phenolics, according to some research [5]. It is believed that these compounds are primarily responsible for the plant's therapeutic effect; identifying and isolating them could result in novel medications to treat a wide range of ailments. Severla studies examining the antibacterial activity and underlying mechanism of *Leucas aspera*, despite the abundance of data regarding its therapeutic efficacy [6].

Several other bacterial and fungal strains, including *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, and Candida tropicalis*, showed antibacterial potential when tested with *Leucas aspera* [6]. Leucas aspera's antibacterial activity and its method of action are detailed below, along with comparisons to other established therapies. This study analyses the phytochemical composition of *Leucas aspera*, determines its antimicrobial activity, and identifies the bioactive phytochemicals that the plant uses to fight against harmful bacteria.

The current study aims to identify the main bioactive chemicals in the plant and test their antibacterial and antifungal activity utilising advanced analytical techniques such as Thin Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FTIR), and enzyme tests. The existing literature on *Leucas aspera* as a new source of antibacterial chemicals may undoubtedly be strengthened its use for medicinal purposes.

This study aims to test the potential of *Leucas aspera* as a natural product source for antimicrobials. This research aims to add to the existing evidence justifying the use of medicinal plants in contemporary pharmacology by performing a comprehensive phytochemical screening, determining the plant's antimicrobial activity, and isolating its bioactive phytoconstituents. To fight against the global problem of antimicrobial resistance, this study's findings could pave the way for the development of novel pharmaceuticals derived from *Leucas aspera*.

Materials and methods

Chemicals and reagents

Amphotericin B and Ciprofloxacin were procured from SRL Chem. India. Merck-Schuchardt of Mumbai, Maharashtra, India, was contacted to purchase ethanol. The Silica gel-G (TLC grade) and petroleum ether were purchased from Loba chemical Pvt. Ltd. in Mumbai, Maharashtra, India. Sri Lankan company SRL Chemicals supplied the Sabouraud dextrose agar (SDA) and Mueller-Hinton agar (MHA) plates. Sigma-Aldrich of Mumbai, Maharashtra, India, served as the source for the potassium pyrophosphate buffer. The microbial type culture collection and gene bank (MTCC) in Chandigarh, Punjab, India, provided the fungal culture (Aspergillus niger, MTCC-281; Candida tropicalis, MTCC 1000) and bacterial culture (Staphylococcus aureus, MTCC96; Pseudomonas aeruginosa, MTCC-3541). The research utilized only analytical-grade chemicals and reagents.

Extraction of plant

The plant was collected from local garden of Bhilai, Chhattisgarh in the month on Nov. 2024. The plant parts were washed, dried and grounded using a grinder. The 900 g of powder that was collected was thereafter immersed in 2.5 litres of ethanol in a conical flask and left to soak for seven days at room temperature (23±0.5) °C. Using Whatman filter paper No. 1, the entire dried plant matter was filtered out. The filtrate was subsequently subjected to additional concentration using a rotary evaporator operating at a temperature below 50°C and reduced pressure. The samples were transferred to Petri plates for subsequent experiments. A solution containing 2.0 mg/mL of extract was prepared by re-dispersing 72 g of dried crude extract in ethanol.

Phytochemical screening

Extract from *Leucas aspera* were subjected to preliminary phytochemical screening in accordance with established methods. Various chemical experiments were conducted on the 90% v/v ethanol extract to identify various phytoconstituents [8]. To detect alkaloids, the extract was treated with Mayer's, Dragendorff's, Wagner's, and Hager's reagents separately. To determine whether carbohydrates were present, various tests were used, including Molisch's test, Fehling's test, Barfoed's test, and Benedict's test. Three tests—Legal's, the Keller-Killiani, and Borntrager's—were used to detect glycosides. Both the stain test and the saponification test were used to evaluate the fixed oils and fats. The ferric chloride, lead acetate, and gelatin solution tests were used to detect tannins and phenolics. Two tests, Salkowski's and Libermann-Burchard's, were used to examine steroids. The foam test was used to detect saponins, whereas Million's test and the ninhydrin test were used to detect proteins. To test the flavonoids, the NaOH water test, the sulphuric acid test, and the Shinoda test. The gum and mucilage were tested using conventional methods. We used Knoller's assay to identify the triterpenoids [8].

Thin Layer Chromatography (TLC)

To determine what chemicals were in *the Leucas aspera* extract, researchers used thin-layer chromatography (TLC). A tiny amount of the extract was applied to a pre-coated silica gel TLC plate to conduct the TLC procedure. To make sure the extract was applied enough, a capillary tube was used to mark the plate. To achieve the highest possible level of compound separation, the mobile phase solvent solution was selected based on the results of the earlier tests. The concentrations of chloroform, methanol, and water were adjusted according to the polarity of the compounds being studied. Compounds were permitted to migrate in accordance with their affinities for the stationary phase (silica gel) and the mobile phase after the TLC plate was placed in a developing chamber with the solvent combination. Following the tests, the plate was taken out and allowed to dry in the air. Since many of the active components of *Leucas aspera* are UV-active, detection was carried out using UV light. The spots' Rf values measured, which are the ratio of the compound's travel time to the solvent front's travel time, by visualising them [9].

FTIR analysis

A typical method for identifying the functional groups and chemical bonds in plant samples, such Leucas aspera, was Fourier Transform Infrared (FTIR) spectroscopy. The process started with the preparation of plant materials, typically in the form of a dried, finely ground powder. The next step is to combine the sample with powdered potassium bromide (KBr) to create a pellet. The FTIR spectrometer was used to analyse. FTIR works by scanning a sample with infrared light and recording the sample's absorption of a specific wavelength caused by vibrations in different molecules. The observed infrared spectra, according to their predicted absorption regions, revealed information regarding the functional groups in the material. Alkaloids, flavonoids, terpenoids, and secondary metabolites were among the bioactive compounds studied in *Leucas aspera* using Fourier transform infrared spectroscopy. To determine the chemical make-up of the plant, the functional groups were located by comparing the FTIR peaks with reference spectra [10]. The method proved useful for identifying the bioactive chemicals responsible for plants' medicinal activity and for characterising the chemical composition of plants.

Enzyme activity assay

MDA

Mix 100 µl of sample with 500 µl of Solution A until well combined. The sample was spun in a centrifuge at 2500 rpm for 5 minutes to separate the proteins, and the resulting supernatant was utilised in further experiments. The mixture was cooked in a boiling water bath for 15 minutes after 100µl of supernatant and 1800µl of TBA Reagent were combined. A blank containing all the chemicals except for the samples was used to measure absorbance at 532 nm [11]. A malondialdehyde concentration of 1.56×10^5 $M^{-1}cm^{-1}$ was determined by using an extinction coefficient.

SOD

The creation of blue crystals of the GSH-Phenazine-Metho-nitro blue tetrazolium formazan assay is inhibited with SOD. The spectrophotometer read 560 nm, the colour that developed at the reaction's finish. 100 microlitres of enzymatic lysate were collected for the bioassay. Pippett the contents of the test sample, blank, standard, and control tubes into a 96-well plate after bringing the reagents and homogenate to a temperature of 37°C. The standard curve was prepared by diluting SOD Enzyme at various concentrations ranging from 6.25 to 100 U/ml. After measuring 1.2 millilitres of potassium pyrophosphate buffer (pH 8.3, 0.025 M), 1 millilitre of PMS (186µM) and 0.3 millilitres of NBT (300µM) were put to a glass cuvette and mixed meticulously. Following this, the sample and blank cuvettes were appropriately mixed with 0.05 ml of enzyme crude extract (10 times diluted) and 05 ml of PBS [12]. After adding 1.15 ml of dH2 to get the reaction volume up to 8 ml, 0.2 ml of GSH (780µM) was used to start the reaction, and readings were taken at an absorbance of 560 nm from 0 to 30 minutes.

Anti-bacterial activity assay

To inoculate the MHA plates, 100 µl of Staphylococcus aureus bacterial culture was spread over them. The inoculum was prepared by adjusting the cell density of 1.5×10^8 CFU/mL from Mueller-Hinton Broth, and then discs containing 10 µl of different concentrations (0 to 100%) were placed on top. As a vehicle control, one disc in each plate was filled with solvent alone, and as a positive control, one disc containing 3 µg of Ciprofloxacin was used. Incubation was carried out at 37 °C for 24 hours on separate plates of *Staphylococcus aureus and Pseudomonas aeruginosa* (Basil Scientific Corp. India) [13]. Measurements and records were made of the resulting clear zones surrounding the disc.

Anti-fungal activity assay

The fungal cultures *Candida tropicalis and Aspergillus niger* were spread onto the SDA plates along with 100 µl of fungal culture. The inoculum was made by adjusting the cell density of 1.5×10^1 CFU/mL from Sabouraud dextrose broth, and each disc contained 10 µl of a different concentration ranging from 0% to 100%. The solvent alone was put onto one disc in each plate to act as a vehicle control, while 50µg of Amphotericin B was used as a positive control. In a controlled environment of 37 °C for 48 hours, the fungal plates were placed in an incubator made by Basil Scientific Corp. India. We measured and documented the clean zones that were generated around disc [14].

Result

Phytochemical screening

Leucas aspera 90% v/v ethanol extract was shown to contain numerous beneficial components, according to the main phytochemical analysis. The presence of alkaloids in the extract was confirmed by positive tests of Mayer, Dragendorff, Wagner, and Hager test. Results from the molisch, Fehling, Barfoed, and Benedict carbohydrate tests all showed the presence of sugar as a component. Glycosides

were confirmed by the assays conducted by Legal, Keller-Killiani, and Borntrager test. Tests with ferric chloride, lead acetate, and gelatin solution, in that order, revealed substantial evidence of tannins and phenolics in the extract (Table 1). There was a high concentration of flavonoids, according to tests for aqueous NaOH, Shinoda, and concentrated sulphuric acid. It was found that saponins exist and used foam tests to validate their presence. However, no fixed oils or fats were detected in the stain or saponification trials, and the results from Salkowski's and Libermann-Burchard's tests were negative. In addition, no proteins were found according to Million's and ninhydrin tests. The absence of gum, mucilage, and triterpenoids was confirmed by a negative Knoller's test. These secondary metabolites, which include alkaloids, carbohydrates, glycosides, tannins, phenolics, flavonoids, and saponins, are believed to be responsible for the pharmacological activity of *Leucas aspera*.

Table 1. Preliminary phytochemical screening of *Leucas aspera* extracts.

S. No.	Chemical Test	Ethanol (90% v/v) extract
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Tannins & Phenolics	+
4.	Glycosides	+
5.	Fixed Oil & Fat	-
6.	Flavonoids	+
7.	Steroids	-
8.	Triterpenoids	-
9.	Proteins	-
10.	Saponins	+
11.	Gum & Mucilage	-

+ Present - Absent

TLC

A Thin Layer Chromatography (TLC) scan of the *Leucas aspera* extract revealed two distinct spots, each suggesting a different set of chemicals. First, we have oleanolic acid, a triterpenoid molecule with an Rf value of approximately 0.31. To find the Rf value, one uses a silica gel plate, which is commonly used for these kinds of experiments, and a mixture of toluene and methanol as a solvent. Oleanolic acid does a lot of climbing up the plate under these settings, which is indicative of moderate polarity. Another bioactive component of the extract is indicated by the second-place finisher at a different Rf value. To better define this molecule's properties, additional research comparing it to different chromatographic methods or existing standards is required (Figure 1).

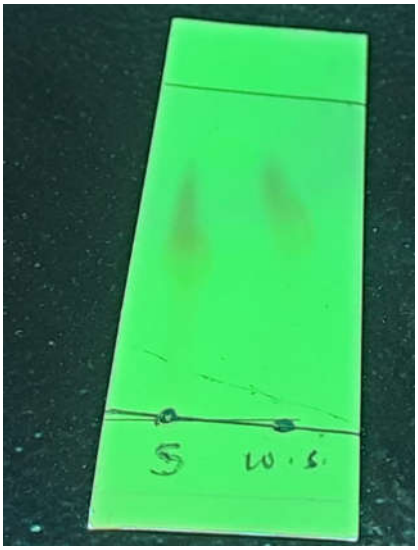

Fig.1. TLC chromatogram of *leucas aspera.*

FTIR

The molecular vibrations of the plant, as shown by the FTIR spectrum, provide crucial information regarding the chemical makeup of *Leucas aspera* extract. Different functional groups found in the plant's bioactive substances are shown by the spectrum's unique absorption peaks. A wide peak at approximately 3400 cm-1 indicates the presence of chemicals containing hydroxyl groups, such as alcohols, phenols, or others, through O-H stretching vibrations. The presence of hydrocarbons is indicated by the presence of C-H stretching vibrations, which are characteristic of alkyl groups, in another noteworthy region about 2920 cm-1. Plant secondary metabolites, such as aldehydes, ketones, or carboxylic acids, are likely to be related with the strong peak about 1730 cm-1, which usually corresponds to C=O (carbonyl) stretching. Possible correlation between peaks at 1600 cm-1 and C=C stretching vibrations suggests aromatic molecules or conjugated systems (Figure 2). Notable features in the 1000-800 cm-1 range, for example, may indicate bending vibrations associated with C-H and C-O groups; these groups are common in polyphenolic chemicals, terpenoids, and flavonoids. In sum, the Fourier transform infrared spectra of *Leucas aspera* reveal its chemical make-up by illuminating functional groups linked to important bioactive compounds that give the plant its therapeutic effects.

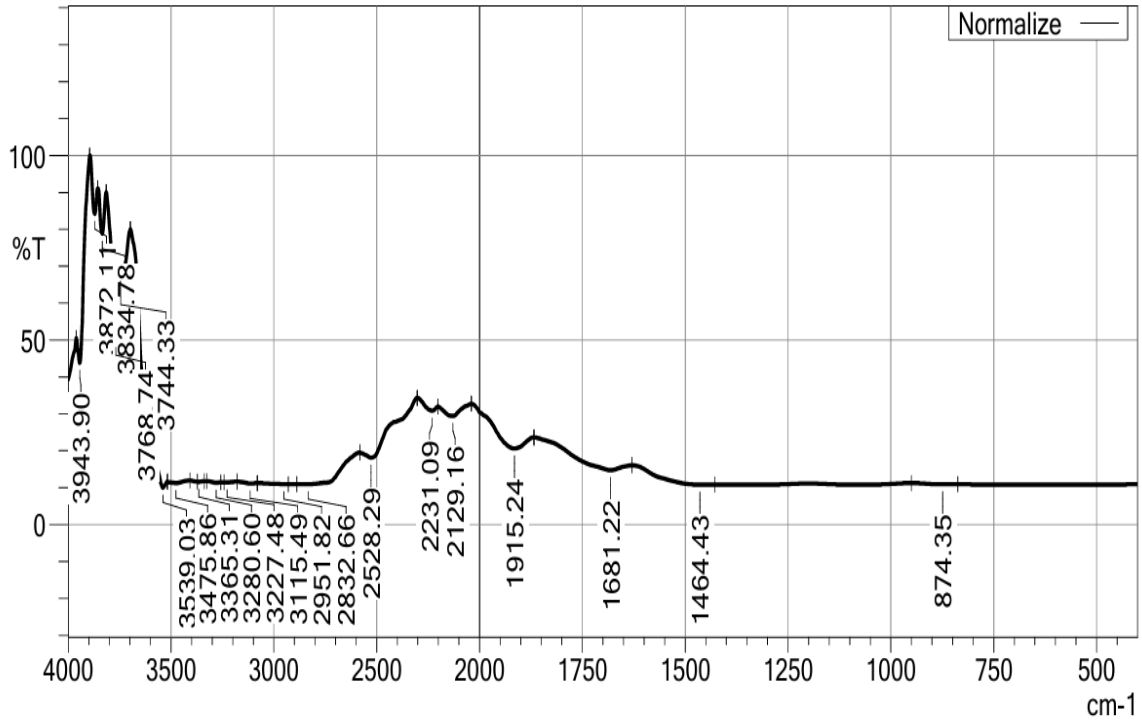

Fig.2. FTIR spectra of *Leucas aspera* extract.

Enzymatic assay activity

Enzymatic assays are essential biochemical methods for determining if a sample contains a particular biomolecule, the activity of an enzyme, or the conversion of a substrate. When studying oxidative stress, one typical enzymatic technique is the lipid peroxidation assay, which quantifies the amount of malondialdehyde (MDA). The peroxidation of polyunsaturated fatty acids produces MDA, a hallmark of oxidative stress. The current investigation revealed that *Leucas aspera* has a moderate to high level of lipid peroxidation, with an MDA concentration of 82.179 nmoles/ml. Environmental variables, ageing, or metabolic imbalances can all lead to elevated MDA levels, which indicate increased oxidative stress. Conversely, robust antioxidant systems are present in plants with lower MDA levels. To safeguard cells from oxidative damage, the superoxide dismutase (SOD) enzyme is essential. *Leucas aspera* has a high potential for antioxidant activity, according to its mean SOD activity value of 3.5087 U/mg.

Antibacterial activity

By utilising the agar well diffusion method, which takes the zone of inhibition into account, the antibacterial activity of *Leucas aspera* was assessed against Staphylococcus aureus and *Pseudomonas aeruginosa*. The Ciprofloxacin (12 µg) showed a zone of inhibition of 28.1 mm against *S. aureus* (Table 2), suggesting potent antibacterial action. In contrast, a 9.3 mm inhibitory zone and limited antibacterial activity against *S. aureus* were observed in the *Leucas aspera* extract at a dosage of 6.25% (Figure 3).

In a similar vein, Ciprofloxacin (12 µg) demonstrated strong antibacterial activity against *Pseudomonas aeruginosa* (Table 2) with a zone of inhibition measuring 24.3 mm. *Leucas aspera* extract, at 6.25% concentration, had a zone of inhibition of 7.5 mm against P. aeruginosa, suggesting that it had significant antibacterial activity than *S. aureus*. Leucas aspera does have antibacterial activity, however, less than Ciprofloxacin, especially against the infamously resistant *P. aeruginosa*. *Leucas aspera* may have antibacterial effects since it contains bioactive phytochemicals as terpenoids, alkaloids, and flavonoids.

Table 2. Antibacterial activity of plant extract against *S. aureus* and *P. aeruginosa.*

S. No.	Sample	Effective Amount	*S. aureus*	*P. aeruginossa*
			Zone of inhibition (in mm)	**Zone of inhibition (in mm)**
1.	Control	-	-	-
2.	Ciprofloxacin	12 µg	28.1±0.13*	24.3±0.44*
3.	*Leucas aspera*	6.25%	9.3±0.45*	7.5±0.77*

Data are represented as mean ± SD (n=3), significantly different at *p<0.05 in comparison to the control group.

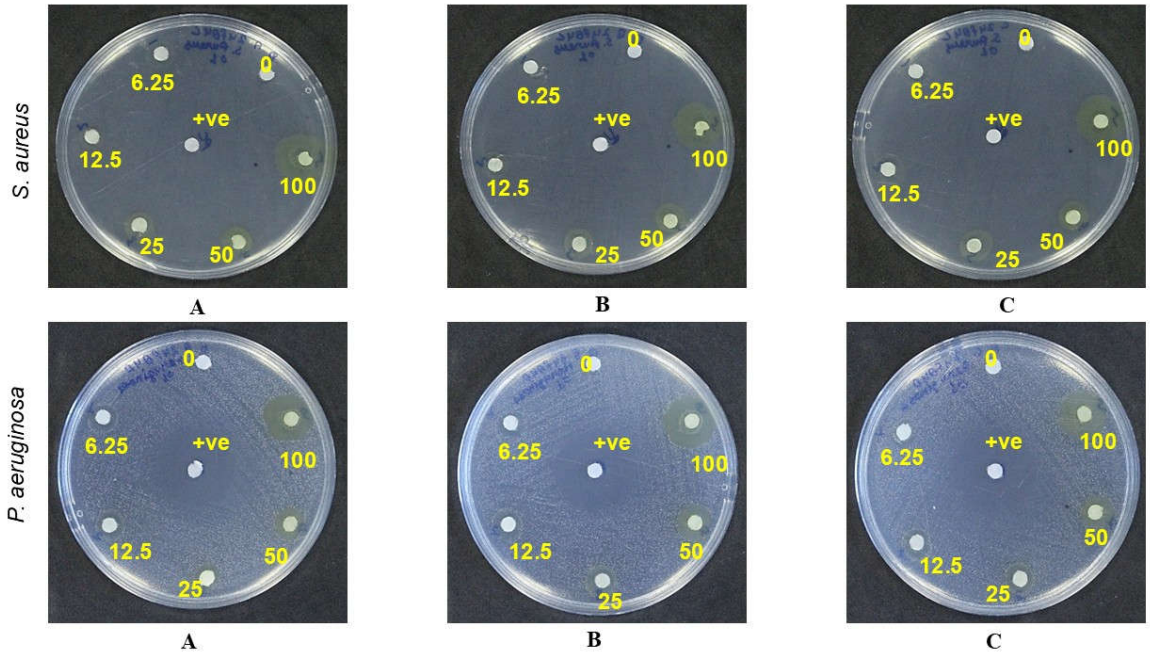

Fig.3. Zone of inhibition by *L. aspera* extract against bacteria.

Antifungal activity

Using an agar well diffusion experiment with Amphotericin B as a positive control, *Leucas aspera* was tested for its antifungal efficacy against *Candida tropicalis* and *Aspergillus niger*. The efficacy of the plant extract was evaluated in comparison to a reference antifungal medicine using the zone of inhibition method. Table 3 shows that the control group did not exhibit any zone of inhibition when tested against *Candida tropicalis*, proving that the medium does not have any inherent antifungal properties. The antifungal activity of Amphotericin B was high, as evidenced by its 16 mm zone of inhibition at 50 µg. To put it in perspective, a 6.25% concentration of *Leucas aspera* extract had a 6.1 mm zone of inhibition, indicating weak antifungal action against *Candida* (Figure 4).

The test conditions were validated because the control group failed to inhibit any zone for *Aspergillus niger* (Table 3). The strong antifungal action was confirmed by the inhibition of a 19.5 mm zone by 50 µg of Amphotericin B. The antifungal activity against *Aspergillus* was demonstrated by the extract of *Leucas aspera*, which, at 6.25% concentration, inhibited a 7.3 mm zone. While the results show that *Leucas aspera* may contain bioactive chemicals with antifungal action, the reported activity lower than that of Amphotericin B. *Leucas aspera* shows significant antifungal activity, slightly stronger against *Aspergillus* than *Candida*, according to the results.

Table 3. Antifungal activity of plant extract against *C. tropicalis* and *A. niger.*

S. No.	Sample	Effective Amount	*C. tropicalis*	*A. niger*
			Zone of inhibition (in mm)	**Zone of inhibition (in mm)**
1	Control	-	-	-
2	Amphotericin B	50 µg	16.2±0.42*	19.5±0.56*
3	*Leucas aspera*	6.25%	6.1±0.39*	7.3±0.35*

Data are represented as mean ± SD (n=3), significantly different at *p<0.05 in comparison to the control group.

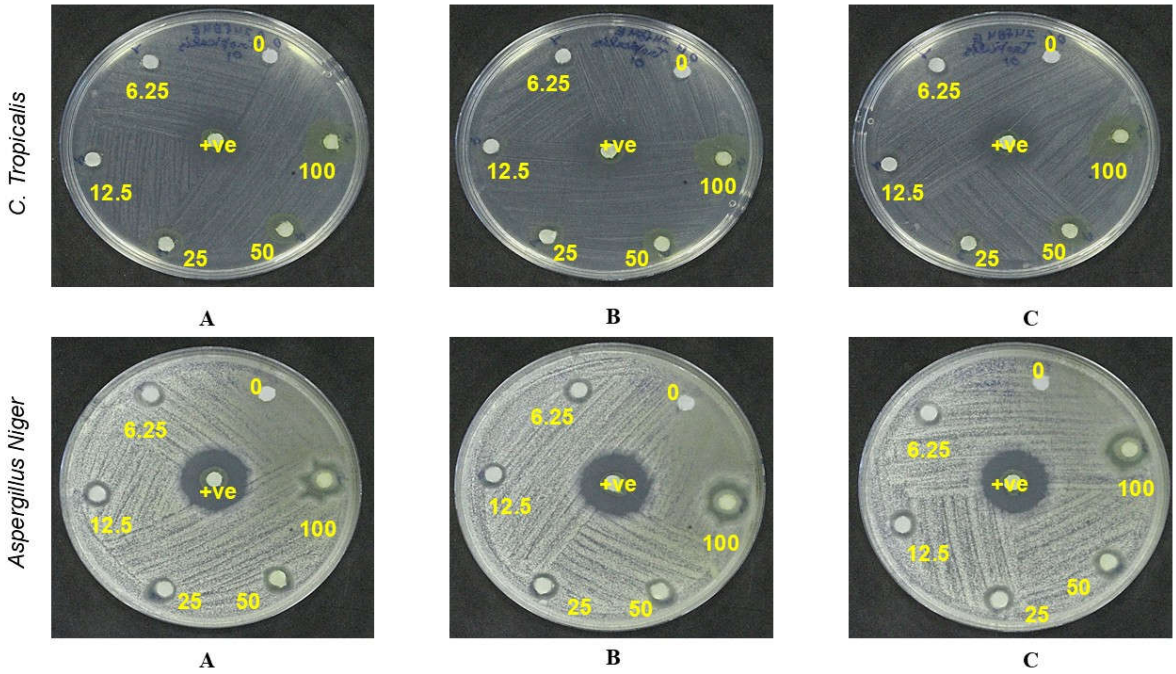

Fig.4. Zone of inhibition by *L. aspera* extract against fungi.

Discussion

The result of this study adheres with reported research showing that *Leucas aspera* has a complex phytochemical composition and exhibits moderate activity against pathogens that cause bacterial and fungal diseases. The existence of many bioactive metabolites in the plant, such as flavonoids, alkaloids, tannins, and glycosides, implies that these compounds are responsible for the plant's therapeutic effects.

This study provides evidence that *Leucas aspera* is an effective antibacterial natural agent, as it aligns with earlier research on medicinal plants that contain the same phytochemicals. Although its antibacterial activity is lower than that of commercial medications like ciprofloxacin and Amphotericin B, the fact that it is nonetheless worth considering as an alternate treatment option is encouraging. In contrast to the significantly higher inhibition values achieved with ciprofloxacin, the extract was observed to be inhibitory against *Staphylococcus aureus and Pseudomonas aeruginosa*, with inhibition values of 9.3 mm and 7.7 mm, respectively. Inhibition zones measuring 6.1 mm against *Candida tropicalis* and 7.3 mm against *Aspergillus niger* were observed, indicating antifungal activity. These values were significantly lower than those of Amphotericin B, which had inhibition zones of 16.2 mm and 19.5 mm, respectively. Although *Leucas aspera* contains bioactive chemicals with antibacterial activity, these results demonstrate that its efficacy is just average when compared to control medications. The potential of plant antimicrobials as adjuvant medicines, particularly in the treatment of drug-resistant illnesses, has, however, been adequately recognised [15].

Oleanolic acid, which has been found to have antibacterial, antioxidant, and anti-inflammatory properties, was one of the main phytochemical components of *Leucas aspera* that was characterised by TLC. In addition, Fourier transform infrared spectroscopy allowed for the identification of functional

groups like hydroxyl, alkyl, and carbonyl compounds in common antibacterial substances. By confirming Leucas aspera's chemical wealth of medicinal value, we find that its antibacterial activity is in line with the prevalence of phytochemical components.

At 82.179 nmoles/ml for MDA and 3.5087 U/mg for SOD, the enzymatic assay likewise revealed high oxidative stress indicators. Based on these findings, it seems that *Leucas aspera* has strong antioxidant properties, which could be related to the fact that it inhibits microbial cell oxidative stress [16]. *Leucas aspera's* pharmacological relevance is further supported by the fact that medicinal plants' capacity to fight oxidative stress is associated with their therapeutic efficacy, which includes antibacterial activity.

Although this work adds to our understanding of Leucas aspera's phytochemical and antibacterial properties, we still need more studies to identify the specific active ingredients that give this plant its antimicrobial properties [17]. Since the emergence of resistance to antimicrobials is increasing, the identification of plant leads like *Leucas aspera* may pave the way for the development of novel therapeutic compounds for the management of infectious diseases.

Conclusion

The results demonstrate that *Leucas aspera* possesses a high level of antibacterial activity, particularly against common bacterial pathogens such as *Staphylococcus aureus and Pseudomonas aeruginosa*, as well as fungi such as *Candida tropicalis and Aspergillus niger*. Despite having little antibacterial action compared to standard treatments like ciprofloxacin and Amphotericin B, the plant extract shows potential as a natural substitute or supplementary treatment, particularly against the backdrop of rising antimicrobial resistance. Finally, due to the rise of antimicrobial resistance, our research highlights the importance of using plant-based medicines in consideration. Because of its great promise as a source of bioactive compounds with antibacterial characteristics, *Leucas aspera* may be included into new therapeutic treatment to against infectious diseases after clinical trials.

Declaration

Authors declared no conflict of interest.

Author credit

KM – writing original draft, ND – method and statistics, TS – proofreading and supervision.

Acknowledgement

We are thankful to the institute for providing all facilities to conduct this study.

Consent

Not applicable

References

1. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MAA. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. Healthcare (Basel). 2023 Jul 5;11(13):1946.
2. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 2021 Sep 27;9(10):2041.
3. Shalini TS, Prathiviraj R, Senthilraja P. Exploring the antimicrobial and antioxidative potential of Leucas aspera (Willd.) link: Phytochemical screening, molecular docking, and HR-LC/MS profiling against SARS-CoV-2 protein 3CLPro, Spike and RDB. Phytomedicine Plus [Internet]. 2025;5(1):100700.
4. Murugesan G, Saha R, Sunmathi D, Nagaraj K, Rathish Kumar S, Subramani K. Leucas aspera mediated SeO nanoparticles synthesis for exploiting its pharmaceutical efficacy. Plant Nano Biol [Internet]. 2022; 2:100013.
5. Mishra R, Dwivedi B, Gupta D. Physicochemical, Phytochemical, Qualitative HPTLC and Antioxidant Study of Medicinal Plant Leucas aspera. Trends Sci [Internet]. 2022 Aug 15;19(16 SE-Research Articles):5646.
6. Rani V, Kannasandra Ramaiah M. Assessment of Antioxidant and Antimicrobial Activity of Leucas Aspera and its Application in Neuroprotection. J Neonatal Surg [Internet]. 2025 Mar 11;14(5S SE-Original Article):388–95.
7. Chew AL, Jessica JJ, Sasidharan S. Antioxidant and antibacterial activity of different parts of Leucas aspera. Asian Pac J Trop Biomed. 2012 Mar;2(3):176-80.
8. Chetia J, Saikia LR. Phytochemical Analysis of Leucas aspera (Willd.) Link. from Dibrugarh. 2020;64(2).
9. Pankaj K. Thin Layer Chromatographic investigation on leave of Leucas Aspera extracted in Ethanol and Dichloromethane. 2015;4(7):69–72.
10. Ojeda JJ, Dittrich M. Fourier transform infrared spectroscopy for molecular analysis of microbial cells. Methods Mol Biol. 2012; 881:187-211.
11. Aliahmat NS, Noor MR, Yusof WJ, Makpol S, Ngah WZ, Yusof YA. Antioxidant enzyme activity and malondialdehyde levels can be modulated by Piper betle, tocotrienol rich fraction and Chlorella vulgaris in aging C57BL/6 mice. Clinics (Sao Paulo). 2012 Dec;67(12):1447-54.
12. Samarghandian S, Afshari R, Sadati A. Evaluation of lung and bronchoalveolar lavage fluid oxidative stress indices for assessing the preventing effects of safranal on respiratory distress in diabetic rats. ScientificWorldJournal. 2014 Feb 20; 2014:251378. doi: 10.1155/2014/251378. Erratum in: ScientificWorldJournal. 2020 Dec 21; 2020:6452878.
13. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal. 2016 Apr;6(2):71-79.

14. Hossain TJ. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. Eur J Microbiol Immunol (Bp). 2024 Apr 22;14(2):97-115.
15. Rahman MA, Islam MS. Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole Leucas aspera extract. Asian Pac J Trop Biomed. 2013 Apr;3(4):273-9.
16. Agnes Nirmala K, Kanchana M. Leucas aspera – A Review of its Biological activity. Syst Rev Pharm. 2018;9(1):41–4.
17. Mohideen AP. Controlling Multidrug-Resistant (MDR) Infections Using Leucas aspera : In Silico Identification of Phytocompounds Inhibiting DNA Gyrase and Tyrosyl-tRNA Synthetase.