

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL, AND ANTIOXIDANT ACTIVITIES OF ROOT AND LEAF EXTRACTS OF *LEUCAS ASPERA*

VASUDHA K, ARCHANA D, MUTYALAMMA B, KISHORI B*

Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati - 517 502, Andhra Pradesh, India.
Email: kktinku@rediffmail.com

Received: 13 August 2018, Revised and Accepted: 26 December 2018

ABSTRACT

Objective: The present study was designed to evaluate the phytochemical screening, antimicrobial, and antioxidant activities from the roots and leaves of *Leucas aspera*.

Methods: The phytochemical screening and Fourier transform infrared (FTIR) analysis of root and leaf extracts were studied using standard methods. The disk diffusion method was performed to analyze the antimicrobial activity of aqueous extract, methanol extract and hexane extract of root and leaf against to selected bacterial and fungal strains. Antibiotics, streptomycin and nystatin were used as standards for bacteria and fungi, respectively. The antioxidant activity of the extracts was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and scavenging of H₂O₂ free radicals.

Results: Preliminary phytochemical screening of extracts exhibited the presence of carbohydrates, proteins, amino acids, steroids flavonoids, terpenoids, saponins, alkaloids, tannins, and phlobatannins. Leaf extracts exhibited effective antibacterial and antifungal activities compared with root extracts against all the tested bacteria and fungi. ME of the leaves exhibited highest antibacterial activity against *Staphylococcus aureus* (12.8±0.31 mm), followed by *Bacillus subtilis* (11.4±0.3 mm), *Escherichia coli* (9.8±0.21 mm), and *Pseudomonas aeruginosa* (7.3±0.29 mm). Leaf extracts of *L. aspera* showed effective scavenging activity compared with root extracts. ME of the leaves showed maximum scavenging activities of 38.39 and 36.85%, respectively, against DPPH and H₂O₂ free radicals with half maximal inhibitory concentration values of 136.17 and 142.42 µg/ml.

Conclusion: Phytochemical analysis and FTIR spectrum revealed that different plant secondary metabolites particularly alkaloids, terpenoids, and flavonoids could be responsible for antimicrobial and antioxidant activities of *L. aspera* leaf extracts.

Keywords: *Leucas aspera*, Antimicrobial activity, Antioxidant activity, Fourier transform infrared analysis.

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INTRODUCTION

Traditional medicine is an integral part of the primary health-care system in many countries such as India, China, and Sri Lanka. Plants are good examples and a number of plant species are known to have medicinal value. India is known for its incredible source of medicinal plants, and large body of evidence has been accumulated to highlight the potential use of medicinal plants against the harmful diseases. Over the past 100 years, development of chemotherapy and technology for the production of synthetic drugs changed the world scenario in medicine. Usage of synthetic drugs against a range of pathogenic microbes is becoming complicated due to non-response/modification of microbes. Moreover, the wide usage of commercial antimicrobial drugs lead to side effects, and most notably, development of drug resistance in the majority of the pathogenic microbes is an another major problem. To minimize the synthetic antimicrobial drugs and to reduce side effects, screening of plants with antimicrobial properties is growing day-by-day.

It is well known that several medicinal plants have been used to treat microbial infections from 1000 years [1,2]. A number of studies reported the antimicrobial properties of plants [3-9]. In addition to medicinal value, there is an increasing interest toward natural antioxidants present in plants. Antioxidant molecules have the capability to quench free radicals and remediate the effects caused by reactive oxygen species and have roles in the prevention of degenerative diseases which caused by oxidative stress [10]. Medicinal plants are rich sources of antioxidant molecules such as flavonoids, triterpenoids, tannins, coumarins, quinones, vitamins, and polyphenolic compounds and it was reported by several studies [11-13].

Numerous studies have investigated the presence of pharmacological properties from several medicinal plants. Since of more importance

to the individual compounds, the recent research is in much focus to isolate and identify the specific compounds using various methods of recent technology. The most popular methods such as high-performance liquid chromatography, gas chromatography-mass spectrometry (MS), matrix-assisted laser desorption ionization time-of-flight, MS-MS, and Fourier transform infrared (FTIR) are in use to isolate the pure compounds from various parts of the plant [14-16].

Leucas aspera, belonging to the family of Lamiaceae or Labiatae, is a common aromatic herb found in Africa, temperate and tropical countries of Asia. It is generally called as "Thumbai," widely distributed throughout India. The plant is reported to contain various phytochemicals such as oleanolic acid, ursolic acid, α -sitosterol, β -sitosterol, reducing sugars, diterpenes, glycosides, (-)isololiolide, linifolioside, nicotine, macelignan, acacetin, apigenin, myristargenol B, machilin C, and (-)chicanine [17]. In addition to the above compounds, steroids, triterpenes, phenols, flavonoids, tannins, and long-chain aliphatic compounds were also reported in *L. aspera* [18-20]. *L. aspera* possesses various medicinal values including, analgesic, anticancer, antidiabetic, antirheumatic, anti-inflammatory, antibacterial, antifungal, cytotoxic, and antioxidant activity [17,20]. There are large body of literature which is available on antibacterial and other medicinal values of *L. aspera*. However, studies on antifungal activity and phytochemical screening different parts of *L. aspera* are in adequate.

Based on the medicinal properties of *L. aspera*, the present study was aimed to evaluate the antimicrobial activity of root and leaf extracts (aqueous extract [AE], methanol extract [ME], and hexane extract [HE]) of *L. aspera* against a range of selected bacterial and fungal strains. The active compounds were also analyzed by FTIR screening and

antioxidant properties were also evaluated in the selected extracts of plant parts.

METHODS

Plant material

L. aspera plants were collected from Seshachalam hills, Eastern Ghats, Tirupati, Andhra Pradesh, India. The plant *L. aspera* was identified by Dr. Madhavashetty, Asst. Professor, Department of the Botany, S. V. University, Tirupati. The roots and leaves were separately kept for shade dry for a week and made them to fine powder. Later the powders of leaves and roots were subjected to Soxhlet extraction with different solvents including aqueous, methanol, and hexane.

Preparation of extracts

Different extracts were prepared using 100 g of powdered samples of leaves and roots were separately taken in 1000 ml of aqueous, methanol, and hexane solvents. Extraction was carried out using Soxhlet extractor at boiling point temperature of aqueous, methanol, and hexane solvents for 12 h. Using Whatman filter paper, the extracts were filtered then the filtrate was subjected for drying and redissolved in 50% (v/v) different solvents separately each containing 2.0 mg/ml extract and stored in airtight container.

Phytochemical screening

Phytochemical screening was performed using standard protocols to identify the presence of carbohydrates, cholesterol, amino acids, proteins, steroids, saponins, flavonoids, alkaloids, tannins, and phlobatannins [21].

FTIR analysis of leaf and root extracts

The dried powders of different extracts were made as pellet by taking 2 mg of the sample mixed with 200 mg KBr (FT-IR grade). The sample pellet kept into the sample holder and recorded the spectra range between 4000 and 450 cm^{-1} in FTIR spectroscopy [22].

Antimicrobial activity

Microorganisms

To screen of antimicrobial activity of *L. aspera* roots and leaves, Gram-positive, Gram-negative bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and fungi *Candida albicans* and *Aspergillus niger* were used. All the cultures were obtained from the Department of Biotechnology, Sri Padmavati Mahila Visvavidyalaya, Tirupati.

Antibacterial activity

Antibacterial activity of the different extracts of roots and leaves of *L. aspera* was evaluated against selected bacteria according to the Kirby-Bauer disk diffusion method [23]. Actively growing bacterial culture of 200 μl was evenly spread on the surface of nutrient agar plates using the sterile glass rod. The extracts were prepared with the concentrations of 25, 50, 75, and 100 $\mu\text{g/ml}$ and antibacterial activity was evaluated by placing the discs of different concentrations on the nutrient agar plates with standard antibiotic, streptomycin and plates were kept for incubation at 37°C for 24 h. The diameters of the inhibitory zones were recorded after incubation.

Antifungal activity

Antifungal activity of the different extracts of roots and leaves of *L. aspera* was evaluated against *C. albicans* and *Aspergillus niger* using disk diffusion method [24]. 200 μl of actively growing fungal inoculum was swabbed on the surface of potato dextrose agar (PDA) plates. The antibiotic discs prepared with 25, 50, 75, and 100 $\mu\text{g/ml}$ concentrations were placed on the PDA plates with standard antibiotic, nystatin. The PDA plates were incubated at 24°C for 72 h. After incubation, the diameters of zone of inhibitions were measured.

Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

In vitro, antioxidant activity of the root and leaf extracts was evaluated by DPPH free radical scavenging assay [25]. 1 ml of each extract

at selected concentrations (25, 50, 75, and 100 $\mu\text{g/ml}$) was mixed with 2 ml of DPPH solution. The reaction mixture was incubated for 30 min at room temperature, and absorbance was recorded at 517 nm. Ascorbic acid was used as a standard. The DPPH scavenging activity was calculated using the following formula:

$$\% \text{DPPH scavenging activity} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test sample})}{\text{Absorbance of Control}} \times 100$$

The amount of sample necessary to decrease the absorbance of DPPH by 50% (half maximal inhibitory concentration [IC_{50}]) was calculated graphically.

H_2O_2 radical scavenging assay

Antioxidant activity of the different extracts of *L. aspera* was further proved by H_2O_2 radical scavenging assay [26]. 2 ml of H_2O_2 solution prepared in 40 mM phosphate buffer (pH 7.4) was added to 1 ml of different concentrations, 25, 50, 75, and 100 $\mu\text{g/ml}$ of extracts separately and kept in the dark at room temperature for 20 min. After incubation, absorbance was measured at 230 nm for each test sample. Ascorbic acid was used as a standard. The percentage of radical scavenging activity (RSA) was calculated using the equation.

$$\text{RSA} (\%) = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test sample})}{\text{Absorbance of Control}} \times 100$$

RESULTS

The present study reports the phytochemical screening, FTIR analysis, antimicrobial, and antioxidant activities of different extracts of roots and leaves of *L. aspera*.

Phytochemical screening

Phytochemical screening of the *L. aspera* suggests the presence of major phytochemicals in the root and leaf extracts (Tables 1 and 2). ME and HE of roots and leaves showed the presence of carbohydrates, cholesterol, proteins, amino acids, steroids, saponins, flavonoids, alkaloids, tannins, and phlobatannins, whereas, AEs of both root and

Table 1: Phytochemical screening in leaf extracts of *L. aspera*

Phytochemical	AE	ME	HE
Carbohydrates	+	+	+
Cholesterol	-	+	+
Steroids	-	+	+
Proteins	+	+	+
Amino acids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phlobatannins	+	+	+

+Indicates the presence; -indicates the absence. *L. aspera*: *Lucas aspera*, AE: Aqueous extract, ME: Methanol extract, HE: Hexane extract

Table 2: Phytochemical screening in root extracts of *L. aspera*

Phytochemical	AE	ME	HE
Carbohydrates	+	+	+
Cholesterol	-	+	+
Steroids	-	+	+
Proteins	+	+	+
Amino acids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phlobatannins	+	+	+

+Indicates the presence; -indicates the absence. *L. aspera*: *Lucas aspera*, AE: Aqueous extract, ME: Methanol extract, HE: Hexane extract

leaf also showed the presence of all phytochemicals except cholesterol and steroids (Tables 1 and 2).

FTIR analysis of the plant sample

FTIR analysis of leaf and root extracts of *L. aspera* was carried out to reveal the functional groups present in the leaves and roots. FTIR spectrum of different extracts leaves of the *L. aspera* (Fig. 1a-c) showed the major peaks at 3334.04, 2924.18, 1618.19, 1322.72, 1251.81, 1029.36, 476.53, and 433.23 cm^{-1} . The broad peak at 3334.04 cm^{-1} is responsible for O-H stretching of polyphenolic compounds such as flavonoids, nonflavonoids, and triterpenoids. The broad peak at 2924.18 could be due to C=O stretching of proteins. The broad peak at 1618.19 cm^{-1} can be corresponding to the amide II group of proteins. The broad peak at 1023.33 cm^{-1} is also corresponding to O-H group of polyphenolic compounds including flavonoids, terpenoids, and nonflavonoids. The remaining other peaks are very narrow which revealed the presence of other compounds such as anhydrides, esters, and carboxylic acids in low amounts.

FTIR spectrum of different extracts of roots of the *L. aspera* (Fig. 2a-c) showed the major peaks at 3403.33, 2926.36, 2137.34, 1604.93, 1415.14, 1263.00, 1032.58, and 533.09 cm^{-1} . The broad peak at 3403.33 cm^{-1} is responsible for O-H stretching of polyhydroxy compounds such as flavonoids, nonflavonoids, and triterpenoids. The broad peak at 2926.36 is responsible for C=O stretching of proteins. The broad peak at 1604.93 cm^{-1} can be corresponding to the amide II group of proteins. The broad peak at 1032.58 cm^{-1} is also corresponding to O-H group of polyphenolic compounds including flavonoids, terpenoids, and nonflavonoids.

Antibacterial activity

Antibacterial activity of the AE, ME, and HE of roots and leaves of the *L. aspera* was evaluated, and zone of inhibition values of all the extracts was presented in Table 3. All the extracts of roots and leaves showed dose-dependent inhibitory activity against all the tested bacteria. All the extracts showed maximum zone of inhibition at the

highest concentration of 100 $\mu\text{g/ml}$ tested. All the prepared extracts of roots and leaves of *L. aspera* displayed the highest inhibitory activity against Gram-positive bacteria compared to Gram-negative bacteria. ME of leaves formed the maximum inhibition zone of 11.4 ± 0.3 mm against *B. subtilis* while HE and AE of leaves showed only 8.1 ± 0.27 and 7.4 ± 0.29 mm, respectively, against the same bacteria. ME of leaves displayed the maximum inhibition zone of 12.8 ± 0.31 mm against *S. aureus* while HE and AE showed only 6.9 ± 0.26 and 7.3 ± 0.25 mm, respectively, against the same bacteria. Similarly, ME of leaves exhibited maximum inhibition zone of 9.8 ± 0.21 mm against *E. coli* while HE and AE of leaves showed only 6.8 ± 0.24 and 6.6 ± 0.22 mm, respectively, against the same bacteria. ME of leaves exhibited maximum inhibition zone of 7.3 ± 0.29 mm against *P. aeruginosa* while HE and AE of leaves showed only 5.8 ± 0.27 and 6.0 ± 0.17 mm, respectively, against the same bacteria.

AE, ME, and HE of roots of *L. aspera* showed the highest inhibitory activity against Gram-positive bacteria compared to Gram-negative bacteria. All the extracts of roots showed the highest antibacterial activity against *S. aureus*, followed by *B. subtilis*, *E. coli*, and *P. aeruginosa*. ME of roots exhibited maximum inhibition zone of 5.6 ± 0.34 mm against *P. aeruginosa* while HE and AE of roots showed only 5.3 ± 0.24 and 4.1 ± 0.23 mm, respectively, against the same bacteria. However, standard drug streptomycin showed 14.2 mm, 15.4 mm, 12.3 mm, and 13.1 mm zone of inhibition against to *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively.

Antifungal activity

Antifungal activity of the AE, ME, and HE of roots and leaves of the *L. aspera* was evaluated against *C. albicans* and *A. niger*; results are represented in Table 4. All the extracts of leaves and roots showed concentration-dependent inhibitory activity against the fungal species. All the extracts of roots and leaves of *L. aspera* showed effective antifungal activity against *A. niger* compared with *C. albicans*. ME of the leaves showed highest antifungal activity compared with HE and AE of leaves. ME of leaves formed the inhibition zones of 10.3 ± 0.27 and

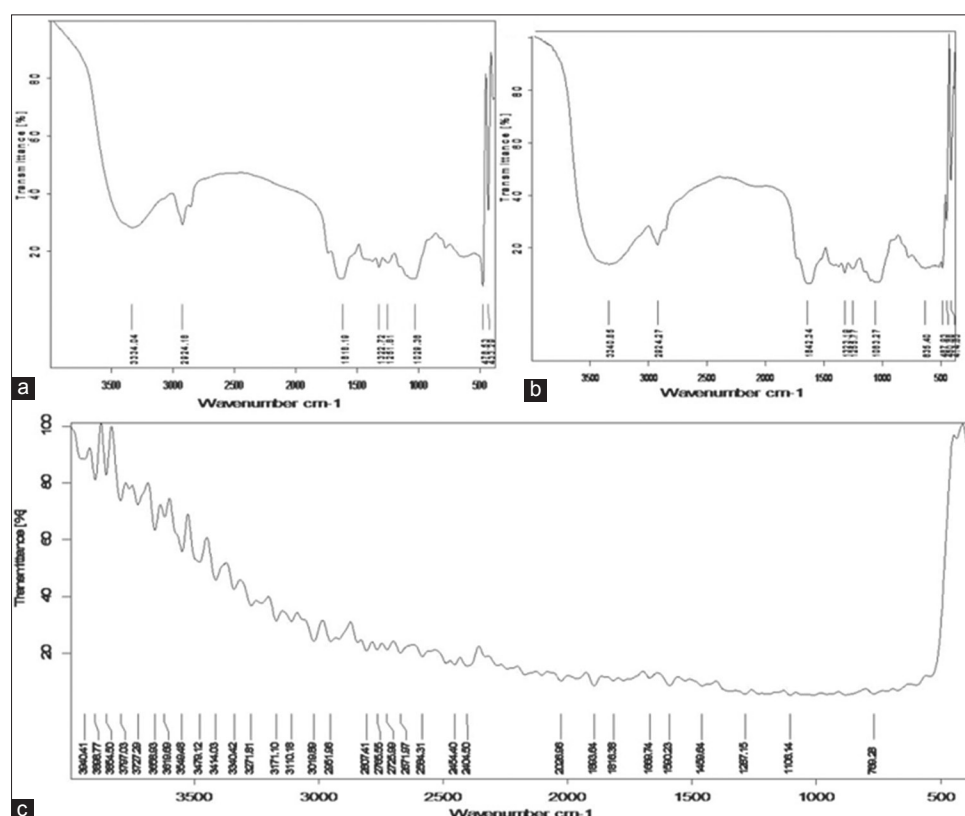


Fig. 1: Fourier transform infrared spectrum of (a) aqueous, (b) hexane, and (c) methanolic extracts of leaves of *Lucas aspera*

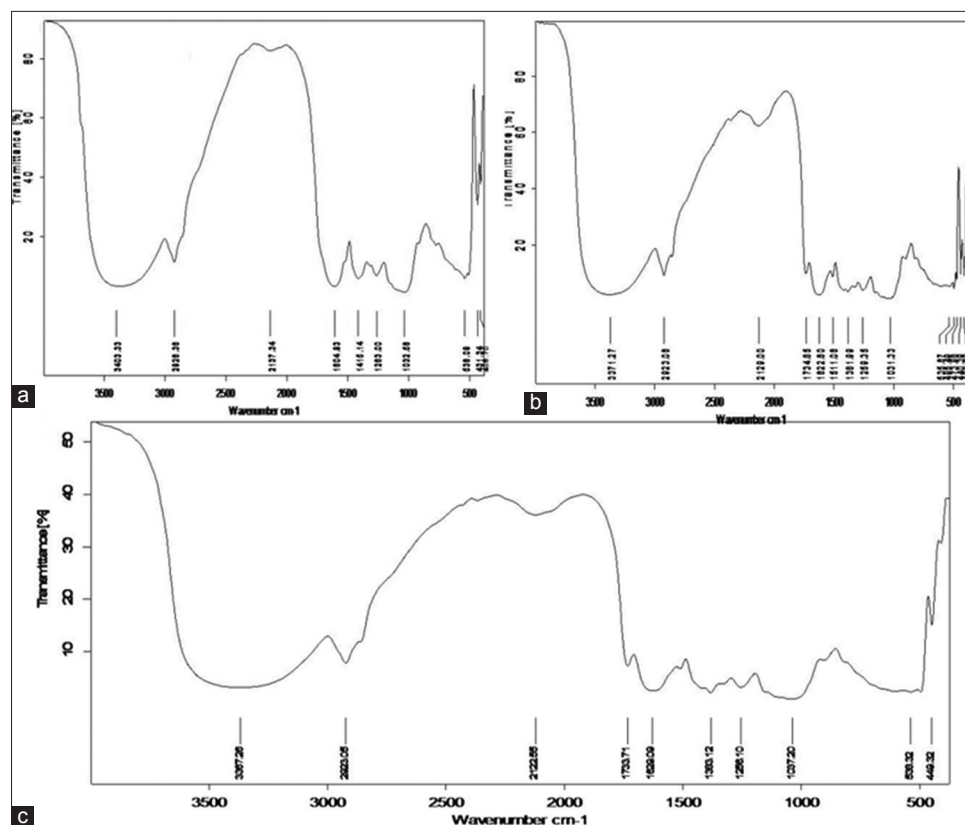


Fig. 2: Fourier transform infrared spectrum of (a) aqueous, (b) hexane, and (c) methanolic extracts of roots of *Lucas aspera*

Table 3: Antibacterial activity of leaf and root extracts of *L. aspera*

Bacterial strains	Conc. (µg/ml)	Leaf			Root			Strp
		AE	ME	HE	AE	ME	HE	
<i>B. subtilis</i>	25	-	2.2±0.12	1.6±0.14	-	1.3±0.18	-	14.2
	50	3.1±0.18	6.3±0.23	4.3±0.21	1.8±0.22	3.3±0.21	2.3±0.19	
	75	5.2±0.27	9.1±0.31	6.8±0.19	3.6±0.18	5.1±0.27	4.1±0.22	
	100	7.4±0.29	11.4±0.3	8.1±0.27	5.1±0.29	6.3±0.22	5.8±0.22	
<i>S. aureus</i>	25	-	3.0±0.21	1.7±0.23	-	1.1±0.2	-	15.4
	50	2.8±0.3	7.1±0.32	4.1±0.18	2.2±0.27	3.2±0.31	2.4±0.21	
	75	5.0±0.22	10.2±0.28	6.1±0.16	4.1±0.3	5.4±0.27	4.2±0.24	
	100	6.9±0.26	12.8±0.31	7.3±0.25	5.3±0.29	6.6±0.32	6.0±0.13	
<i>E. coli</i>	25	-	1.4±0.14	-	-	1.3±0.23	-	12.3
	50	2.1±0.25	5.1±0.22	3.0±0.19	1.4±0.2	2.8±0.2	1.6±0.21	
	75	5.1±0.19	7.3±0.18	5.3±0.17	3.1±0.18	4.6±0.24	4.0±0.24	
	100	6.6±0.22	9.8±0.21	6.8±0.24	4.7±0.25	5.8±0.21	5.6±0.19	
<i>P. aeruginosa</i>	25	-	-	-	-	1.1±0.2	-	13.1
	50	2.6±0.28	2.8±0.2	2.5±0.19	1.3±0.17	2.4±0.14	1.5±0.21	
	75	4.2±0.29	4.8±0.33	4.4±0.22	2.8±0.14	4.1±0.22	4.1±0.17	
	100	6.0±0.17	7.3±0.29	5.8±0.27	4.1±0.23	5.6±0.34	5.3±0.24	

Zone of inhibition (mm) values was represented. Values are mean±SD (n=3). AE: Aqueous extract; ME: Methanol extract; HE: Hexane extract; Str: Streptomycin, *B. subtilis*: *Bacillus subtilis*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*

11.2±0.19 mm, respectively, against *C. albicans* and *A. niger*. HE of leaves formed the inhibition zones of 7.1±0.27 and 7.4±0.3 mm, respectively, against *C. albicans* and *A. niger*. AE of leaves formed the inhibition zones of 6.2±0.21 and 6.7±0.31 mm, respectively, against *C. albicans* and *A. niger*. The standard drug nystatin 12.2 mm and 13.8 mm zone of inhibition against to *C. albicans* and *A. niger*, respectively.

Antioxidant activity

Antioxidant activity of different extracts of roots and leaves of *L. aspera* was evaluated by DPPH radical scavenging and H₂O₂ radical scavenging assays. All the extracts of leaves and roots showed RSA against DPPH and H₂O₂ free radicals (Figure 3a-d). Increase in the

concentration of extracts from 25 to 100 µg/ml showed increase in the scavenging activity. All the extracts showed maximum scavenging activity at the highest concentration (100 µg/ml) of the extract. ME of the leaves exhibited maximum scavenging activity of 38.39±1.25% and 36.85±1.11%, respectively, against DPPH and H₂O₂ radicals while MEs of roots exhibited maximum scavenging activity of 33.52±0.37% and 28.16±0.49%, respectively, against DPPH and H₂O₂ radicals. The concentrations of the extracts required to scavenge 50% of the radicals (IC₅₀ values) were determined based on the linear regression curves (R²=0.9). IC₅₀ values were represented in Table 5. The IC₅₀ concentrations of ME of the leaves against DPPH and H₂O₂ radicals were determined as 136.17 and 142.42 µg/ml, respectively. The IC₅₀

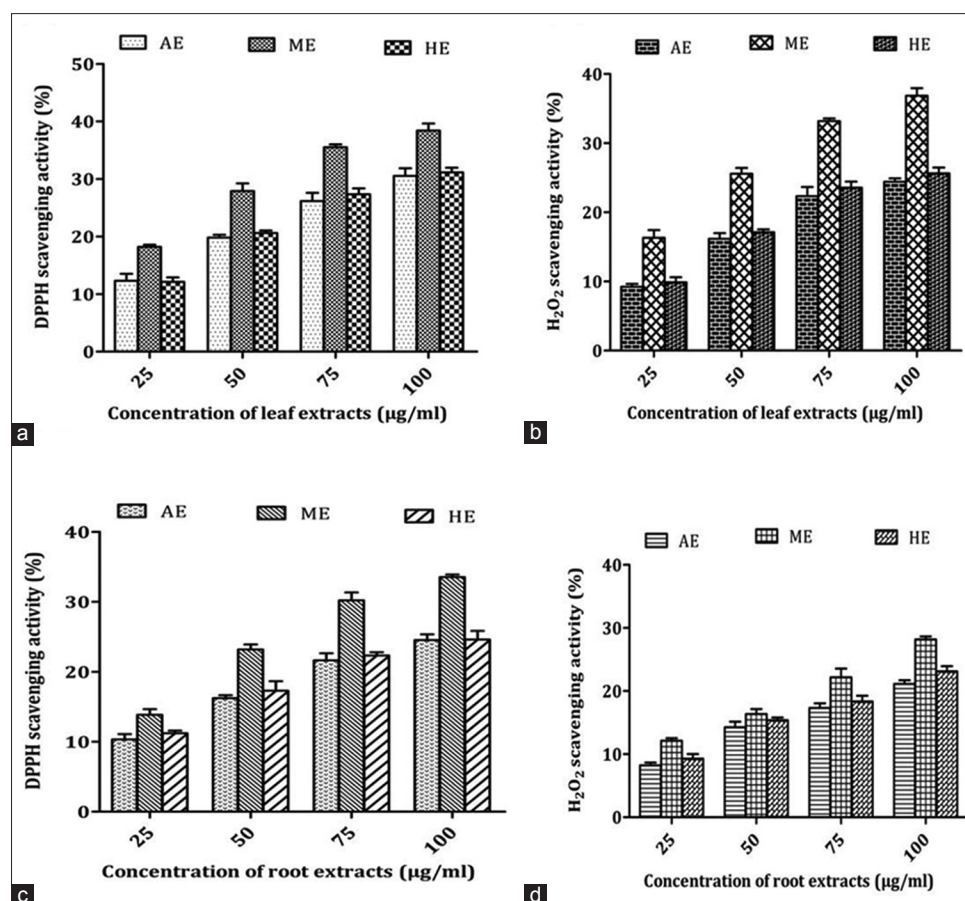


Fig. 3: Free radical scavenging activities of different extracts of leaves and roots of *L. aspera*. (a) DPPH radical scavenging activity (RSA) of leaf extracts, (b) H₂O₂ RSA of leaf extracts, (c) DPPH RSA of root extracts, (b) H₂O₂ RSA of root extracts

Table 4: Antifungal activity of leaf and root extracts of *L. aspera*

Fungal strains	Conc. (µg/ml)	Leaf			Root			Nyst
		AE	ME	HE	AE	ME	HE	
<i>C. albicans</i>	25	-	2.3±0.22	1.2±0.1	-	-	-	12.2
	50	1.8±0.17	5.7±0.31	3.1±0.22	1.3±0.1	2.1±0.24	1.7±0.15	
	75	4.3±0.22	8.1±0.19	5.7±0.29	3.8±0.24	4.6±0.31	3.9±0.2	
<i>A. niger</i>	100	6.2±0.21	10.3±0.27	7.1±0.27	5.0±0.21	6.2±0.22	5.1±0.18	13.8
	25	-	3.0±0.23	1.6±0.27	-	-	-	
	50	2.1±0.23	5.9±0.28	3.8±0.28	1.6±0.11	2.3±0.31	1.9±0.11	
	75	4.6±0.27	8.3±0.27	6.1±0.22	4.1±0.28	5.3±0.28	4.5±0.22	
	100	6.7±0.31	11.2±0.19	7.4±0.3	5.3±0.27	6.9±0.27	5.7±0.19	

Zone of inhibition (mm) values were represented. Values are mean±SD (n=3). AE: Aqueous extract, ME: Methanolic extract, HE: Hexane extract, Nyst: Nystatin, SD: Standard deviation, *C. albicans*: *Candida albicans*, *A. niger*: *Aspergillus niger*

Table 5: IC₅₀ concentrations (µg/ml) of different extracts of *L. aspera*

Antioxidant assays	IC ₅₀ concentrations of extracts (µg/ml)					
	Leaf			Root		
	AE	ME	HE	AE	ME	HE
DPPH RSA	177.09	136.17	169.17	228.35	156.53	235.72
H ₂ O ₂ RSA	217.88	142.42	207.38	270.65	202.75	251.89

AE: Aqueous extract; ME: Methanolic extract and HE: Hexane extract, IC₅₀: Half maximal inhibitory concentration, RSA: Radical scavenging activity

concentrations of HE of the leaves against DPPH and H₂O₂ radicals were determined as 169.17 and 207.38 µg/ml, respectively. The IC₅₀ values of AE of the leaves against DPPH and H₂O₂ radicals were determined as

177.09 and 217.88 µg/ml, respectively. From the RSA values and IC₅₀ concentrations, it is determined that MEs showed 1.2–1.5-fold more antioxidant activity compared to HE and AE. The IC₅₀ concentrations of ME of the roots against DPPH and H₂O₂ radicals were determined as 156.53 and 202.75 µg/ml, respectively. The IC₅₀ concentrations of ME of the roots against DPPH and H₂O₂ radicals were determined as 156.53 and 202.75 µg/ml, respectively. The IC₅₀ concentrations of HE of the roots against DPPH and H₂O₂ radicals were found to be 235.72 and 251.89 µg/ml, respectively. The IC₅₀ concentrations of AE of the roots against DPPH and H₂O₂ radicals were calculated as 228.35 and 270.65 µg/ml, respectively.

DISCUSSION

In the present study, the phytochemical screening and FTIR analysis of root and leaf extracts of *L. aspera* revealed that the presence of

carbohydrates, cholesterol, steroids, proteins, amino acids, saponins, flavonoids, alkaloids, terpenoids, tannins, and phlobatannins in ME and HE in AE except cholesterol and steroids remaining all were detected. In earlier studies, the presence of different phytochemicals was reported in *L. aspera* whole plant in different extracts. In that series Mangathayaru et al. [27] identified the alkaloids and nicotine in methanolic extract of *L. aspera* whole plant (without roots). The presence of alkaloids, glycosides, saponins, tannins, terpenoids, and flavonoids were reported in ethanol, hexane, and ethyl acetate extracts [28]. High amounts of glycosides, tannins, and flavonoids observed in ethanolic extract, while the other two extracts contain moderate amount of the chemical constituents [28]. In ethanol extract of the whole plant reported with all major phytochemicals [29]. In another study, except flavonoids and tannins, others phytochemicals were reported in the whole plant of *L. aspera* in MEs [30]. The presence of different phytochemicals in the same plant is due to solvents which were used to extract preparations and plant parts selected. The occurrence of these secondary metabolites suggests that the plant might be of medicinal importance. It was reported that the presence of phenols, tannins, and flavonoids exhibits different pharmacological activities such as antimicrobial [31,32], antioxidant [33,34], anti-inflammatory [35,36], and anticancer [33,37].

Antimicrobial activity of *L. aspera* leaf and root extracts observed in this study, antibacterial studies of *L. aspera* showed that leaf extracts exhibited effective activity compared with root extracts. Among the different extracts, MEs showed potential activity compared with HE and AE. Among the different bacteria used in this study, Gram-positive bacteria were more susceptible than the Gram-negative bacteria to *L. aspera* extracts. The results of the antibacterial activity are very effective and are in line with many earlier antimicrobial studies of plant extracts. Earlier ME of leaf showed potent antibacterial activity against to *E. coli*, *Klebsiella*, *Pseudomonas*, and *Staphylococcus aureus* [38]. Similarly, ME of root, leaf, stem, and flower showed 7.0–11.0 nm zone of inhibition against selected bacteria [39]. Among all parts, root showed high antibacterial activity against *E. coli*, *S. aureus*, *S. choleraesuis*, *S. typhimurium*, *S. flexneri*, and *P. aeruginosa* and leaf extract showed against to *S. aureus*, *S. choleraesuis*, *S. typhimurium*, and *S. flexneri* [39]. In another study, whole plant ethyl acetate and MEs of *L. aspera* showed moderate to high and concentration-dependent antibacterial activity against all the tested bacterial strains [30]. Similarly, whole plant ME and ethanol showed antibacterial activity against to *B. cereus*, *B. megaterium*, *B. subtilis*, *P. aeruginosa*, *S. paratyphi*, *S. typhi*, *S. dysenteriae*, *S. sonnei*, and *S. aureus* [40]. Leaf extract of *L. aspera* from methanol, ethyl acetate, and petroleum ether showed potential antibacterial activity at 1mg/disc in other study [41]. Many scientists tried to elucidate the plausible mechanism of action of the antimicrobial activity of plant extracts. Triterpenoids or saponins present in the extracts could form pore-like structures and cause the dissipation of membrane electrical potential or membrane proton motive force, and thus membrane destruction occurs [42].

MEs of roots and leaves of *L. aspera* showed highest inhibitory activity against selected fungi than HE and AE. The present results agreement with the earlier study, methanol, ethyl acetate, and petroleum ether leaf extract of *L. aspera* showed potential antifungal activity at 1 mg/disc against to *Aspergillus fumigates*, *Botryodiplodia theobromae*, *Colletotrichum corchori*, *Curvularia lunata*, and *Fusarium equiseti* but not to *C. albicans* and *Saccharomyces cerevisiae* [41]. Similar to this in another study, methanol and ethanol of whole plant extract showed potent antifungal activity against to *F. equiseti*, *B. theobromae*, and *C. corchori* [40]. FTIR analysis and phytochemical screening revealed that plant secondary metabolites present in the extracts can perturb the fungal cell wall and also caused the release of cellular components such as ions and intracellular proteins which, in turn, halt the growth of fungi. It had long been documented that plants metabolites saponins, tannins, and alkaloids are known for antimicrobial activity [43].

Antioxidants or radical scavengers are the compounds of bio or synthetic origin that protect the cells from various damaging effects

and diseases caused by reactive oxygen species. In addition, naturally occurring antioxidants can be formulated to give nutraceuticals, which can help to prevent oxidative damage from occurring in the body. Assays associated with lipid peroxidations is a common method for estimation of antioxidant activity; however, estimation of stable free radical DPPH is other method used in plants other natural sources [44,45]. In the present study, free radical scavenger levels of H₂O₂ and DPPH were observed in ME, AE, and HE leaf and root extract of *L. aspera*. The levels of free radical scavengers increased with dose-dependent in all extracts and ME of leaf showed high antioxidant activity compared with root extracts. Antioxidant or RSA of the different extracts is mainly due to the presence of hydrogen-donating molecules such as proteins, flavonoids, and terpenoids and other biomolecules are present in the extracts. Antioxidant activity of *L. aspera* with different extracts produced in this study is consistent with earlier reports [28,38,39,46]. Apart *L. aspera*, numerous plant components have proven to show antioxidants activity [28,47]. Previous reports emphasized that tannins and flavonoids are natural antioxidants that could play the main role in the antioxidant activity of the plant extracts. Besides, these alkaloids are natural antioxidants used for medicinal and commercial needs [48]. The presence of alkaloids, terpenoids, and flavonoids could be responsible for the antioxidant activities of the *L. aspera* extracts.

CONCLUSION

All the extracts showed antibacterial and antifungal activity with maximum inhibition against to selected microorganisms. MEs of leaf exhibited maximum antibacterial, antifungal, and highest RSA. Phytochemical screening and FTIR analysis revealed that alkaloids, triterpenoids, and flavonoids could be responsible for the antimicrobial and antioxidant activities, respectively, of the *L. aspera* extracts. From the present results, it is concluded that compounds of *L. aspera* can be used as antimicrobial agents and ingredients in the antioxidant formulations in different food and pharmaceutical fields.

ACKNOWLEDGMENTS

The authors are thankful to the Head, Department of Biotechnology, Tirupati, for providing the laboratory facilities. Authors are also thankful to DST-CURIE, Central Instrumentation Facility, Sri Padmavati Mahila Visvavidyalayam (Women's University) Tirupati, for providing permission to utilize FTIR for analysis of plant material.

AUTHORS' CONTRIBUTIONS

BK conceived the idea, participated in designing, and supervised the work. KV and MB conducted the experiments; AD monitored the work progress. BK, KV and DA drafted the manuscript for publication. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The author(s) declare that they have no conflicts of interest with respect to the research work, authorship, and publication of this article.

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