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Unveiling the Bioactive Profile of Leucas aspera Leaves: A Study on Antimicrobial and Antioxidant Activities

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ABSTRACT

Leucas aspera, a traditionally valued medicinal plant was examined for its phytochemical constituents, antibacterial activity and antioxidant potential. Qualitative screening of ethanolic, methanolic and aqueous leaf extracts revealed a broad spectrum of secondary metabolites including alkaloids, flavonoids, phenols, saponins, steroids and terpenoids demonstrating solvent dependent variability. Among the extracts, the ethanolic fraction evinced the strongest antibacterial effect against seven pathogenic bacteria, particularly Staphylococcus aureus and Escherichia coli with inhibition zones reaching 18 mm. Bioassay guided column chromatography enabled the isolation of active fractions enriched in alkaloids and phenolic compounds. FTIR and UV-Vis spectral analyses confirmed the presence of functional groups characteristic of these bioactive constituents, while GC-MS analysis identified potent alkaloids such as 2-Ethylacridine in the most active fraction. The ethanolic extract also exhibited notable hydrogen peroxide scavenging ability, achieving 63.5% inhibition at 100 µg/mL in a concentration dependent manner. Overall, the findings provide scientific validation for the ethnomedicinal use of L. aspera leaves highlighting their potential as a promising source of natural antimicrobial and antioxidant agents.

KEYWORDS: Leucas aspera, Phytochemical, Antibacterial activity, GC-MS analysis, FTIR spectroscopy, Antioxidant potential.

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INTRODUCTION

Herbal medicines constitute a vital component of traditional healthcare systems worldwide due to their accessibility, cultural acceptance and the diverse bioactive compounds exhibiting antioxidant, antibacterial and antifungal properties. Plants have historically served as the basis for many modern medicines, with natural products and their derivatives representing over 50% of drugs currently in clinical use [1]. The escalating problem of antibiotic resistance has intensified the search for new antibacterial agents, positioning medicinal plants like *Leucas aspera* as promising sources of novel bioactive compounds [2]. Ethnobotanical knowledge plays a crucial role in identifying medicinal plants with therapeutic potential as many bioactive secondary metabolites such as alkaloids, saponins, terpenoids, phenolics and flavonoids contribute both to plant defense mechanisms and human health benefits including antimicrobial and antioxidant activities [2]. Phytochemicals in plants have demonstrated significant antioxidant potential which helps mitigate oxidative stress-related diseases emphasizing the therapeutic value of plant derived compounds [3]. *Leucas aspera* (Lamiaceae), commonly known as Thumbai or Dronapushpi, is a perennial herbaceous plant widely distributed in tropical regions, particularly India, where its leaves have been traditionally employed in folk medicine for treating wounds, skin infections, cough, fever, snakebites and inflammatory conditions. Pharmacological studies have validated these ethnomedicinal uses revealing significant antimicrobial activity against pathogens such as *Staphylococcus aureus*, *Escherichia coli* and dermatophytes, alongside potent antioxidant effects through DPPH scavenging and ferric reducing power attributed to its rich phytochemical profile of flavonoids, phenols, alkaloids, terpenoids and diterpenes [4]. Amidst rising antimicrobial resistance, which renders conventional antibiotics ineffective against multidrug resistant clinical isolates, there is urgent need for natural alternatives with broad-spectrum activity and multi-target mechanisms. Despite promising preliminary data, comprehensive evaluation of *L. aspera* leaf extracts across multiple solvents against diverse bacterial strains remains limited [5]. This study aimed to investigate the antibacterial and antioxidant activities of ethanol, methanol and aqueous extracts from *Leucas aspera* leaves using agar well diffusion and hydrogen peroxidase assays respectively to validate traditional claims and identify solvent-specific bioactive profiles for potential therapeutic applications.

METHODOLOGY

Plant Collection and Storage

The leaves of the *Leucas aspera* were collected from the local region of Tirunelveli district. Fresh leaves were dried under shade, ground into a coarse powder and mixed with solvents like ethanol, methanol and aqueous. The solvents were kept in dark condition for 72 hours. It was subjected to filtration using filter paper (Whatman No.1). A rotary evaporator was used to evaporate the solvent and the dried extract was placed in refrigerator at 4° C until use.

Preliminary Phytochemical Analysis

The extracts obtained after successive solvent extraction were qualitatively tested for the presence of various phytochemicals. The preliminary phytochemical screening was carried out as described by Harborne (1991) and Kokate (1995) for the Alkaloids, Saponins, Amino acids, Terpenoids, Reducing Sugars, Flavonoids, Steroids and Phenolics test [6], [7].

Primary Antibacterial Activity

Antibacterial activity was carried out against four selected Gram positive (*S. aureus, S. mutans, E. faecalis* and *S. pneumoniae*) and three Gram negative pathogens (*E. coli, V. vulnificus* and *A. hydrophila*). In order to assess the biological significance and ability of the plant part, the minimal inhibitory activity was determined by agar well diffusion method. Nutrient agar medium plates were seeded with 18-24 hours cultures of microbial inoculum (a standardized inoculum of 10^8 CFU ml^{-1}). Four wells with a diameter of 5 mm each were cut into the agar media with a sterilized cork borer to which the plant extracts of 50µl concentration of ethanol, methanol and aqueous extract were poured into the wells. An antibiotic and DMSO (24µl per well) were also poured into one well each as a positive and negative control respectively. Inoculated plates were incubated at 37ºC for 24 hrs and the zones of inhibition were measured in mm. Three replicates were prepared for each bacterial culture [8].

Isolation and Purification of Bioactive Compound

Column Chromatography

The phytoconstituents from the ethanol extract of *Leucas aspera* leaves were synthesized by placed in a vertical glass column (40 mm width and 60 mm length) made of borosilicate material for the fractionation. The column was rinsed well with acetone and was completely dried before packing. A piece of glass wool was placed at the bottom of the column with the help of a glass rod. Sea sand (50-70 particle size) was added to the top of the glass wool to 1 cm height. The sand particles were rinsed down using the solvent. Hexane was poured into the column up to 3/4th level by closing the stopcock. About 200 g of silica gel (60-120 mesh size) was used as the packing material. Silica slurry was prepared with hexane and was poured from the top of the column approximately 2/3rd of the column with simultaneous draining of the solvent to aid proper packing of the column. Sea sand was added to the top of the silica slurry to 1 cm height and the sand particles were rinsed down with the solvent. About 20 g of ethanol extract was mixed with minimum quantity of hexane and was poured down from the top of the column along the sides and was rinsed down with the solvent. Sea sand was added to the top of the extract to 1 cm height. Solvent level 6 cm from above the extract was maintained to prevent drying of the column. Gradient elution method was followed to separate the fractions from ethanol extract by using solvents from low polarity to high polarity (i.e., hexane to methanol) in varying ratios. The flow rate was adjusted to 5 ml/min and 40 ml solvent was collected for each fraction [9].

Thin Layer Chromatography

Column chromatography of *Leucas aspera* ethanol leaf extract was performed to isolate and purify bioactive phytochemicals. Briefly, dried extract (5-10 g) was adsorbed onto silica gel (60-120 mesh) as a dry powder slurry and loaded onto a glass column (diameter 3-5 cm, length 40-60 cm) packed with silica gel slurry (60-120 mesh) pre-wetted with the initial solvent. The column was eluted sequentially with solvents of increasing polarity, typically starting from non-polar n-hexane followed by gradually increasing polarity with mixtures of hexane: ethyl acetate, ethyl acetate and finally methanol. Fractions (20-30 mL) were collected systematically and monitored by TLC to identify similar fractions with matching Rf values and spot patterns. Similar fractions were pooled and concentrated under reduced pressure using a rotary evaporator for further analysis. This chromatographic separation facilitated enrichment of target compound classes such as terpenoids, flavonoids and phenolics for subsequent characterization and bioactivity testing [10].

Antibacterial Activity of Isolated Bioactive Compound

Overnight fresh cultures (adjusted to standard of 0.5 McFarland turbidity) were inoculated on Muller Hinton agar plates. In each plate, ten column chromatography fractions of *Leucas aspera* plant were poured in separate well. All the plates were incubated at 37°C for 24 h and zones of inhibition were measured in mm. Three replicates were prepared for each microorganism [11].

Characterization of the Potential Bioactive Compound

UV-Visible Spectroscopic Analysis

The presence of the isolated compounds was confirmed by UV- Visible spectroscopic analysis. One ml of Fraction LF6 was taken and absorbance was measured by using UV-visible spectrophotometer between 200-600 nm [12].

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The isolated bioactive compound fraction LF6 was subjected to FTIR analysis for the determination of present functional groups. Fourier transform infrared spectrometry (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany) equipped with OMNIC software was used in the analysis. The plant samples were analysed to obtain IR spectra in the scanning wave number ranging from 500-4000 cm^{-1} with a resolution of 4 cm^{-1} [13]. The spectra of the observed compounds, group frequencies and characteristics bonds from the extracts were compared with the table of expected absorption bands for the molecule's various groups and bonds.

GC-MS Analysis of Fraction LF6

The phytochemical investigation of the isolated bioactive compound fraction LF6 was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 40°C raised to 250°C at 5°C/min and injection volume was 1µl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme [14].

***In vitro* Antioxidant Assay**

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging ability of the *Leucas aspera* ethanolic extract was determined according to the method described by Ruch *et al*., (1989). The extract was dissolved in phosphate buffer (0.1nM, pH 7.4) at various concentrations and mixed with 600µl of hydrogen peroxide solution. Ascorbic acid was used as the reference compound. The concentration of the hydrogen peroxide was measured by reading the absorbance values of the reaction mixtures at 230nm after 10minutes. Hydrogen peroxide was determined using molar absorptivity for hydrogen peroxide [15].

RESULT AND DISCUSSION

Phytochemical Analysis

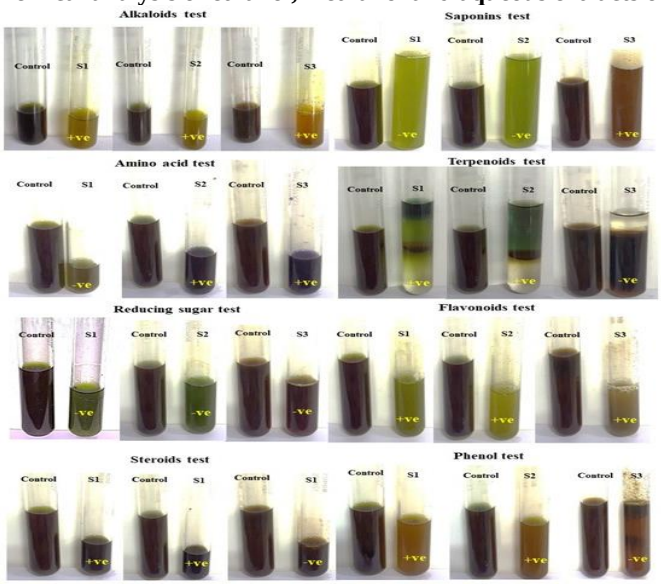
Phytochemical screening revealed that the ethanolic extract of *Leucas aspera* leaves contained alkaloids, flavonoids and phenols, while the methanolic extract exhibited a broader spectrum including alkaloids, amino acids, flavonoids, steroids and phenols. The aqueous extract was distinctive in showing saponins, amino acids, terpenoids, flavonoids and steroids. This solvent dependent variation aligns with established principles of phytochemical solubility, where polar solvents like ethanol and methanol effectively extract phenolics, flavonoids and alkaloids, whereas water preferentially solubilizes saponins and certain terpenoids due to their amphiphilic nature [16]. The results of the phytochemical analysis of *L. aspera* are represented in Table 1 and Figure 2. The consistent presence of flavonoids and phenols across all extracts underscores their prominence in *L. aspera* leaves supporting the plant's documented antioxidant potential through DPPH scavenging and FRAP assays reported in prior studies. These polyphenolics probably aid in metal chelation and free radical neutralization, which explains their traditional applications against oxidative stress-related illnesses, wounds and inflammatory diseases. Previous observations of activity against *Staphylococcus aureus* and dermatophytes (MIC 5-10 mg/mL) indicate that alkaloids in ethanol and methanol extracts offer antibacterial activities, possibly through membrane disruption or enzyme inhibition [17].

Table 1 Phytochemical analysis of ethanol, methanol and aqueous extract of *L. aspera*

S. No.	Phytoconstituents	Ethanol extract	Methanol extract	Aqueous extract
1	Alkaloids	+	+	+
2	Saponins	-	-	+
3	Amino acid	-	+	+
4	Terpenoids	+	+	-
5	Reducing sugar	-	-	-
6	Flavonoids	+	+	+
7	Steroids	+	+	-
8	Phenols	+	+	-

+: = Present: = Absent

Figure 2 Phytochemical analysis of ethanol, methanol and aqueous extracts of *L. aspera* leaves



S1: Ethanol extract. S2: Methanol extract. S3: Aqueous extract
+ve: Positive. –ve: Negative

Primary Antibacterial Activity

The ethanol extract from *Leucas aspera* leaves exhibited strong antibacterial effects across all seven bacterial strains tested with the biggest zones of inhibition against *Staphylococcus aureus* (18±0.80 mm) and *Escherichia coli* (18±0.77 mm). This is because ethanol works by pulling out medium-polarity compounds like flavonoids, phenols and alkaloids compounds that's been found in the test plant before and is known to inhibit bacterial cell walls, block key enzymes or even damage their DNA. What's really promising is how it worked against both Gram-positive organism like *S. aureus* and Gram-negative *E. coli* and *Vibrio vulnificus*, probably these compounds teaming up to inhibit cell membrane and blocked the bacterial pumps similar to what other studies have seen with MICs around 5-10 mg/mL [18]. The methanol extract was moderately effective showing 6-12 mm zones against *Aeromonas hydrophila* (12±0.55 mm) and *V. vulnificus* (11±0.60 mm) likely because it grabs steroids and extra alkaloids that slows down bacterial growth by targeting protein production. Aqueous extracts left behind only mildly active (7-10 mm) on a few strains which fits since aqueous extracts mostly have saponins and water-loving terpenoids that mainly act on the surface rather than getting inside Gram-negative bacteria (Chew *et al.*, 2012). *Leucas aspera* extract hit 30-60% level of inhibition role of ciprofloxacin (32-35 mm) which indicates the emergence of resistance against clinical strains like *Enterococcus faecalis* and *Streptococcus pneumoniae* suggesting that they could pair well with antibiotics to fight resistance (Table 2 & Figure 3). This backs up with the traditional healers usage of *L. aspera* for infections and wounds, tying the activity straight to its flavonoid and phenol levels and there is an urge to purify those bioactive compounds that exhibited MICs under 50 µg/mL. Checking minimum inhibitory concentrations and combined effects with standard drugs would show its clinic potential against resistant bugs [19].

Table 2: Antibacterial efficacy of ethanol, methanol and aqueous extracts of *L. aspera*

S. No.	Test Bacterial Strains	Positive Control (mm) (Ciprofloxacin)	Negative Control (DMSO)	Diameter of Zone of Inhibition (mm)		
				Ethanol extract	Methanol extract	Aqueous extract
1	*Enterococcus faecalis*	32±1.38	-	11± 0.58	10±0.47	-
2	*Streptococcus pneumoniae*	34±1.38	-	13±0.62	06±0.52	-
3	*Streptococcus mutans*	35±1.38	-	12±0.55	8±0.40	7±0.35
4	*Staphylococcus aureus*	32±1.38	-	18±0.80	8±0.45	10±0.50
5	*Escherichia coli*	35±1.38	-	18±0.77	10±0.48	-
6	*Vibrio vulnificus*	33±1.38	-	10±0.50	11±0.60	09±0.42
	Aeromonas hydrophila	35±1.38	-	15±0.70	12±0.55	09±0.40

Values are expressed as mean ± SEM from triplicate determinations. Values are non-significant

Figure 3 Antibacterial efficacy of *Leucas aspera* extract

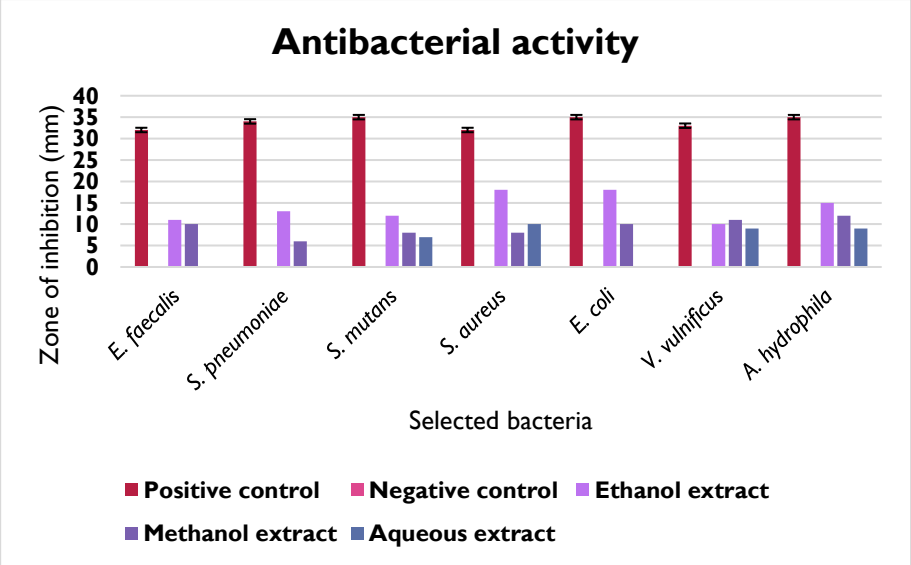
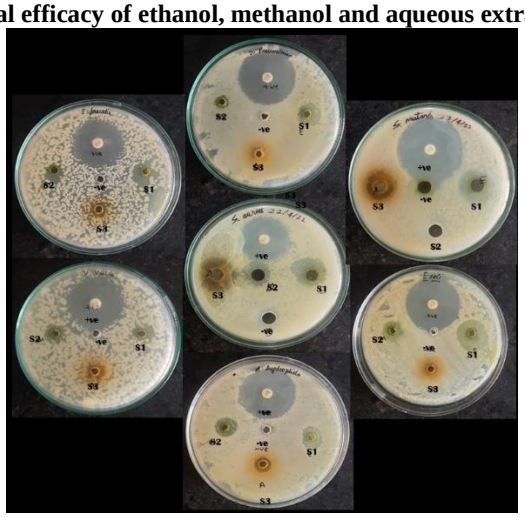


Figure 4 Antibacterial efficacy of ethanol, methanol and aqueous extracts of *Leucas aspera*



Column Chromatography & TLC

For column chromatography, the ethanolic leaves exact of *Leucas aspera* was used. Column chromatography helps to separate the bioactive compounds from the extract. Column chromatography works on the principle of separation of compounds from a mixture. A column chromatography contains the stationary phase, allowing the mobile phase to pass through it. The column could separate substances based on different adsorption of compounds to the adsorbent. In this experiment, the stationary phase used in this column was silica gel (pore size 100-200) and n-hexane, ethyl acetate and ethanol in sequence were used as a mobile phase. The interaction occurs between the polar stationary phase and nonpolar mobile phase. When the mobile phase collides with the stationary phase, each component interacts with the polar stationary phase or the nonpolar mobile phase more preferentially. The mixture will get separated and travel along with the eluting solvent. Therefore totally 10 fractions were obtained from the ethanolic leaves extract of *Leucas aspera*. The 10 fractions were named as LF1, LF2, LF3, LF4, LF5, LF6, LF7, LF8, LF9 and LF10 (Table 5). The fractions obtained from the column chromatography was checked by TLC to detect the presence of active compound after which R_f value was calculated. Nine different Rf values were obtained. TLC profiling of the isolated column chromatography fractions exhibited diverse nature of Rf values. The n- Hexane fraction 1 and 2 had Rf value of 0.4 and 0.18. Ethyl acetate fraction (3 & 4) had Rf value of 0.62 and 0.55. Among 6 fractions of ethanolic eluting solvent, 3 fractions had compounds in TLC with Rf value 0.76 (fraction- 5) 0.75 (fraction-6) and 0.70 (fraction-10). The 7,8,9 fractions have Rf values of 0.68, 0.80 and 0.66 respectively which indicated the presence of alkaloids, flavonoids and phenol compound in the isolated column chromatography fraction. Among the ten fractions LF1, LF6, LF8 and LF10 represented potent antibacterial activity against *Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli* and *Aeromonas hydrophila.* This is because of the presence of alkaloids, flavonoids and phenol compound in the *Leucas aspera* leaves extract. The result was compared with the study of Kaur and Sawant in 2021. Their study evinced the presence of bioactive alkaloid (2%) in *Adhatoda vasica.* On TLC plates, three spots were noticed by bioautography which separated at 0.4, 0.86 and 0.92 Rf values in chloroform: methanol (80: 20) solvent system. One of the spots was identified as alkaloid, while the chemical nature of the other 2 spots could not be determined. *Adhatoda vasica* has also been recognized for its diverse health benefits along with their antimicrobial potential. They possessed good antimicrobial activity against respiratory pathogens *S. aureus* and *E. coli* due to the presence of alkaloids and flavonoids in *Adhatoda vasica [20]*.

Table 5 Column Chromatography and TLC Analysis of ethanolic extract of *Leucas aspera* Leaves

Fraction No.	Eluting Solvent	Weight of the Fraction (g)	R_f value
1	n- Hexane-1	Trace	0.4
2	n- Hexane-2	Trace	0.18
3	Ethyl acetate-1	0.1	0.62
4	Ethyl acetate-2	0.2	0.55
5	Ethanol-1	0.36	0.76
6	Ethanol-2	0.41	0.75
7	Ethanol-3	0.6	0.68
8	Ethanol-4	0.7	0.80
9	Ethanol-5	0.15	0.66
10	Ethanol-6	0.3	0.70

Antimicrobial Activity of the Isolated Fractions

Antibacterial activity of the partially purified chromatographic fractions LF1 to LF 10 against the test bacteria are presented in Table 6. Among the ten chromatographic fractions LF1 to LF10, only LF1, LF6, LF8 and LF10 rendered potent antibacterial activity. LF1 depicted activity against *S. pneumoniae, E. coli* and *A. hydrophila* and the inhibition was recorded between 7 to 10mm. Poor zone of inhibition was recorded between 5 to 12mm for LF2, LF3, LF4, LF5, LF7 and LF9 against *S. pneumoniae, S. aureus, E. coli* and *A hydrophila.* The inhibition range was recorded between 7 to 10 mm for LF1, LF8 and LF10 and 10-14 mm for LF6 respectively. Therefore, LF6 which depicted the higher zone of inhibition was selected for further study. Similar studies were conducted by Hafeez *et al.,* in 2020 who studied the antibacterial activity of different column chromatography

fractions of *Aloe vera* gel against the pathogenic bacteria. Two fractions of. *A vera* gel rendered maximum antibacterial activity up to 22mm. Moderate antibacterial activity (06-11mm) of six fractions (E-J) were observed in the ethanol extract and no activity was observed with the remaining eight fractions (A, B, K-P). Similarly, two fractions (73 and 74) of the methanol extract exhibited a maximum antibacterial activity followed by ten fractions (16, 40, 46, 48, 113, 114, 123, 125, 126 and 128) with moderate activity and the remaining ten fractions (80, 83, 94, 95, 100, 103, 104, 105, 117 and 127) with no activity [21].

Table 6 Antibacterial activity of isolated column chromatography fractions from the ethanol extract of *Leucas aspera*

S.No.	Fraction	Test organism Diameter of zone of inhibition (mm)			
		Streptococcus pneumoniae	***Staphylococcus aureus***	***Escherichia coli***	***Aeromonas hydrophila***
1	LF 1	7±0.40	-	10±0.30	7±0.55
2	LF 2	-	6±0.30	-	-
3	LF 3	-	8±0.55	5±0.45	-
4	LF 4	6±0.45	-	-	-
5	LF 5	9±0.55	-	-	-
6	LF 6	14±0.70	12±0.65	10±0.30	13±0.30
7	LF 7	5±0.20	8±0.45	-	-
8	LF 8	10±0.54	7±0.40	-	7±0.40
9	LF 9	-	6±0.32	12±0.30	-
10	LF 10	9±0.40	10±0.45	7±0.45	-

Values are expressed as mean ± SEM from triplicate determinations. Values are non-significant.

Figure 6 Antibacterial activity of isolated column chromatography fractions from the ethanol extract of *Leucas aspera*

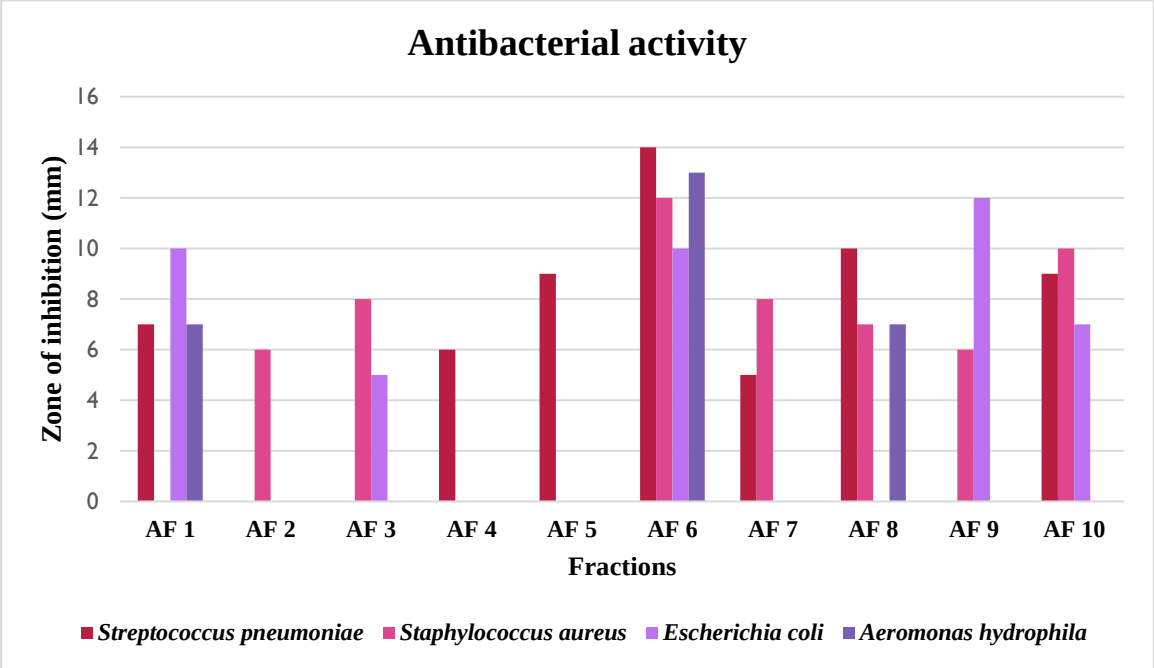
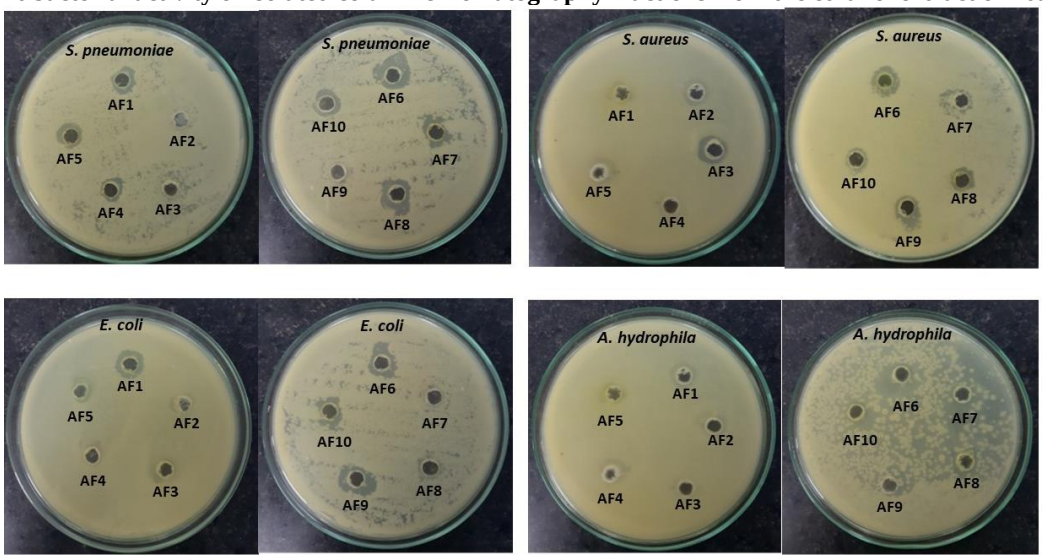


Figure 7 Antibacterial activity of isolated column chromatography fractions from the ethanol extract of *Leucas aspera*



FTIR Analysis

FTIR spectroscopic analysis of the chromatographic fraction LF6 revealed the presence of different functional groups of bioactive compounds in the form of peaks, when the extract passed in the FTIR region, the functional groups were separated based on its bonding positions, the resulting 11 peaks confirms the presence of wide range of functional groups of bioactive compounds. The results of FTIR spectrum of chromatographic extract LF6 of *Leucas aspera* confirmed the presence of alcohols with a peak at 1338.51 cm^{-1}, 3443.66 cm^{-1}, 3741.65 cm^{-1} and 3879.55 cm^{-1} corresponded to hydroxyl and O-H bonding frequency respectively. The peak at 1643.24 cm^{-1} and 2883.38 cm^{-1} assigned to the C-H stretching indicating the presence of some alkene compounds. The peak value at 517.85 cm^{-1} confirms Halo compounds. The peak value at 1057.88 cm^{-1} confirms primary alcohol. The peak value at 1510.16 cm^{-1} confirms nitro compound, the peak value at 2360.71 cm^{-1} confirms carbon dioxide, and the peak value at 2975.96 cm^{-1} confirming carboxylic acids are given in Figure 8 and Table 7. In the present study, a wide range of functional groups of bioactive compounds such as alcohols, alkanes, primary alcohol, carboxylic acids, nitro compounds and halo compound were found in the chromatographic extract (Figure 8 & Table 7). This study was similar with the absorption spectra of *J. azoricum* L., which exhibited a peak at 2960.73 representing the presence of alkane (C-H stretch) and carboxylic acid (O-H stretch). The peak at 1739.40 showed the presence of ester (C-O stretch) and aldehyde (C-O stretch). The peak at 1462.04 referred the presence of aromatic (C=C stretch). The peak at 1380.36 confirms the presence of nitro (N-O stretch) and alkane (-C-H bending). The peak at 1022.12 represented the presence of amines (C-N stretch), esters (C-O stretch) and alkyl halide (C-F stretch). The peak at 769.59 exhibited the presence of esters (S-OR stretch), amines (N-H stretch), alkyl halide (C-Cl stretch) and alkene (=C-H bending) also reported that the stretches such as C-H, C=O, C=C, N-O, C-N and S-OR with the nearest range representing the same functional groups [22].

Figure 8 FTIR spectrum of chromatographic fraction LF6 of *Leucas aspera* leaves extract

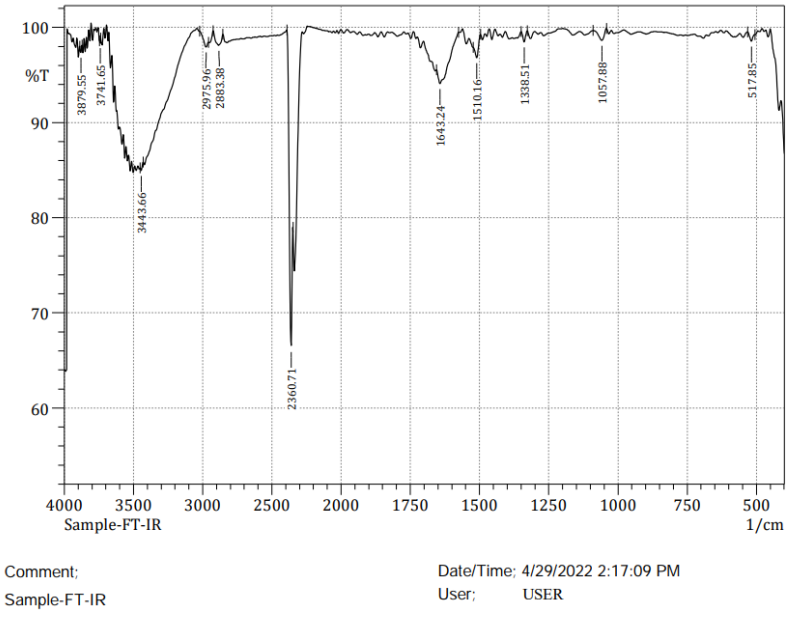


Table 7 FTIR - Band position and their functional groups of chromatographic fraction LF6 of *Leucas aspera* leaves extract

S. No.	Band Position (cm^{-1})	Functional Groups
1	517.85	Halo compound
2	1057.88	Primary Alcohols
3	1338.51	Alcohol
4	1510.16	Nitro compound
5	1643.24	Alkene
6	2360.71	Carbon dioxide
7	2883.38	Alkane
8	2975.96	Carboxylic Acids
9	3443.66	Alcohol
10	3741.65	Alcohol
11	3879.55	Alcohol

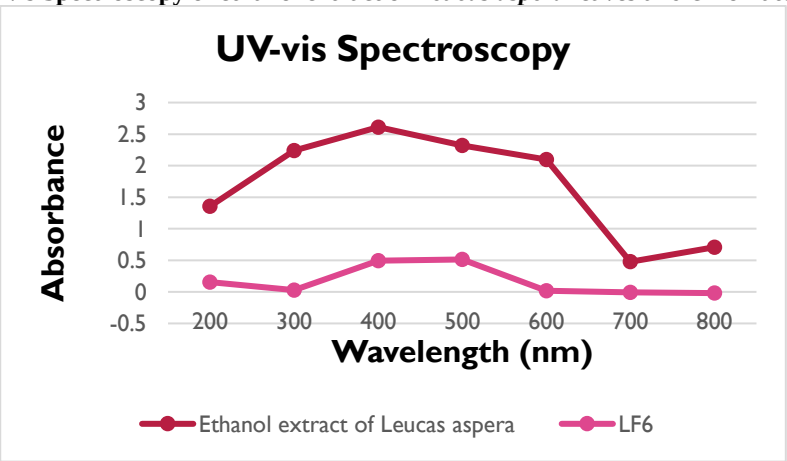
UV Vis Spectroscopy

The UV-VIS analysis preformed for the identification of phytoconstituents present in column chromatographic fraction LF6 and ethanol leaves extract of *Leucas aspera*. The qualitative UV-VIS profile of ethanolic extract and chromatographic fraction LF6 of *Leucas aspera* leaves extract was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 200, 300, 400, 500, 600, 700 and 800 nm with the absorption 1.3573, 2.2366, 2.6107, 2.3205, 2.0964, 0.4771and 0.7061 respectively for ethanol leaves extract. The absorption was 0.156786, 0.025863, 0.493165, 0.51351, 0.017088, -0.008705 and -0.019492 for chromatographic fraction LF6. Figure 6 shows the absorption spectrum of ethanol extract and these are almost transparent in the wavelength region of 300-800 nm. Absorption bands observed pertaining to *Leucas aspera* plant extract are displayed in Table 8. In the UV-VIS spectra, the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O [23]. The UV-Vis spectroscopy helps to determine the total phenolic extract (280 nm), flavones (320 nm), phenolic acids (360 nm), and the total anthokyanids (520 nm). This technique is not time-consuming and presents reduced cost compared to other techniques [24].

Table 8 UV-vis Spectroscopy of ethanol extract of *Leucas aspera* leaves and chromatographic fraction LF6

S. No.	Wavelength (nm)	Absorbance of ethanol extract	Absorbance of LF6
1.	200	1.3573	0.156786
2.	300	2.2366	0.025863
3.	400	2.6107	0.493165
4.	500	2.3205	0.51351
5.	600	2.0964	0.017088
6.	700	0.4771	-0.008705
7.	800	0.7061	-0.019492

Figure 9 UV-vis Spectroscopy of ethanol extract of *Leucas aspera* leaves and chromatographic fraction LF6



GCMS Analysis of the Isolated Pure Compound

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of LF6 fraction revealed the presence of four compounds (phytochemical constituents) that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their), molecular formula, molecular weight (MW), structure and therapeutic activity are presented in Table 9 and Figure 10. This confirms the presence of pharmacologically important bioactive compounds viz., 2-Ethylacridine, Benz[h]quinoline, 2-4-dimethyl-, 5-metyl-2-phenylindolizine and Silicic acid and diethyl bis(trimethylsilyl) Ester. They possess antimicrobial activity, antioxidant activity,

anti-tumour activity etc. The nature of the compound is heterocyclic, alkene and alkaloids. The highest peak was given by 2-Ethylacridine which confirms that the isolated compound to be alkaloids.

Figure 10 GCMS Analysis of LF6 fraction

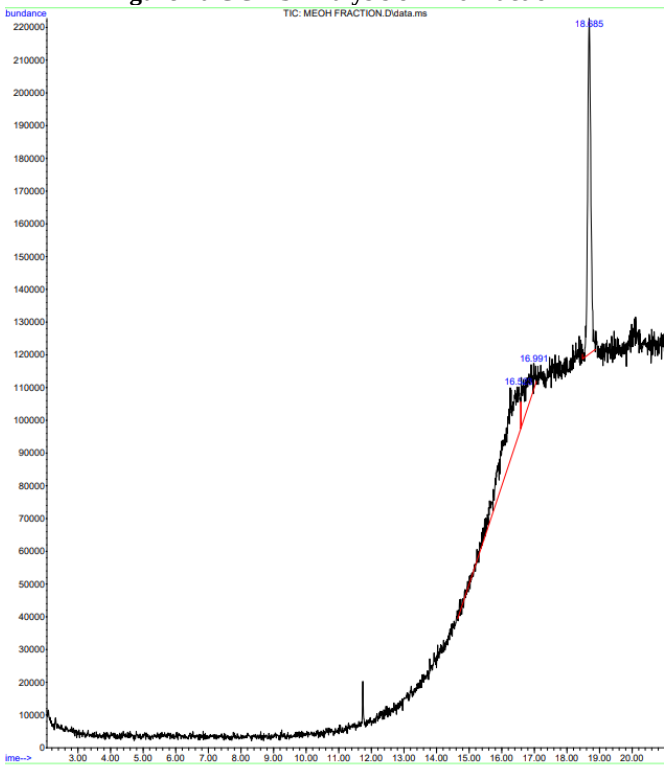
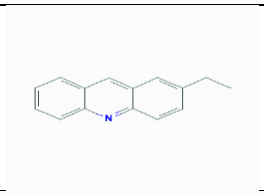
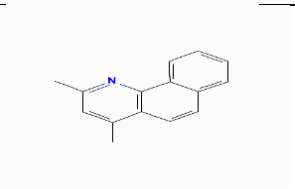
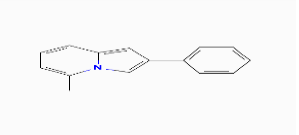
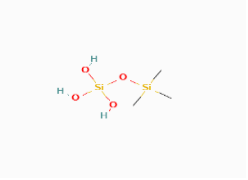


Table 9 GCMS Analysis of LF6 Fraction

S. No.	Peak Name	Molecular Formula	Molecular Weight	Structure	Therapeutic Activity
1.	2-Ethylacridine	$C_{15}H_{13}N$	207.27		Anti-bacterial, antifungal
2.	Benz[h]quinoline, 2-4-dimethyl-	$C_{15}H_{13}N$	207.27		**Anti-bacterial, anti-viral, anti-fungal, anti-inflammatory and anti-tumor drugs**
3.	5-metyl-2-phenylindolizine	$C_{15}H_{13}N$	207.27		antileukemic activity, antioxidant and antimicrobial
4.	Silicic acid, diethyl bis(trimethylsilyl) Ester	C3H12O4Si2	168.30		Cosmetics, antiviral, antimicrobial, anti-inflammatory and anti-tumoral activity

***In vitro* Antioxidant Assay**

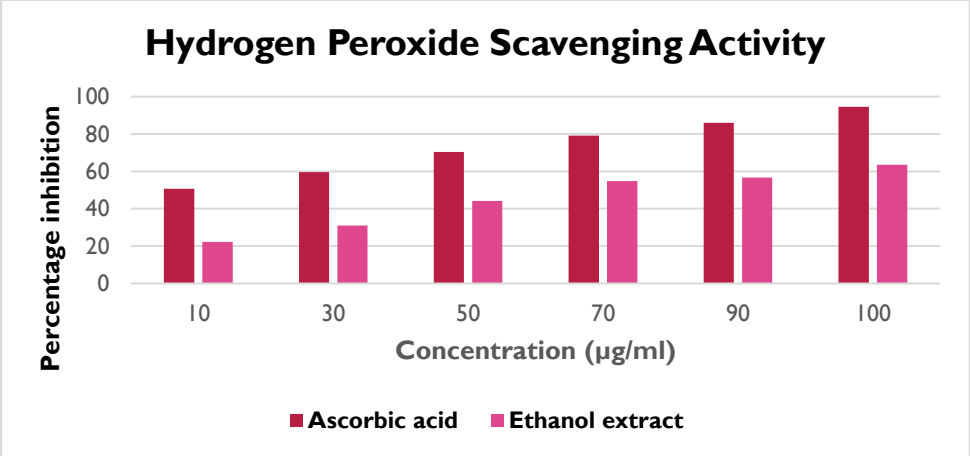
Hydrogen Peroxide Scavenging Activity

The ability of the plant extracts to scavenge hydrogen peroxide is shown in Table 11 and Figure 8. The plant sample exhibited hydrogen peroxide decomposition activity in a concentration dependent manner. At extract concentrations of 10 to 100 µg/ml /mL, *Leucas aspera* exhibited 22.3±0.51, 31±0.37, 44.20±0.82, 54.8±0.38, 56.6±0.33 and 63.5±0.05 respectively. The 100 µg/ml concentrations indicated a higher percentage of inhibition than the standard ascorbic acid (Table 10 and Figure 11). The result was compared with the study of Keser *et al.*, (2012). The study stated that the *C. monogyna* extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. 100 µg of water and ethanol extracts of *C. monogyna* exhibited 15.44-30.13% scavenging activity on hydrogen peroxide. Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems [25].

Table 10 Hydrogen Peroxide Scavenging activity of ethanol Leaves extract of *Leucas aspera* at different concentrations

S. No.	Percentage Inhibition		
	Concentration (µg/ml)	Ascorbic acid	Ethanol Extract
1	10	50.7±3.6	22.3±0.51
2	30	59.6±4.5	31±0.37
3	50	70.3±3.1	44.20±0.82
4	70	79.2±2.6	54.8±0.38
5	90	86±0.9	56.6±0.33
6	100	94.5±2.5	63.5±0.05

Figure 11 Hydrogen Peroxide Scavenging Activity of ethanol Leaves extract of *Leucas aspera* at different concentrations



CONCLUSION

The present study demonstrates that *Leucas aspera* leaves contain a diverse array of bioactive phytochemicals with notable antibacterial and antioxidant properties. Among the tested extracts, the ethanolic fraction exhibited the most pronounced inhibitory activity, particularly against *Staphylococcus aureus* and *Escherichia coli* which can be attributed to the presence of phenols, flavonoids and alkaloids. Bioassay guided purification further enabled the identification of potent antibacterial fractions with LF6 emerging as the most active. Spectral analyses including FTIR and UV-Vis supported the presence of key functional groups while GC-MS confirmed alkaloid constituents such as 2-Ethylacridine, reinforcing its therapeutic potential. In addition to its antimicrobial efficacy, the extract displayed substantial hydrogen peroxide scavenging activity, highlighting its relevance in managing oxidative stress. Overall, the findings provide scientific validation for the traditional use of *L. aspera* in treating infections and promoting wound healing. The dual antibacterial and antioxidant activities locate this plant as a promising candidate for developing natural therapeutic agents. Future research should involve MIC/MBC profiling, *in vivo* studies, toxicity evaluation and structural characterization of the lead compounds to support the development of standardized and clinically applicable formulations.

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