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ORIGINAL ARTICLE

Phytochemical Screening and Bioactive Potential of Pod Seed Extracts of *Leucaena leucocephala* Linn

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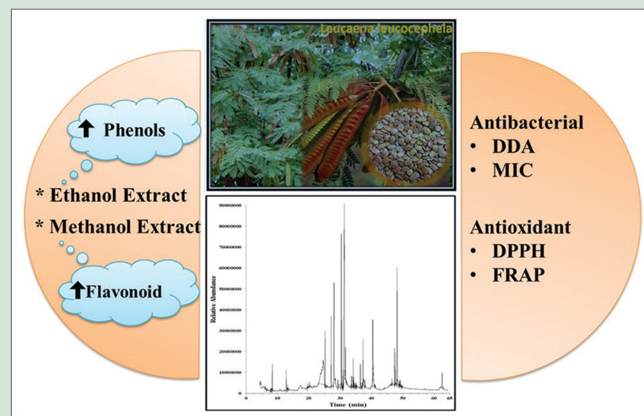
ABSTRACT

Background: Free radicals generated during injury lead to the development of various diseases such as diabetes, myocardial infarction, cerebrovascular disease, and cancer. Antioxidants present in plants can prevent the deleterious effect of these free radicals. Among various plants, *Leucaena leucocephala* is a mimosoid, fast-growing, nitrogen-fixing small tree having pods with various medicinal properties. **Objective:** Hence, the present study was designed to determine the bioactive potential of ethanolic and methanolic extracts of *L. leucocephala* pod seeds. **Materials and Methods:** We have assessed the antioxidant and antibacterial activities of the extract. In addition, the presence of various metabolites and other compounds was also evaluated though gas chromatography-mass spectrometry (GC-MS) analysis. **Results:** The results indicated that the methanol extract had relatively higher antibacterial and antioxidant properties than ethanol extract. Furthermore, GC-MS data revealed the presence of various active constituents in the methanolic extract. **Conclusion:** Thus, the bioactive potential of various compounds present in methanol extracts of plant parts could be responsible for its antibacterial and antioxidant properties.

Key words: Antibacterial, antioxidant, gas chromatography-mass spectrometry, *Leucaena leucocephala*, phytochemicals, plant extracts

SUMMARY

- This study investigated the seeds of commonly available North Indian shrub *Leucaena leucocephala*. In this study, the flavonoid and polyphenol contents were measured following standard protocol in the methanol and ethanol extracts of *L. leucocephala*. The antimicrobial potential was observed, and the minimal inhibitory concentrations of methanol and ethanol extracts were measured on seven pathogenic bacteria (both Gram positive and Gram negative). Compound profiling through gas chromatography-mass spectrometry confirmed the presence of various components in pod seed of *L. leucocephala,* which confirms their antibacterial and antioxidant potential. Presence of various metabolites and chemical compounds in the methanol extract of *L. leucocephala* showed that it may be further used as an antidiabetic, anti-inflammatory, and immunomodulatory agent, which needs further investigations.



Abbreviations Used: *L. leucocephala: Leucaena leucocephala*; GC-MS: Gas chromatography-mass spectrometry; RT: Room temperature; DDA: Disc diffusion assay; DMSO: Dimethyl sulfoxide; MIC: Minimum inhibitory concentration; LB: Luria–Bertani; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing ability of plasma

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DOI: 10.4103/pr.pr_49_20

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INTRODUCTION

Plants play an essential role in human life by providing dietary benefits as well as various medicines. For many centuries, different plant parts such as shrubs, herbs, and roots have been incorporated in our daily life because of their bioactive and pharmaceutical properties.[1] Recent advances in medicine focus on the use of plants to purify important components to design drugs with specific metabolic intermediates. More than 50,000 plant species are believed to be used for medicinal purposes worldwide.[2] Various Indian medicinal plants have been used from old times to treat different diseases because of their beneficial properties.[3-5] Volatile oils, secondary metabolites, polypeptides, polysaccharides, and other natural plant products are used because of their anticancerous, antidiabetic, anti-inflammatory, antibacterial, antimicrobial, antifungal, antioxidant, as well as wound-healing properties.[6-9] Various studies have reported that extracts from plants exhibit antibacterial activity against various Gram-positive and Gram-negative bacteria.[10-12] Many of these extracts have equivalent or better antibacterial activity to that of standard antibiotics.

Secondary metabolites including tannins, terpenes, polyphenols, glycosides, flavonoids, alkaloids, and few other pigments present in plants provide protection from diseases and stressful environment and help in maintaining health status.[13] These active constituents of plants help to improve the digestive, nervous, respiratory, excretory, circulatory, and immune systems of humans as well as other animals.[14,15] The

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Cite this article as: Prakash O, Malik S, Rani KV, Verma VK. Phytochemical screening and bioactive potential of pod seed extracts of *Leucaena leucocephala* linn. Phcog Res 2020;12:361-7.

Submitted: 05-Jun-2020 **Revised:** 15-Jul-2020
Accepted: 24-Aug-2020 **Published:** 23-Jan-2021

amount of these active constituents changes in different plant parts at different conditions.[16-19] Hence, it is important to discover plants which are rich source of these active components and can be used for further research processes.

Leucaena leucocephala is a fast-growing leguminous tree (lead tree), belonging to family Fabaceae (Leguminosae). It is grown for a variety of uses, such as green manure, livestock fodder, and soil conservation. This tree is native to southern tropical America but now present in Africa, Asia, Australia, southern USA, southern Europe, and many oceanic islands with warm climate. In India, *L. leucocephala* is found throughout the country and many regional people from eastern and northeastern states use this for medicinal purposes indicating its ethno-pharmacological importance. *L. leucocephala* is also used as fodder for cattle since long. Studies suggested that *L. leucocephala* has antidiabetic and antinematicidal potential.[20,21] The use of the plant parts has been increased from methane production to quality food for different animals.[22] Seeds of *L. leucocephala* have high protein content (24.5%–46%), various essential amino acids, and β-carotene.[23,24] Hence, this is an excellent source of quality protein animal feed.

A study by Benjakul *et al*. (2013) explored the antioxidant potential of water extract of pod seed of *L. leucocephala* by oxygen radical absorbance capacity as well as by estimating hydroxyl radical, singlet oxygen, hydrogen peroxide, and hypochlorous acid scavenging activities as well as through β-carotene-linoleic acid system.[25] Literature suggests that seeds of *L. leucocephala* contain galactomannan and its lectin derivative that constitutes a glycoside, which is a known antidiabetic agent.[26,27]

Although *L. leucocephala* is used for various purposes since traditional times, very few studies have investigated its active constituents and their biological activities. Hence, in the present study, we have selected *L. leucocephala* pod seed for evaluating the presence of active constituents and determined their antioxidant and antibacterial potential.

MATERIALS AND METHODS

Chemicals

Ampicillin and chloramphenicol discs were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, Maharastra, India. All other chemicals were purchased from Sigma Aldrich, Saint Louis, Missouri, USA, and were of analytical grade.

Plant material and extraction

Pod seeds of *L. leucocephala* were collected from the campus of Sri Venkateswara College, University of Delhi (New Delhi, India). The collected pod seeds were then thoroughly washed with water and dried in shade at room temperature (RT). The dried pod seeds were crushed and passed through 1-mm sieve. Ten grams of the sieved powder was dissolved in 100 mL of methanol and ethanol and stirred overnight using a magnetic stirrer (200 rpm) at RT. After that, it was filtered through Whatman® no. 41 filter paper and the filtrates were dried with a rotary evaporator (Labconco Digital rotary evaporator, Cole-Parmer India Pvt. Ltd., Mumbai India) at 40°C and stored at – 20°C until use.

Antibacterial assay

Culture of bacteria

Bacterial isolates were inoculated in 250-mL conical flasks containing 50-mL Luria-Bertani (LB) culture media (pH 7.4) and 1% or 2% NaCl concentration for freshwater bacteria (*Aeromonas hydrophila* [MTCC 1739], *Escherichia coli* [MTCC 1575], *Enterococcus faecalis* [MTCC 2729], *Pseudomonas aeruginosa* [MTCC 1034], and *Staphylococcus aureus* [MTCC 3160]) and marine water bacteria (*Vibrio anguillarum* [kind gift from Debra L. Milton, Professor, Department of Molecular Biology, Umea University, Umea, Sweden] and *Vibrio harveyi* [MTCC 7954]) respectively. The inoculated bacterial flasks were allowed to grow overnight at 37°C under gentle orbital shaking conditions.

Measurement of antibacterial activity

The antibacterial activity of pod seed extracts was determined by disc diffusion assay (DDA) against selected bacterial strains. The bacteria were seeded with a standard inoculum of 1×10^8 cells in sterilized LB agar plates (1.5%) prepared with 1% or 2% NaCl (for freshwater and marine water bacteria) and placed on agar plates. Sterile circular paper discs (thickness 1 mm; diameter 6 mm) were impregnated with 40-μL plant extract prepared at two different concentrations (200 and 100 μg/disc) in 0.2% dimethyl sulfoxide (DMSO). For negative and positive controls, 0.2% DMSO and antibiotics (ampicillin [10 μg/disc] and chloramphenicol [30 μg/disc]) were used, respectively.

Minimum inhibitory concentration (MIC) was performed in a 96-well U-shaped microtest plate. The final concentration in wells was adjusted to 250–0.244 mg/mL in a sequel of double dilution. The 1×10^6 cells of respective bacterial inoculum were added to each well. The concentration of both the extracts was the final concentration in the solution including bacterial inoculum. The LB broth was taken as negative control and DMSO was taken as positive control for each bacterium. The plates were incubated at 37°C in a plate orbital shaker for 24 h. The absorbance of plates was taken at 600 nm using a microtest plate reader. The MIC was confirmed after spreading of 20 μL broth onto LB agar plate and incubated overnight at 37°C.

Antioxidant assay

2,2-diphenyl-1-picrylhydrazyl assay

The antioxidant property of methanol and ethanol extracts was determined by the method of Brand-Williams *et al*. (1995) which was modified by Miliauskas *et al*. (2004).[28,29] For this, 10 μL of freshly prepared respective extract (0.5 mg/mL) was added to 300 μL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (6×10^{-5} M in methanol) in a 96-well microtiter plate and incubated at 37°C for 20 min. The absorbance was recorded at 515 nm. The methanol and ethanol solutions were used as respective controls. The free radical scavenging property of the plant extract was calculated as percentage inhibition using the standard formula: $([A_B - A_S]/A_B]) \times 100$, where A_B is the absorbance of blank and *As* is the absorbance of sample.[30,31] Serial double dilution of ascorbic acid, butylated hydroxy toluene (BHT), gallic acid, and quercetin was used as a positive standard (20–0.078 mg/mL). The samples were processed in quadruplicates in this assay.

Ferric-reducing ability of plasma assay

A volume of 30 μL of distilled water and 300 μL of fresh ferric-reducing ability of plasma (FRAP) solution (containing 10 parts of 300 mM acetate buffer [pH 3.6], 1 part of 10 mM [2,4,6-tripyridyl triazine] in 40 mM HCl, and 1 part of 20 mM ferric chloride) was added to the 10 μL of respective pod seed extract solution (0.5 mg/mL). The samples were then incubated at 37°C for 30 min. A standard curve was prepared by serial double dilution of ferrous sulfate (20.0–0.009 mg/mL) as substrate. Similar to DPPH assay, serial double dilution of ascorbic acid, BHT, gallic acid, and quercetin (20–0.078 mg/mL) was used as the positive control. For control, acetate buffer was used in the place of sample. The absorbance was recorded at 593 nm, and the reducing activity of extract was expressed in millimoles of Fe^{2+}/mg of the plant extract.

Estimation of total phenolic content

Phenolic content in methanol and ethanol extracts was determined according to the method by Djeridane *et al*. (2006).[32] One milliliter of

the extract (2 mg/mL) was dissolved in 0.5-mL Folin–Ciocalteu's phenol reagent and to this, 1.5 mL-distilled water was added. After 1 min, 20% sodium carbonate solution (1.25 mL) was added. The mixture was incubated for 2 h in dark at 25°C with intermittent shaking and the absorbance was recorded at 760 nm. A standard curve was obtained using serial double dilutions of gallic acid (20–0.5 μg/mL) as standard.[31] The total phenolic content was represented as the microgram of gallic acid equivalent present per milligram of the extract.

Determination of total flavonoids

Flavonoid content of both the extracts of pod seeds was measured with Dowd method as modified by Arvouet-Grand *et al*. (1994).[33] For this, 1 mL of the plant extract (10 mg/mL) was mixed with 1 mL of 2% aluminum tri-chloride solution (prepared in methanol). After 10 min incubation at RT, the absorbance was recorded at 415 nm. The methanol/ethanol solution was used as control (carrier blanks).[31] Concentration of flavonoids in the extracts was calculated using serial double dilution of quercetin (8.33–0.032 mg/mL) as standard and expressed as microgram of quercetin equivalent flavonoids present per milligram of the extract.

Gas chromatography-mass spectrophotometry analysis

Preparation of samples

Methanolic extract of the pod seeds was dissolved in 1-mL high-performance liquid chromatography-grade methanol and then filtered through a 0.22-μm syringe filter. A volume of 1 μl of the sample was injected by an automatic syringe injector into the apparatus for gas chromatography-mass spectrometry (GC-MS) analysis. GC-MS analysis was conducted at the Advanced Instrumentation Research Facility, JNU, New Delhi.

Gas chromatography-mass spectrometry chromatographic conditions

GC-MS analysis was conducted on a thermal desorption TD-20 system, GCMSQP-2010 Plus (Shimadzu, Nakagyo-ku, Kyoto Japan). The gas chromatograph was interfaced to a mass spectrometer instrument employed with RTx-5MS column (30 m × 0.25 mm × 0.25 μm) operating in an electron impact mode at 70 eV. Helium gas (99.99%) was used as the carrier gas in the instrument with a constant flow rate of 1.2 mL/min. The column's initial oven temperature was 80°C (isothermal for 4 min) with a gradual increase of 5°C/min to 310°C, the flow rate was 1.21 mL/min, and the column pressure was 81.7 kPa. A mass spectrum was prepared at a scan interval of 0.50 s with a mass scan from 40 to 650 *m/z*.

Compound identification

NIST/NIH/EPA Mass Spectral Database (National Institute of Standards and Technology) MS program v. 2.0d with NIST05 and WILEY08 libraries were used for GC-MS data interpretation. The spectrum of unknown components was determined with NIST spectrum and Wiley libraries as per their retention time. The names, chemical formulas, molecular mass, and structure of components of the identified compounds were also ascertained. With the help of Dr. Duke's phytochemical and ethano-botanical databases, NCBI-Pubchem, ChemSpider from the Royal Society of Chemistry and various literatures, biological and chemical activities of the identified compounds were determined.

Statistical analysis

The statistical and numerical values were presented in mean ± standard error of the mean. Student's *t*-test and ANOVA test were used for analysis of the data from experiments; the data of significance were analyzed using Sigma Plot 12.0 software San Jose, USA. $P < 0.05$ was considered statistically significant.

RESULTS

Antimicrobial activity

The antibacterial activity of crude methanol and ethanol extracts of *L. leucocephala* pod seeds was assessed by DDA on agar plate. Table 1 represents the zone of inhibition of both extracts against Gram-positive bacteria such as *E. faecalis* and *S. aureus* as well as Gram-negative bacteria such as *A. hydrophila*, *E. coli*, *P. aeruginosa*, *V. anguillarum,* and *V. harveyi*. The methanol extract at both the concentrations (200 and 100 μg/disc) exhibited statistically significant ($P < 0.05$) antibacterial activity against five bacteria as compared to the respective ethanol extract. At 200 μg/disc concentration, the methanol extract showed maximum inhibition against *E. faecalis* (23.50 ± 0.33), whereas at 100 μg/disc, it showed the maximum activity against *V. anguillarum* (21.25 ± 0.17). On the other hand, the ethanol extract showed maximum activity against *V. harveyi* at both concentrations [Table 1]. However, all the extracts showed antibacterial activity against these human pathogenic bacteria.

Furthermore, MIC of both the extracts was also determined. The methanol extract of pod seeds showed MIC against all the tested bacterial strains in the range of 1.0–15.6 mg/mL, while the ethanol extract showed at 1.9–31.2 mg/mL concentration. Both the extracts showed maximum inhibitory activity against *V. anguillarum* and least activity against *E. coli* [Table 2].

Antioxidant activity

The antioxidant potential of the plant extracts was calculated through DPPH and FRAP assays. DPPH assay was used to measure the radical scavenging property of the extract and FRAP assay assessed the reducing ability of the extracts. In the present study, the methanol extract showed statistically significantly ($P < 0.05$) higher antioxidant activity in comparison to the ethanol extract but less than the standard antioxidants [Table 3].

Table 1: Antibacterial activity of methanol and ethanol extracts of *Leucaena leucocephala* pod seed by disc diffusion assay

Bacterial strain (Gram +/–)	Methanol extract (200 μg/disc)	Ethanol extract (200 μg/disc)	Methanol extract (100 μg/disc)	Ethanol extract (100 μg/disc)	Ampicillin	Chloramphenicol	Control/solvent
Aeromonas hydrophila (–)	15.30±0.08*	13.25±0.20	12.60±0.25*	10.10±0.20	6	9	6
Escherichia coli (–)	11.20±0.17*	10.33±0.05	10.60±0.05*	8.25±0.67	14	22	6
Enterococcus faecalis (+)	23.50±0.33*	15.33±0.67	16.50±0.17*	12.75±0.33	6	27	6
Pseudomonas aeruginosa (–)	19.33±0.10*	16.50±0.50	13.33±0.40*	11.33±0.15	22	27	6
Staphylococcus aureus (+)	16.67±0.15	19.10±0.44*	15.80±0.18	17.33±0.67*	6	28	6
Vibrio anguillarum (–)	22.35±0.67*	20.10±0.14	21.25±0.17*	18.33±0.026	11	24	6
Vibrio harveyi (–)	17.75±0.89	21.50±0.10*	15.25±0.67	19.25±0.56*	7	26	6

Values are represented as mean±SEM. *Statistically significant values between extracts. SEM: Standard error of mean

Total phenolic and flavonoid contents

Figure 1 shows the presence of phenolic and flavonoid contents in the plant extracts. The results showed that methanol extract had statistically significantly higher phenolic content than ethanol extract ($P < 0.05$) [Figure 1a]. However, the ethanol extract of *L. leucocephala* pod seed showed relatively higher flavonoid content than that of methanol extract [Figure 1b].

From the above results, we found that methanolic extract of pod seed had more phenolic contents and exhibited higher antioxidant and antibacterial activities. Thus, we conducted GC-MS analysis of methanolic extract to check the presence of responsible phytochemicals.

Gas chromatography-mass spectrometry analysis of methanol extract of *Leucaena leucocephala*

Chromatogram representing GC-MS analysis of methanol extract of pod seed of *L. leucocephala* is depicted in Figure 2. The chromatogram showed 58 total peaks, indicating the presence of various compounds in the methanol extract. On mass spectrometry analysis using NIST library tool, individual phytocomponents of the methanol extract have been characterized and identified [Table 4 and Figure 3]. The peaks of myo-inositol (17.25%), palmitic acid (10.9%), linoleic acid methyl ester (5.76%), linoleic acid (28.73%), ethyl linoleate (4.34%), and β-sitosterol (4.64%) were observed, which constitute the major proportion of the methanol extract.

Table 2: Minimum inhibitory concentration of methanol and ethanol extracts of *Leucaena leucocephala* pod seed

Bacterial strain	*Leucaena leucocephala* pod seed (mg/ml)	
	Methanol extract	Ethanol extract
Aeromonas hydrophila (−)	7.8*	15.6
Escherichia coli (−)	15.6*	31.2
Enterococcus faecalis (+)	3.9*	7.8
Pseudomonas aeruginosa (−)	7.8	7.8
Staphylococcus aureus (+)	7.8	3.9*
Vibrio anguillarum (−)	1.0*	1.9
Vibrio harveyi (−)	3.9*	7.8

Values are represented as mean±SEM. *Statistically significant values between extracts. SEM: Standard error of mean

Table 3: 2,2-diphenyl-1-picrylhydrazyl and ferric-reducing ability of plasma assay in methanol and ethanol extracts of *Leucaena leucocephala* pod seed

	DPPH assay (% scavenging activity)	FRAP assay (mM Fe^{2+}/mg extract)
Methanol extract	35.32±0.70*	14.71±4.46
Ethanol extract	19.40±0.32	115.60±2.57*;
Ascorbic acid	92.65±1.01	9070.51±211.54
BHT	54.61±0.59	8057.68±121.80
Gallic acid	85.80±3.76	18608.97±250.00
Quercetin	82.90±0.27	15070.51±929.49

Values are represented as mean±SEM. *Statistically significant values between extracts. DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric-reducing ability of plasma; BHT: Butylated hydroxy toluene; SEM: Standard error of mean

DISCUSSION

Before the use of modern medicines, the most prevalent method to treat or cure illness/disease was through the existing plants. This study subjects to the plant *L. leucocephala*, popularly known as kubabul in India. A previous study has evaluated the antioxidant activity and estimated the total flavonoid and phenolic contents of various extracts of *L. leucocephala* leaves.[34] It has been found that seeds of this plant have higher protein value compared to the leaves of the plant itself and thus the plant's seed can be useful for humans as a medicinal component. In this study, we evaluated the total flavonoid and phenolic contents along with the antibacterial and antioxidant potential of methanol and ethanol extracts of *L. leucocephala* pod seeds. We found that the methanol extract had higher phenolic content and has shown good antioxidant and antibacterial activities. The methanol extract inhibited the growth of human pathogenic Gram-positive as well as Gram-negative bacteria, which was evaluated by DDA. In addition to this, the methanol extract showed better MIC against bacteria in comparison to the ethanol extract.

Measurement of the antioxidant property through various assays is conducted to evaluate the plant extract's ability to inhibit peroxidation, which in term represents their pharmacological effect.[35] The reactive oxygen species can damage the protein, DNA, and lipids, which leads to various diseases. The WHO has recommended the use of natural antioxidants that can delay or inhibit the lipids or other molecule's oxidation. The enhancement of the already-existing defense mechanism by various means such as enzymes, nutrients, and secondary dietary or other metabolites can neutralize the damaging effects of the freely available oxygen intermediates. Radical scavenging activities are mostly dependent on both the reactivity and concentration of the antioxidants, which can be assessed by DPPH and FRAP assays. The DPPH assay mainly focuses on the free radical scavenging ability of the compound and FRAP assay evaluates the reducing potential of the compound. In our study, we have evaluated the antioxidant potential of extracts with DPPH and FRAP assays and found that the methanol extract has higher antioxidant activity than the ethanol extract.

Phytoconstituents such as phenols and flavonoids have been reported to have multiple biological effects. Phenolic compounds contribute to

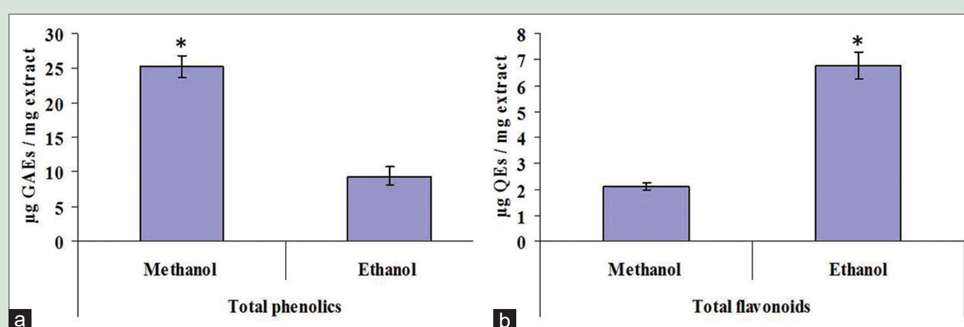

Figure 1: Total phenolic (a) and flavonoid (b) contents in the methanol and ethanol extracts of *Leucaena leucocephala* pod seed. (*) represent statistically significant values. GAEs: Gallic acid equivalent; QEs: Quercetin equivalent

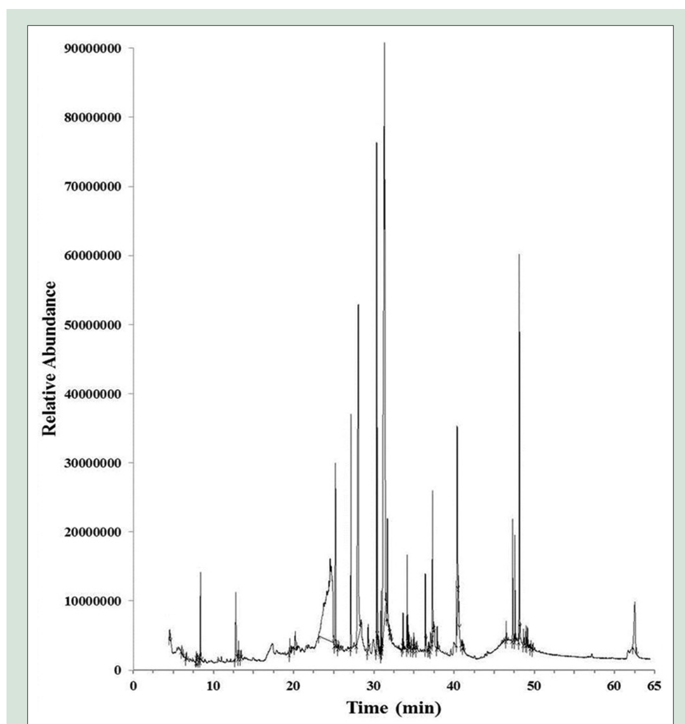

Figure 2: Gas chromatogram of methanol extract of *Leucaena leucocephala* (pod seed)

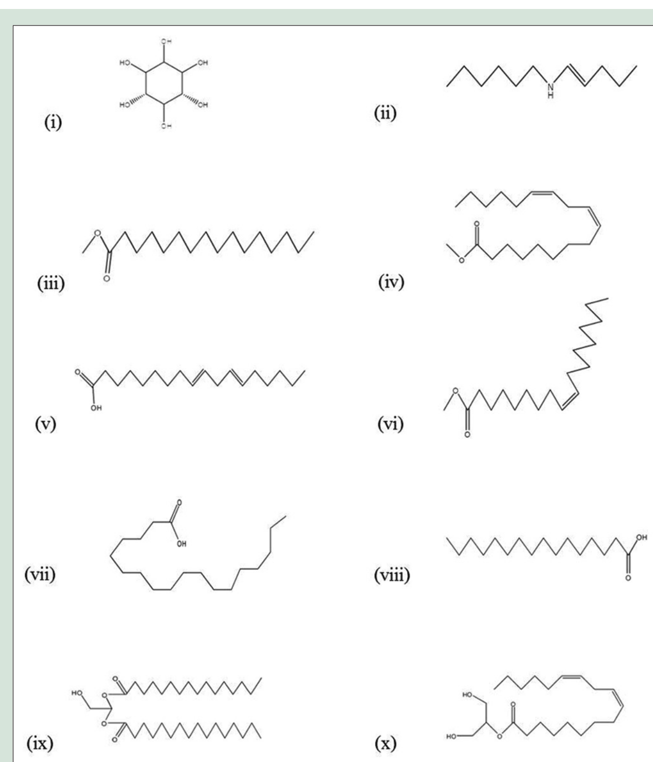

Figure 3: Chemical structure of major compounds in the methanol extract of *Leucaena leucocephala* (pod seed): (i) Mome inositol; (ii) N-(2-heptynyl)-n-hexylamine; (iii) palmitic acid methyl ester; (iv) linoleic acid methyl ester; (v) linoleic acid; (vi) methyl oleate; (vii) stearic acid; (viii) palmitic acid; (ix) dipalmitin; (x) β-monolinolein

quality and nutritional value in terms of modifying color, taste, aroma, and flavor along with health beneficial effects.[36] Recently, various studies have focused on the usefulness of phenols and flavonoids present in plant parts. These compounds exhibit numerous properties such as antioxidant, anticataract, antibacterial, cardioprotective, hepatoprotective, antiviral, and antifungal.[13] Phenolic compounds act as a radical scavenger due to the presence of hydroxyl group in their structure, are hydrogen donators, and can act as reducing agents.[37] Flavonoids also have hydroxyl group in their structure and thus act as natural antioxidants.[38] In this study, we determined the phenolic and flavonoid contents of methanol and ethanol extracts of pod seeds. We found that the methanol extract had more phenolic and moderate flavonoid contents, which could be responsible for its antioxidant activities.

The GC-MS of methanol extract showed the presence of alkaloids, flavonoids, various phenols, terpenoids, phytosterols, saturated and unsaturated fatty acids, and many more including the sugar-like inositol. Inositol is a major component present in the methanol extract of pod seed of *L. leucocephala* (17.24%). Inositol is a vitamin-like substance (pseudovitamin) but a natural sugar and acts as a good immunostimulant. Studies suggest its beneficial effects in polycystic ovarian disease and regulation of cholesterol levels.[39,40] Along with this, inositol has antioxidant, anti-inflammatory, and antidiabetic activities.[41-43] The antidiabetic activity of inositol is due to the stimulation of glucose uptake by the skeletal muscle. On the basis of this, earlier studies showed the antidiabetic potential of pod seed of *L. leucocephala*.[20]

Fatty acids such as palmitic acid, linoleic acid, and ethyl-linoleate present in the methanol extract also have anti-acne,[44] anti-arthritic, anti-inflammatory,[45,46] anti-atherosclerosis,[47] anticancer, hepatoprotective, anti-hypercholesterolemic, immunomodulatory, and wound-healing activities, as mentioned by Dr. Duke's phytochemical and ethano-botanical databases.

The other class of secondary metabolites present in methanol extract includes phytosterols, saponins, tannins, terpenoids including monoterpenes, sesquiterpenes, diterpenes, and triterpenoids, which have well-established antiviral, antibacterial, antioxidant, anticancer, anti-apoptotic, anti-inflammatory, anti-arthritic, and anti-asthma activities (Dr. Duke's phytochemical and ethano-botanical databases).

CONCLUSION

The results of the present study indicate that *L. leucocephala* pod seed has antioxidant and antibacterial potential when evaluated *in vitro*. The methanol extract has high antibacterial and antioxidant potential due to the presence of various beneficial phenolic, flavonoids, and other secondary metabolites evaluated through GC-MS analysis. The presence of various important metabolites showed that this can be used as a potential therapeutic candidate if further investigated. These findings can be further confirmed using animal studies.

Acknowledgements

The authors are thankful to the technical staff for conducting GC-MS analysis. The authors are also grateful to Mr. Deepak Sharma, Technician, Department of Pharmacology, AIIMS, New Delhi, for his tireless support.

Financial support and sponsorship

Nil.

Table 4: Chemical composition of methanol extract of pod seed of *Leucaena leucocephala* through gas chromatography-mass spectrophotometry

Peak number	Retention time	Area (%)	Chemical formula	Common name
2	4.481	0.27	$C_3H_8O_3$	1,2,3-Propanetriol
3	5.999	0.17	$C_6H_8O_3$	Furaneol
7	8.099	0.11	$C_5H_8O_3$	Levulinic acid
8	8.355	1.82	$C_6H_8O_4$	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one
9	12.774	1.34	C_8H_{16}	Propylcyclopentane
10	13.117	0.19	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
12	19.534	0.22	$C_{10}H_{12}O_3$	3',5'-Dimethoxyacetophenone
13	20.204	0.43	$C_{12}H_{14}O_4$	Diethyl phthalate
14	24.61	17.24	$C_7H_{14}O_6$	Mome inositol
15	25.257	3.24	$C_{11}H_{21}N$	Cyclohexylpiperidine
16	25.569	0.15	$C_{20}H_{29}FO_4$	6-β-Hydroxyfluoxymesterone
17	27.195	2.52	$C_{17}H_{34}O_2$	Palmitic acid methyl ester
18	28.113	10.9	$C_{16}H_{32}O_2$	Palmitic acid
19	29.347	0.46	$C_{12}H_{24}O$	Dodecanal
20	30.42	5.76	$C_{19}H_{34}O_2$	Linoleic acid methyl ester
21	30.516	2.26	$C_{19}H_{36}O_2$	Methyl oleate
23	30.735	0.07	$C_{20}H_{40}O$	Phytol
24	30.974	0.65	$C_{19}H_{38}O_2$	Methyl stearate
25	31.064	0.13	$C_{15}H_{26}O$	Viridiflorol
26	31.406	28.73	$C_{18}H_{32}O_2$	Linoleic acid
27	31.768	1.56	$C_{18}H_{36}O_2$	Stearic acid
28	32.075	0.18	$C_{16}H_{33}NO$	Palmitamide
31	34.236	0.98	C_9H_{19}	Dihydropinidine
32	34.326	0.31	$C_{18}H_{37}NO_2$	Hydroxyethylpalmitamide
33	34.455	0.26	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
34	34.723	0.23	$C_{18}H_{31}ClO$	Linoleoyl chloride
36	35.076	0.22	$C_{20}H_{40}O_2$	Arachidic acid
38	36.508	0.73	$C_{10}H_{21}N$	Benzedrex
41	37.185	0.38	$C_{14}H_{19}N$	p-Heptylbenzonitrile
42	37.416	1.99	$C_{21}H_{42}O_4$	Alpha-monostearin
44	38.005	0.29	$C_{24}H_{38}O_4$	Dioctyl phthalate
45	40.506	4.34	$C_{20}H_{36}O_2$	Ethyl linoleate
46	40.64	0.39	$C_{10}H_{16}O$	Limonene oxide
49	46.633	0.41	$C_{29}H_{50}O_2$	Vitamin E
50	47.464	1.45	$C_{28}H_{48}O$	Dihydrobrassicasterol
51	47.739	1.12	$C_{29}H_{48}O$	Stigmasta-5,22-dien-3-ol
52	48.288	4.64	$C_{29}H_{50}O$	β-Sitosterol
54	49.125	0.24	$C_{30}H_{50}O$	Lanosterol
58	62.754	2.24	$C_{22}H_{40}O_2$	Linoleic acid, butyl ester

Conflicts of interest

There are no conflicts of interest.

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