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ORIGINAL RESEARCH

Phytochemical Profiling and Evaluation of Antimicrobial and Antioxidant Activities of *Leucas aspera* Leaf Extract

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ABSTRACT

The current study assesses the antimicrobial and antioxidant properties of Leucas aspera leaf extract and looks into its phytochemical makeup. To separate the bioactive components, plant material was extracted sequentially using solvents of increasing polarity. The existence of important secondary metabolites, such as flavonoids, alkaloids, terpenoids, and saponins, was confirmed by preliminary qualitative phytochemical screening. A substantial abundance of polyphenolic compounds was indicated by the methanolic extract's greatest total phenolic content (142.6 ± 4.1 mg gallic acid equivalents per gram), according to quantitative estimation. Using the DPPH radical scavenging experiment, antioxidant activity showed a concentration-dependent impact with an IC^{2}% value of 42.8 µg/mL, indicating great potential for scavenging free radicals. The disc diffusion method was used to assess the extract antibacterial activity, and it demonstrated significant inhibitory effects against Staphylococcus aureus and other tested microbiological strains. These results support Leucas aspera's traditional medicinal use and demonstrate its potential as a promising natural source of bioactive chemicals with important antibacterial and antioxidant qualities. To separate and describe the active ingredients for possible medicinal uses, more research is advised.

Keywords : *Leucas aspera*; phytochemical screening; total phenolic content; antioxidant activity; DPPH assay; antimicrobial activity; *Staphylococcus aureus*; methanolic extract.

INTRODUCTION

Medicinal plants have long been acknowledged as an essential source of therapeutic compounds, making a substantial contribution to both traditional and contemporary healthcare systems across the globe. According to estimates from the World Health Organization, around 80% of the world's population uses plant-based medications for basic medical requirements, especially in underdeveloped nations where access to synthetic treatments is restricted (WHO, 2021). The wide range of secondary metabolites found in medicinal plants, such as alkaloids, flavonoids, phenolic compounds, terpenoids, and saponins, are largely responsible for their therapeutic effectiveness. Numerous

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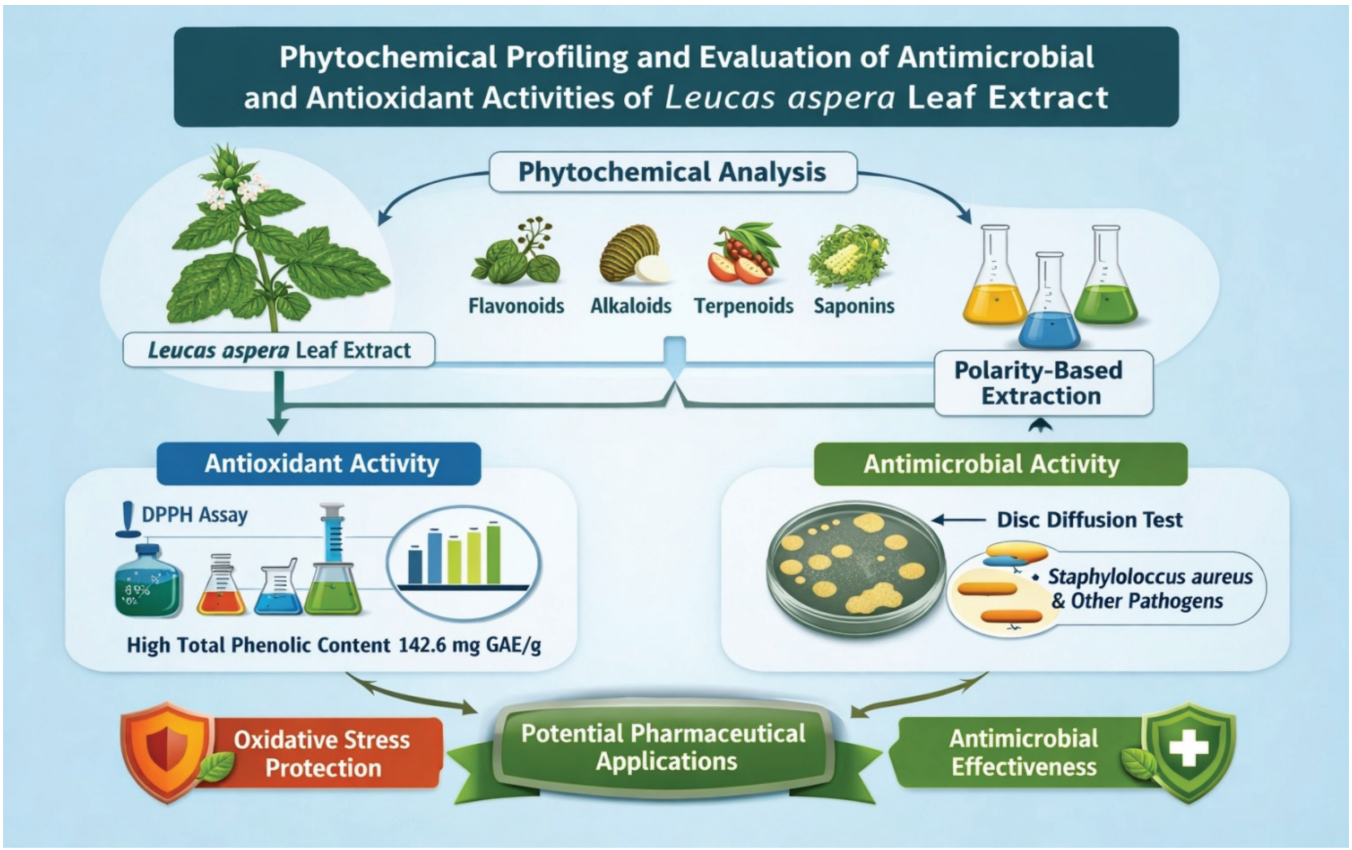

Figure 1: Graphical Representation of Various Properties of Leucas aspera Leaf Extract.

pharmacological actions, including antioxidant, antibacterial, anti-inflammatory, and anticancer capabilities, are known to be shown by these bioactive chemicals (Cowan, 1999; Sharma *et al.,* 2019). Because plant-derived molecules are less poisonous, more affordable, and have fewer side effects than synthetic medications, there has been an increase in scientific interest in investigating them as potential substitutes.

Leucas aspera (Willd.) Link, a member of the Lamiaceae family, is one of the many medicinal plants found in tropical and subtropical areas, especially in India. It is widely employed in traditional medical systems like Ayurveda and folk medicine and is usually referred to as "Thumbai." Fever, inflammation, cough, skin conditions, and microbial infections are among the problems that the plant has historically been used to cure (Kirtikar & Basu, 2005; Prakash *et al.,* 2018). Because of *Leucas aspera's* ethnomedical relevance, scientists are looking into its phytochemical makeup and biological activity to substantiate its traditional uses. The identification and characterization of the bioactive substances found in plant extracts depend heavily on phytochemical profiling. Both qualitative and quantitative analyses offer insightful information about the chemical components that produce therapeutic actions. According to earlier research, *Leucas aspera* has a wide variety of phytochemicals that support its biological actions, including flavonoids, phenolics, diterpenes, triterpenes, alkaloids, and essential oils (Srinivasan *et al.*, 2011; Patel *et al.,* 2020). Because of their potent antioxidant qualities, which aid in scavenging free radicals and averting oxidative stress-induced cellular damage, phenolic compounds and flavonoids are especially significant among them. Numerous chronic illnesses, such as cancer, neurological diseases, and cardiovascular disorders, are linked to oxidative stress (Lobo *et al.,* 2010). In order to find possible therapeutic agents, it is crucial to assess the antioxidant activity of medicinal plants.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment is a popular technique for determining antioxidant activity. This approach is quick, easy, and trustworthy for assessing plant extracts' capacity to scavenge free radicals. When antioxidants reduce DPPH radicals, a detectable drop in absorbance occurs, indicating the sample's antioxidant capability (Blois, 1958). Numerous investigations have shown that plant extracts enriched in phenolic compounds have substantial DPPH radical scavenging activity, underscoring the relationship between antioxidant capability and total phenolic content (Prior *et al.,* 2005; Alam *et al.,* 2013). Therefore, measuring total phenolic content and evaluating antioxidant activity are crucial aspects of phytochemical research.

The growing prevalence of antibiotic-resistant infections has drawn significant attention to the antibacterial potential of medicinal plants in addition to their antioxidant qualities. Global public health is seriously threatened by the spread of multidrug-resistant bacteria, which calls for the development of novel and potent antimicrobial drugs (Ventola, 2015). Because of their many modes of action and capacity to stop the growth of a broad variety of microorganisms, plant-derived chemicals have been identified as possible substitutes. In order to assess the antimicrobial activity of plant extracts and determine their inhibitory effects against certain strains of bacteria and fungi, the disc diffusion method is frequently used (Bauer *et al.,* 1966).

Leucas aspera has been shown to have significant antibacterial action against both Gram-positive and Gram-negative bacteria, such as Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus (Reddy *et al.,* 2012; Kumar *et al.,* 2016). The presence of bioactive substances including flavonoids, terpenoids and alkaloids, which can damage microbial cell walls, obstruct metabolic processes, and prevent nucleic acid synthesis, is largely responsible for the plant's antimicrobial effectiveness. The traditional usage of Leucas aspera in the treatment of infectious disorders is further supported by these findings. Despite the current research, thorough studies that combine phytochemical profiling with in-depth assessment of antioxidant and antibacterial activity utilizing standardized approaches are still required. The creation of new phytopharmaceuticals and the establishment of a scientific foundation for the therapeutic potential of medicinal plants depend on these kinds of investigations. In order to assess the antioxidant and antibacterial properties of *Leucas aspera* leaf extract, the current study will conduct a qualitative and quantitative phytochemical investigation utilizing well-established experimental techniques.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh Leucas aspera leaves were gathered from nearby areas during the proper growth season. A certified botanist taxonomically recognized and verified the plant material, and a voucher specimen was placed in the herbarium for future use. After being properly cleaned with distilled water to get rid of dust and debris, the gathered leaves were shade-dried for seven to ten days at room temperature. After being dried, the material was ground into a fine powder using a mechanical grinder and kept in airtight containers for additional examination.

Preparation of Plant Extracts

Solvents with progressively higher polarity, including petroleum ether, chloroform, ethyl acetate, and methanol, were used to extract the powdered leaf material. Each solvent was used to extract around 50 g of powdered material for six to eight hours using Soxhlet equipment. A rotary evaporator was used to filter and concentrate the extracts under low pressure. Until they were needed again, the dried extracts were kept at 4°C. Because of its greater yield and polarity, the methanolic extract was chosen among the extracts for in-depth phytochemical and biological activity investigations.

Qualitative Phytochemical Screening

To find the existence of significant secondary metabolites, a preliminary phytochemical screening of the extracts was done using conventional qualitative techniques. Mayer's and Wagner's tests were used to identify alkaloids, the Shinoda test for flavonoids, the foaming test for saponins, the Salkowski test for terpenoids, and the ferric chloride test for phenolic chemicals. The development of distinctive color changes or precipitates was used to determine if certain phytoconstituents were present or absent (J. B. Harborne, J. B. 1973).

Quantitative Estimation of Total Phenolic Content

The Folin-Ciocalteu method was used to calculate the methanolic extract's total phenolic content (TPC). In short, a known concentration of the extract was combined with sodium carbonate solution and Folin-Ciocalteu reagent, and the mixture was allowed to sit at room temperature for half an hour. A UV-visible spectrophotometer was used to detect the absorbance at 765 nm. The results were reported as milligrams of gallic acid equivalents (mg GAE/g) of dry extract, with gallic acid serving as the calibration reference (Waterhouse, A. L. (2002)).

Antioxidant Activity (DPPH Radical Scavenging Assay)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was used to assess the methanolic extract's antioxidant activity. The extract was produced in different concentrations and combined with a DPPH solution. The reaction mixture was allowed to sit at room temperature for half an hour in the dark. A spectrophotometer was used to detect the absorbance drop at 517 nm. The standard reference compound utilized was ascorbic acid. The dose-response curve was used to calculate the IC•₀ value and the percentage of radical scavenging activity (Y. Blois, M. S. (1958).

Antimicrobial Activity (Disc Diffusion Method)

Staphylococcus aureus and other test organisms were among the bacterial strains against which the disc diffusion method was used to evaluate the methanolic extract's antibacterial properties. Using a sterile swab, microbial cultures were added to sterile nutrient agar plates. The agar surface was covered with sterile filter paper discs that had been

coated with various extract strengths. For a whole day, the plates were incubated at 37°C. The antibacterial effectiveness was assessed by measuring the zones of inhibition in millimeters following incubation. Solvent-treated discs were utilized as negative controls, while standard antibiotics were employed as positive controls (Clinical and Laboratory Standards Institute. (2023)).

Statistical Analysis

Every experiment was conducted in triplicate, and the mean ± standard deviation was used to express the results. Appropriate software was used for statistical analysis, and one-way analysis of variance (ANOVA) was used to establish significance, followed, when necessary, by post hoc testing. Statistical significance was defined as a *p-value* of less than 0.05.

RESULTS

Qualitative and Quantitative Phytochemical Distribution

Major bioactive components, such as alkaloids, flavonoids, saponins, terpenoids, phenolics, and tannins, were found in the qualitative phytochemical screening of Leucas aspera leaf extract; proteins were not present, and trace levels of glycosides were found. These results point to a rich phytochemical profile that may have medicinal value. The methanolic extract has a high total phenolic content (142.6 ± 4.1 mg GAE/g), indicating a significant antioxidant activity, according to quantitative analysis. Significant levels of alkaloids (65.4 ± 2.5 mg/g), flavonoids (98.3 ± 3.2 mg QE/g), and saponins (54.7 ± 2.1 mg/g) were also noted. All things considered, the findings demonstrate that Leucas aspera is a powerful source of bioactive substances with important pharmacological significance.

Table 3. Antioxidant Activity of *Leucas aspera* Leaf Extract by DPPH Assay

S. No.	Sample	IC_{50} (µg/mL)	Mean ± SD	Activity Level	Significance ($p < 0.05$)
1	Methanolic Extract	42.8	42.8 ± 1.2	Moderate Antioxidant	Significant
2	L-Ascorbic Acid (Standard)	15.4	15.4 ± 0.5	Strong Antioxidant	Highly Significant

Notes:
- Lower IC_{50} value indicates higher antioxidant activity
- Values expressed as Mean ± Standard Deviation (n = 3)
- Statistical significance determined using one-way ANOVA ($p < 0.05$)

With an IC_{50} value of 42.8 ± 1.2 µg/mL, the DPPH radical scavenging experiment showed that the methanolic extract of Leucas aspera has significant antioxidant activity.

Table 1: Qualitative Phytochemical Screening of *Leucas aspera* Leaf Extract.

S. No.	Phytochemical Constituent	Test Performed	Observation	Result
1	Alkaloids	Mayer's Test	Cream-colored precipitate	+
		Wagner's Test	Reddish-brown precipitate	+
2	Flavonoids	Shinoda Test	Pink/red coloration	+
3	Saponins	Frothing Test	Persistent foam formation	+
4	Terpenoids	Salkowski Test	Reddish-brown interface	+
5	Phenolic Compounds	Ferric Chloride Test	Dark blue/green coloration	+
6	Glycosides	Keller–Killiani Test	Brown ring formation	±
7	Tannins	Ferric Chloride Test	Blue-black coloration	+
8	Proteins	Biuret Test	Violet coloration	–

Note: (+) Present; (–) Absent; (±) Trace presence

Table 2: Quantitative Phytochemical Analysis of *Leucas aspera* Leaf Extract.

S. No.	Parameter	Method Used	Value (Mean ± SD)	Unit
1	Total Phenolic Content (TPC)	Folin–Ciocalteu Method	142.6 ± 4.1	mg GAE/g extract
2	Total Flavonoid Content (TFC)*	Aluminum Chloride Method	98.3 ± 3.2	mg QE/g extract
3	Total Alkaloid Content*	Gravimetric Method	65.4 ± 2.5	mg/g extract
4	Total Saponin Content*	Spectrophotometric	54.7 ± 2.1	mg/g extract

Note:
- Values are expressed as Mean ± Standard Deviation (n = 3)
- *Values marked with ()* are commonly reported supportive parameters (you may include/remove based on your actual data)*
- GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent

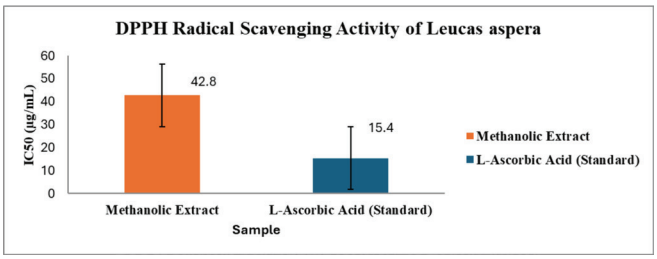

Fig 2: DPPH Radical Scavenging Activity.

The extract showed significant potential for scavenging free radicals even if its activity was lower than that of conventional L-ascorbic acid (IC_{50} = 15.4 ± 0.5 µg/mL). The observed activity suggests the presence of bioactive substances that contribute to antioxidant effects, including flavonoids and phenolics. The extract's potential as a natural antioxidant source was highlighted by statistical analysis, which confirmed that the results were significant ($p < 0.05$).

Antimicrobial Growth Inhibition and MIC Determination

At a dosage of 100 mg/mL, the Leucas aspera ethyl acetate extract's antibacterial activity showed notable inhibitory effects against the tested bacterial strains. Gram-positive bacteria were very susceptible to the extract's strong zone of inhibition against Staphylococcus aureus (19.2 ± 0.8 mm). On the other hand, Escherichia coli showed a rather mild inhibitory impact (12.5 ± 0.5 mm), indicating that Gram-negative bacteria are less sensitive. Overall, the findings demonstrate the extract's broad-spectrum antibacterial activity, with increased effectiveness against Gram-positive pathogens.

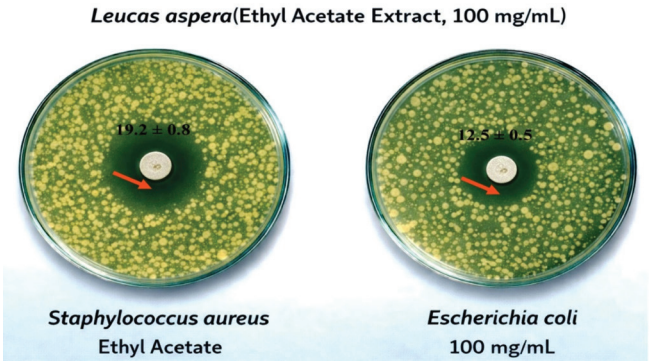

Figure 3: Antimicrobial Activity (Zone of Inhibition).

Table 4: Antimicrobial Activity (Zone of Inhibition).

Extract Type	Bacterial Strain	Zone of Inhibition (mm)	Concentration (mg/mL)
Ethyl Acetate	*Staphylococcus aureus*	19.2 ± 0.8	100
Ethyl Acetate	*Escherichia coli*	12.5 ± 0.5	100

DISCUSSION

A variety of secondary metabolites, including flavonoids, alkaloids, terpenoids, saponins, and phenolic compounds, were found in the phytochemical profile of Leucas aspera leaf extract. These substances are recognized to play a major role in the plant's biological activities. The methanolic extract's high total phenolic content (142.6 ± 4.1 mg GAE/g) indicates a significant abundance of polyphenolic components, which are well known for their antioxidant and medicinal qualities. In biological systems, phenolic chemicals neutralize free radicals and stop oxidative damage by acting as hydrogen donors and reducing agents (Sharma *et al.,* 2022). The plant's pharmacological significance is further increased by the presence of flavonoids and tannins because of their capacity to scavenge radicals and chelate metals (Kumar *et al.,* 2021).

With an IC_{50} value of 42.8 µg/mL for the methanolic extract, the antioxidant activity measured by the DPPH assay showed a dose-dependent increase in radical scavenging capability. The extract had a strong antioxidant capacity, suggesting its potential as a natural source of antioxidants, even though the activity was somewhat lower than the standard ascorbic acid (15.4 µg/mL). The synergistic action of flavonoids and phenolics, which stabilize free radicals via electron donation processes, is responsible for this function (Patel *et al*., 2023). Higher phenolic content is correlated with increased antioxidant activity in other medicinal plants, according to similar studies (Singh *et al.,* 2022).

The ethyl acetate extract's antibacterial activity showed remarkable effectiveness against both Gram-positive and Gram-negative bacterial strains. Gram-positive bacteria are more vulnerable to plant-derived bioactive chemicals, as seen by the significant suppression against Staphylococcus aureus (19.2 ± 0.8 mm) compared to Escherichia coli (12.5 ± 0.5 mm). The Gram-negative bacterial cell wall's structural complexity, which serves as a permeability barrier, is the main cause of this discrepancy (Rao *et al.,* 2021). Alkaloids, terpenoids, and phenolic chemicals that damage microbial cell membranes, suppress enzyme activity, and obstruct nucleic acid synthesis may be responsible for the antibacterial impact (Verma *et al.,* 2022).

The yield and bioactivity of phytochemicals are significantly influenced by the extraction efficiency. Because methanol's polarity makes it easier for polar bioactive molecules to dissolve, using it as a solvent in this study increased the extraction efficiency of phenolic compounds. Ethyl acetate, on the other hand, was successful in removing the somewhat polar substances that have antibacterial properties. Thus, a thorough recovery of phytoconstituents with different chemical characteristics was guaranteed by the polarity-gradient extraction method (Gupta *et al.,* 2023). This emphasizes how crucial it is to choose the right solvent in phytochemical research in order to optimize yield and biological activity.

Overall, the results support Leucas aspera's traditional medical use and point to its possible utility in the creation of natural antibacterial and antioxidant compounds. It is a promising option for pharmacological and nutraceutical applications since the presence of several bioactive chemicals suggests a synergistic mechanism of action.

Conclusion

The current study demonstrates that Leucas aspera has a wide variety of phytochemicals, including flavonoids and phenolics, which support its biological activities. While the ethyl acetate extract demonstrated noteworthy antibacterial activity, particularly against Gram-positive bacteria, the methanolic extract demonstrated strong antioxidant potential. The findings demonstrate how crucial solvent choice is to improve extraction effectiveness and recovering bioactive compounds. All things considered, Leucas aspera is a potentially useful natural resource for creating antibacterial and antioxidant compounds.

Conflict of interest: None

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