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Original Article

LCMS-Based Phytochemical Screening of *Albizzia lebbeck*

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ABSTRACT

Background and Objectives: Potential antidepressant properties are shown by the ethanolic extract of *Albizzia lebbeck* (L.), a member of the Fabaceae family. Triterpenes, glycosides, saponins, flavonoids, and indole alkaloids are among the many phytoconstituents that are abundant in the plant. Numerous pharmacological responses, including anti-inflammatory, antioxidant, antiepileptic, anticonvulsant, and antifungal, are highlighted in a review of the literature. A wide range of semi-polar components and important secondary metabolites are responsible for the effects that have been reported, these bioactive components might have therapeutic value in the treatment of depression. **Materials and Methods:** After being verified, the plant samples were extracted using ethanol and methanol, examined phytochemically, and then examined using LC-MS. The extracts' antioxidant activity was assessed by the use of DPPH, Assessments for scavenging superoxide anion and hydroxyl group activists. **Results:** Several Flavonoids, alkaloids, carbolic acid, steroids, tannic acid, glycosides, and saponins are examples of phytochemicals, were examined by the phytochemical Analysis in the ethanolic (AL) and ethanolic extracts. 58 and 81 chemicals were found in ALEN, ALMN, respectively, by LC-MS analysis. *Albizzia lebbeck* showed greater radical scavenging activity, as indicated by IC_{50} values, even though the data showed that both extracts had strong antioxidant qualities. **Conclusion**: According to these results, *Albizzia lebbeck* (L.) may contain bioactive substances and be a useful source of organic antioxidants for medicinal purposes. This study for future investigations to isolate and discover new chemicals that may have uses in medication development and therapy.

Keywords: *Albizia lebbeck*, Antidepressant, Depression, Ethanol, LC-MS, Phytochemical, etc.

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INTRODUCTION

According to estimations from the Creation Well-being Group, hopelessness is among the most shared psychological strength situations in the world (Kulwicka *et al*., 2024) distressing over 280 million people worldwide and contributing significantly to disability (Zulfiqar, S., *et al*., 2024). Only in the US the National Institute of Mental Health (NIMH) estimates that 21 million adults-nearly 8.3% of the adult population-had at least one major depressive episode during the preceding 12 months (Nehme, 2024). Since many people are looking for alternatives to conventional pharmacological interventions, this high prevalence has increased demand for effective treatments, such as innovative and natural antidepressants *A. lebbeck* has long been used to treat stress, respiratory issues, allergies, and cognitive loss because of its calming, anti-inflammatory, and adaptogenic properties. Its varied phytochemical components, which include alkaloids, saponins, flavonoids, and glycosides, were also emphasized in some recent studies. These components collectively account for the different pharmacological effects. Additionally, preclinical data suggests that *A. lebbeck* regulates dopamine and serotonin neurotransmitters. reduces neuroinflammation and oxidative stress, two of the basic mechanisms linked to depression. Conventional medicines such as Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs), are helpful for a limited period of time but can have unfavourable side effects and may not work for everyone (Chu *et al*., 2024). Furthermore, studies reveal that some medications may take weeks to start easing symptoms, which can be distressing for those who need relief right away. Because of these drawbacks and the stigma attached to synthetic drugs, there is a lot of interest in discovering natural antidepressants, especially those with shorter half-lives, fewer side effects, and long-term, sustainable effectiveness (Keller, 2025).

The demand for natural antidepressants is driven by several factors

Effectiveness of Certain Natural Compounds: These substances frequently affect neurotransmitters including dopamine, serotonin, and norepinephrine, which are also targeted by

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conventional antidepressants but frequently have fewer adverse effects (Dobrek, *et al*., 2023).

Preference for Holistic and Plant-Based Treatments: More people are drawn to natural remedies for mental health and prefer holistic health solutions. The inclination for holistic and plant-based therapies is consistent with the growing acceptance of wellness regimens and natural supplements for mental health management (Lavretsky, 2009).

Interest in Psychedelic Plant Medicine: Even though these substances are now illegal and not generally available in many locations, research indicates that some patients may benefit from their quick and long-lasting effect (Nichols, *et al*., 2021).

The Role of Lifestyle and Diet in Mental Health: lifestyle modifications can significantly affect mental health. Because they provide a natural, easily available method of supporting mental health, interest in nutraceuticals and functional foods that promote brain health is growing (Heidari, *et al*., 2023).

Significance of *Albizia lebbeck*

The tree species *Albizia lebbeck*, which belongs to the Fabaceae family, is original to the tropics and semitropical sections of South and Southeast Asia. It is frequently referred to as the "Siris tree" or "Indian walnut" (Rajora, 2024). It has long been used in outdated drug, particularly in Ayurvedic and Unani therapies, due to its numerous therapeutic advantages (Verma, 2013). Numerous ailments, such as infections, inflammation, skin conditions, respiratory conditions, and allergies, are treated with the bark, leaves, seeds, and roots of the *A. lebbeck* tree (Brewbaker, 2004).

MATERIALS AND METHODS

Plant material

Fresh leaves of *Albizzia lebbeck* were collected from the been on irritation and dried, The sample was authenticated by Dr. V. Ram Rao, Dept of botany, Uttara Halli (Hobli), Kanak Pura main road Bangalore-560109. Ref. RRCBI-1637. The collected dried leaves powered is used for the study.

Chemicals

We bought ethanol, methanol, H_2SO_4, NaOH, $FeCl_3$, HCl acetone, glacial acetic acid, ammonia, sodium bicarbonate, chloroform, and Bangalore, India.

Solvent extracts

To get ethanolic (AL) extract, 10 g of shade-dried entire *Albizzia lebbeck* plant material was crushed and macerated for 8 hr. in a Soxhlet device with 100 mL of 95% ethanol and methanol. A rotary evaporator was used to evaporate the filtrate at 40°C after the crude liquid extract had been filtered through Whatman no. 1 paper. For later usage, dried crude *Albizzia lebbeck* were stored in a desiccator in airtight containers.

Qualitative phytochemical analysis

Standard protocols were used to screen for *Albizzia lebbeck*. (Shaikh and Patil, 2020) 10 mL of Benedict's reagent were added to the crude extracts, heated, and the presence of carbohydrates was verified by the appearance of a reddish-brown precipitate. Dragendorff's reagent was added after the plant extracts were boiled for 2 min with 2% H_2SO_4 and filtered Alkaloids are present when a reddish-brown precipitate is present. After applying Millon's reagent to the crude extracts, the development of a white precipitate indicates the presence of protein. When crude extract was added to a $FeCl_3$ (2%) solution in the ferric chloride test, Phenols and tannins were present because a blue-green precipitate formed. Crude extract, For the zinc-HCl decrease test, a small amount of zinc dust and a few drops of durable HCl were collective. Flavonoids are present when a magenta colour is produced. Extracts were combined with concentrated H_2SO_4 and chloroform for Salkowski's test. and the creation of a golden yellow or greyish colour shows the existence of triterpenes or steroids. In the Froth test, after vigorously mixing the extracts with A few drops of a solution of sodium bicarbonate, they were left for 3 min. The development of a honeycomb-like foam indicates the presence of saponin. Excerpts remained combined with glacial acetic acid, drops of FeCl3, and concentrated H_2SO_4 for the Keller-Kilani test. The presence of glycoside is shown by the development of a brown ring. Chloroform and ammonia were applied to the extracts for treatment. The presence of anthracene derivatives is indicated by a pink, red colour development. When sodium hydroxide was added to the crude extract, quinone was detected by the production of a reddish-green colour (Bontrager's test). For the paper test, a drop of extracts was undisturbedly positioned between two filter papers.

LC-MS analysis

Sample Preparation

10 mg of the sample extract are dissolved in 2 mL of ethanol, filtered, and then injected.

Method

0.1% formic acid in liquid (aqueous phase, A) and acetonitrile (organic phase, B) were current in the portable phase. By means of the following program, A flow rate of 0.2 mL/min was used for gradient elution: 2% B was maintained for the first minute. From 1 to 6 min, it increased linearly to 50% B; and from 6 to 12 min, it increased linearly to 95% B. From 12 to 16 min, the composition was maintained at 95% B. From 16 to 17 min, it was re-equilibrated at 2% B, and it was maintained there for three more minutes. After inoculating a 5 µL sample amount, During the research, the support oven was maintained at a constant temperature of 22°C. An Electrospray Collective Ionization (ESCi) source that works in both positive and negative ionization modes was used to achieve mass spectrometric detection. The following instrument

settings were optimized: probe temperature at 450°C, 150° "The source temperature was maintained at [specify °C if needed], with a nitrogen cone gas flow of 50 L/h and a desolation gas flow of 750 L/h. Data acquisition and processing were performed using Mass Lynx software (version 4.1, Waters Corporation, Milford, MA, USA) (Shaikh, *et al*., 2020).

Antioxidant Assays

Another way to describe antioxidants is compounds that capture toxic forms of oxygen and stop them from causing cell damage. Mechanistic definitions of antioxidants usually Centre on their capacity to donate electrons or hydrogen. Tests based on a single electron transfer reaction or hydrogen transfer tests are the two main categories into which many commonly used tests for antioxidant capacity fall. These tests evaluate the sample's capacity to scavenge or reduce free radicals rather than its ability to function as an antioxidant defense mechanism (Apak, 2018).

DPPH radical scavenging assay

2 mL of methanol and 2 mL of 0.1 mM DPPH were mixed. The absorbance was immediately measured at the 517 nm control wavelength. 2 mL of test extracts and 2 mL of DPPH were combined and shaken well. The test samples were incubated for half an hour. Methanol was used as a blank to measure the wavelength of absorption at 517 nm. The antioxidant activity of the extracts was reported as Mean±SEM, and all tests were conducted in triplicate. The IC_{50} was computed together with the percentage of scavenging activity. 0.1 mM DPPH + Methanol Test as a control: 0.1 mM DPPH15 + Acrbic Acid Standard or Sample (SLNA) (Mandal, *et al*., 2009).

Super oxide free radical scavenging assay

5 mL of Tris-HCl buffer (16 mM, pH 8.0) including 2 mL of sample solution, 2 mL of Tris-HCl, 1 mL of NBT (300 µM) solution, and one mL of NADH (936 µM) solution were used to create superoxide radicals. 1 mL of PMS solution (120 µM) was added to the mixture to initiate the reaction. After incubation at 25°C for 5 min, the absorbance of the reaction mixture was measured at 560 nm using a spectrophotometer against a blank. L-ascorbic acid served as the reference standard. All experiments were conducted in triplicate, and the antioxidant activity of the extracts was expressed as Mean±SEM. The percentage scavenging activity was calculated, and the IC_{50} values were determined. The reaction mixture consisted of Tris-HCl buffer, NBT, NADH, and PMS. For test samples (SLNA) or the ascorbic acid standard, the mixture included Tris-HCl buffer, NBT, NADH, PMS, and the respective sample. Tris-HCl buffer alone was used as the blank (Chang, *et al*., 1996).

Hydroxyl radical scavenging assay

Different concentrations of *Albizzia lebbeck* and gallic acid (125, 250, 500, 1000, and 2000 µg/mL) were mixed with KH_2PO_4 buffer, pH 7.4 (0.05 M), "The reaction mixture contained deoxyribose (2.8 mM), EDTA (0.1 mM), H_2O_2 (1 mM), and $FeCl_3$ (0.1 mM). After incubation at room temperature for 30 min, thiobarbituric acid and trichloroacetic acid (2.8% w/v) were added. The mixture was then incubated in a water bath for 30 min, cooled, and the Optical Density (OD) was measured at 532 nm (Halliwell *et al*., 1987).

Nitric oxide scavenging assay

"1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of the test or reference solution in Phosphate-Buffered saline (pH 7.4) at varying concentrations. The mixture was incubated at 25°C for 150 min. Subsequently, 1 mL of the incubated solution was withdrawn and treated with 1 mL of Griess reagent, consisting of 0.1% N-(1-naphthyl) ethylenediamine, 2% o-phosphoric acid, and 1% sulfanilamide in 1% sulfuric acid. The reaction mixture was then kept at room temperature for 10 min. After diazotization of nitrite with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine, the absorbance of the resulting chromophore was measured at 548 nm. The percentage inhibition was calculated by comparing the test samples with the control (Oyaizu, 1986).

RESULTS

Preliminary Phytochemical Screening

Table 1 displays the findings of *Albizzia lebbeck's* phytochemical analysis. It illustrates how *Albizzia lebbeck* includes steroids, terpenoids, alkaloids, phenols, flavonoids, proteins and amino acids, tannins, quinones, glycosides, fixed oils, resins, coumarins, and carbohydrates.

LC-MS analysis: *Albizzia lebbeck* (L.) is abundantly rich in bioactive compounds, according to the LC-MS chromatogram spectra acquired for ALEN and ALMN. Figures 1 and 2 display a selection of the bioactive compounds exhibiting a range of pharmaceutical activities from the LC-MS spectrum for ALEN and ALMN, which displayed 30 and 65 peaks and indicated 35 and 65 compounds. These bioactive compounds' spectra were

Table 1: Preliminary phytochemical analysis of ALEN and ALMN.

Sl. No.	Constituents	AL
1	Alkaloids	+
2	Carbohydrates	+
3	Glycosides	+
4	Quinone	+`
5	Terpenoids	+
6	Tannins	+
7	Flavonoids	+
8	Protein and amino acid	+
9	Phenols	+

+ve indicates no particles are degraded

compared to the LC-MS software from the NIST library. Table 2 for ALEN and Table 3 for ALMN displayed the compound's retention time, nature, molecular weight, peak area, and molecular formula.

Evaluation of the antioxidant activity of the extracts

DPPH radical-scavenging assay

The main purpose of the DPPH assay is to lessen the capacity of various extracts and compounds that rely on the presence of hydrogen-donating stimulants. Table (2) and Figure (1) show the findings of the DPPH test, which gauges ALEN's ability to scavenge free radicals. Albizzia lebbeck (ALEN, ALMN) "The extract exhibited strong DPPH radical scavenging activity, with an IC_{50} value of 52.81±0.17 µg/mL, compared to 3.07±0.04 µg/mL for ascorbic acid, which served as the reference standard. Although the IC_{50} values of the extracts were higher than that of ascorbic acid, they still fell within an effective range. These findings suggest that both extracts possess the potential to inhibit free radical generation.

Super oxide free radical scavenging assay

Table (3) and Figure (2) demonstrate the free essential scavenging activity of ALEN and ALMN using the Superoxide anion anion thorough scavenging assay; nevertheless, Figure 3 demonstrates a substantial antioxidant activity in ALEN in comparison to those ALMN. Compared to ascorbic acid, which was employed as a reference and had an IC_{50} value of 60.51±0.13 µg/mL, ALEN and ALMN were shown to be potent scavengers of superoxide anion radicals, with an IC_{50} of 390.37±0.09 µg/mL.

Hydroxyl radical scavenging assay

The test for scavenging hydroxyl radicals in the hydroxyl radical scavenging assay, which is used to show the free radical scavenging activity of ALEN, SLNA was found to be a powerful scavenger of hydroxyl radicals, in contrast to ascorbic acid, which was employed as a reference and had an IC_{50} of 65.57±0.21 µg/mL (Table 1).

DISCUSSION

The goal of phytochemical screening is to find bioactive substances that may be useful in the synthesis of medicinal substances (Yang, *et al*., 2019). According to our current findings the ethanolic and methanolic whole-plant extracts were found to contain proteins and amino acids, alkaloids, tannins, phenols, flavonoids, steroids/terpenoids, saponins, glycosides, quinones, fixed oils, resins, coumarins, and carbohydrates (Table 1). Madhavan *et al*., provide support for these findings (Madhavan, *et al*., 2013) who used phytochemical analysis to show that the alcoholic extract of *Albizzia lebbeck* leaves included flavonoids, phenols, sugars, alkaloids, glycosides, tannins, and phytosterols. The pharmacological actions of plants are caused by the combined effects of these phytochemicals (Uma *et al*., 2009), which are produced by several physiological processes. Plant extracts have a significant impact on preventing chronic illnesses like cancer, cardiovascular disease, and neurodegenerative diseases through a number of biological processes (Al-Owaisi, 2014). The most popular method for measuring the active ingredients found in plants used in the food, pharmaceutical, and cosmetics industries is LC-MS analysis (Uma, 2009). Different phytochemical constituents were identified by a number of peaks in the ethanolic and methanol whole plant extract of *Albizzia lebbeck* (Figures 1 and 2) (Tables 2 and 3). The main chemical constituents of *Albizzia lebbeck* crude extracts were found to contain phenols, steroids, terpenoids, saponins, alkaloids, coumarins, quinolones, naphthalenes, fatty acid derivatives, vitamin E, pyridine, phthalates, alkanes, esters, and organosilicon compounds. Several of these compounds are reported to possess multiple pharmacological activities, irrespective of their concentration. Notably, most of the identified compounds have been associated with antimicrobial and antioxidant properties" (Taher, 2020), Preethamo, 2020). According to research, the HYA assay is used to evaluate the antioxidants' capacity to reduce free radicals, while the DPPH and superoxide assays are used to evaluate the antioxidants' ability to quench free radicals (Brand-Williams, W., 1995 Re, R., *et al*., 1999; Benzie, 1996). We used the DPPH assay

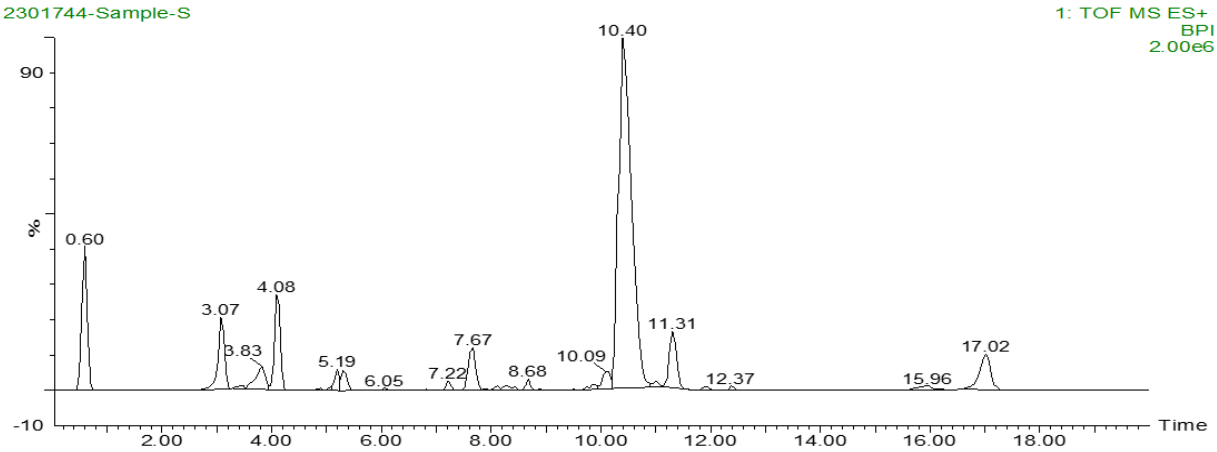


Figure 1: LC-MS chromatogram of ALEN.

Table 2: Compounds detected for ALEN in LCMS analysis.

Sl. No.	Retention time (min)	Name of the compound	Molecular formula	Molecular Ion (m/z [M-H]⁻)	Nature of the compound
1	0.497	Mycosporine glutaminol	$C_{13}H_{22}N_2O6$	303.1495	Cyclohexenones
2	0.548	Lactobionic Acid	$C_{12}H_{22}O_{12}$	381.1202	Mono-and disaccharide fatty acyl glycosides
3	0.548	Salvinorin A	$C_{23}H_{28}O_8$	455.1655	Diterpene lactones
4	0.649	Poncirin	$C_{28}H_{34}O_{14}$	595.2145	Flavonoid-7-O-glycosides
5	2.619	Methyl cinnamate	$C_{10}H_{10}O2$	163.0682	Cinnamic acid esters
6	2.922	3-Trimethylsilylpropionic acid	$C_6H_{14}O_2S$	147.0732	Carboxylic acids
7	3.073	Poncirin	$C_{28}H_{34}O_{14}$	595.2145	Flavonoid-7-O-glycosides
8	3.326	Chromen-9-yl) oxybutan-2-three-hydrox y-2-methyl-4-(7-oxofuro[3,2-g]	$C_{21}H_{22}O_7$	773.2724	Psoralens
9	3.376	Acetyloxyethyl	$C_{19}H_{28}O_{13}$	465.1578	Terpene glycosides
10	3.427	Hesperidin	$C_{28}H_{34}O_{15}$	611.2091	Flavonoid-7-O-glycosides
11	3.477	Thept-2-yl acetate [2.2.1] 1,7,7-trimethylbicyclo	$C_{12}H_{20}O_2$	197.1493	Bicyclic monoterpenoids
12	3.528	Euphodendroidin N	$C_{40}H_{46}O_{13}$	757.2837	Jatrophane, cyclojatrophane diterpenoids
13	3.629	Reynosin	$C_{15}H_{20}O_3$	249.1543	Eudesmanolides, secoeudesmanolides, derivatives
14	3.679	Actephilol C	$C_{36}H_{34}O_8$	595.2245	Phenanthrols
15	3.831	methylbut-2-enoate	$C_{24}H_{26}O_7$	449.1554	Angular furanocoumarins
16	3.831	Satratoxin F	$C_{29}H_{34}O_{10}$	565.2039	Trichothecenes
17	3.882	Mangostin	$C_{24}H_{26}O_6$	433.1604	8-prenylated xanthones
18	3.932	Luteolin C-glucoside C-xyloside	$C_{26}H_{28}O_{15}$	581.207	Flavonoid 8-C-glycosides
19	4.084	14-hydroxy-5,9-dimethyl-14	$C_{25}H_{40}O_5$	421.2947	Kaurane diterpenoids
20	4.185	Aprepitant (MK-0869)	$C_{23}H_{21}F_7N_4O_3$	535.1548	Phenylmorpholines
21	4.235	Anthothecol	$C_{28}H_{32}O_7$	519.1595	Limonoids
22	4.437	Lichexanthone	$C_{16}H_{14}O_5$	287.0902	Xanthones
23	4.589	4-hydroxy-3-(3-methylbut-2-enyl) benzoic aci	$C_{12}H_{14}O_3$	207.1028	Hydroxybenzoicacid derivatives
24	4.841	DereplicatorIdentification - E'Surugamide_B'	$C_{47}H_{79}N_9O_8$	898.6036	Oligopeptides
25	4.892	1-oxobutan-2-yl 1-methoxy-3-methyl	$C_{60}H_{95}N_3O_{19}$	1162.67	Triterpene saponins
26	5.094	Caffeic acid	$C_9H_8O_4$	181.1536	Hydroxycinnamic acids
27	5.195	Notoginsenoside S	$C_{63}H_{10}6O_30$	1365.677	Triterpenoids
28	5.296	3,4'-dihydroxy-	$C_{25}H_{35}NO_5$	468.2174	Isoindolones
29	5.346	Naratriptan HCl	$C_{17}H_{26}ClN_3O_2S$	336.1738	3-alkylindoles
30	5.649	14-(Hydroxymethyl)-5,9-dimethyl tetracyclo-hexadecan-5-ol	$C_{19}H_{32}O_2$	275.2374	Kaurane diterpenoids

to test ALEN and ALMN's capacity to neutralize radicals, and we discovered that the DPPH radical had a concentration-dependent scavenging effect. The DPPH method measures the capacity of individual or combination antioxidant compounds to scavenge radicals. It is a simple, quick, affordable, and repeatable assay (Gulcin, *et al*.., 2023). The outcome is consistent with other *Albizzia lebbeck* species' scavenging DPPH assay results, indicating that ALEN has a potent antioxidant function. The DPPH radical assay is frequently used to determine a crude extract's overall antioxidant capacity (Dong, 2015; Dong, 2014). Generates potent and hazardous oxidants, including hydroxyl radicals and singlet oxygen. The reaction between the hydrogen peroxide radical and the superoxide anion radical produced strong reactive oxygen species, singlet oxygen, and OH radicals. Ascorbic acid, ALEN, and ALMN all showed a dose-dependent increase in their capacity to scavenge superoxide radicals, indicating that ALEN's Ascorbic acid-like scavenging action was observed. It can initiate auto-oxidation by generating double bond addition, electron transfer, radical production, hydrogen withdrawal, and other reactions. Polymerization, and fragmentation reactions in a variety of biomolecules (Senthilkumar, *et al*., 2024). The hydroxyl radical is a strong ROS that damages biological membrane lipids, alters the base and sugar in oxidative DNA lesions, breaks strands, and breaks DNA-protein bonds by targeting purines, pyrimidines, and deoxyribose sugar backbone in DNA, as well as the creation of different oxidation products by targeting several amino acids in proteins (tryptophan is transformed into kynurenine, while lysine produces leucine, valine, and α-aminoadipic semialdehyde (Martemucci, 2022). The findings showed that OH radicals are eliminated and additional harm is prevented when ALEN is exposed to the reactant. Therefore, scavenging hydroxyl radicals is essential for protecting living systems (Yang, 2008). In the assay for ferric ion reducing power (Shiddhuraju *et al*., 2002). A substance's reducing capacity is related to its likely antioxidant activity. The current study's findings showed that ascorbic acid, ALMN, and ALEN all improved their ferric reducing capacities in a dose-dependent manner. A substance's capacity to donate electrons is gauged by its reduction potential. (Moreno, 2002) Compared to ALMN, ALEN ought to be a more effective free radical scavenger.

CONCLUSION

Several active components were identified by phytochemical and LCMS analysis of the ethanolic and n-hexane extracts of *Albizzia lebbeck* in the current study. They were found to possess antimicrobial, anti-diabetic, anti-inflammatory, and anti-cancer qualities, indicating the plant's enormous therapeutic potential. Additionally, their strong radical scavenging properties were

Table 3: Compounds detected for ALMN in LCMS analysis.

Sl. No.	Retention time (min)	Name of the compound	Molecular formula	Molecular Ion (m/z [M-H]⁻)	Nature of the compound
1	5.902	tsas#9	$C_{23}H_{33}NO_9$	468.2218	Sugar acids and derivatives
2	6.054	Androst-5-ene-3,17-diol	$C_{19}H_{30}O_2$	291.2317	Androgens and derivatives
3	6.256	FA 18:3+1O	$C_{18}H_{30}O_3$	277.2206	Medium-chain fatty acids
4	6.71	2-heptadecanone	$C_{17}H_{34}O$	277.2512	Ketones
5	6.811	1,2,6,7,8,8a-hexahydronaphthalen-1-yl-7-[2,6-dimethyl-8-(2-methylbutanoyloxy)]Acid -3,5-dihydroxyheptanoic	$C_{24}H_{38}O_6$	445.2558	Medium-chainhydroxy acids and derivatives
6	7.215	Androst-5-ene-3,17-diol	$C_{19}H_{30}O_2$	291.2317	Androgens and derivatives
7	7.266	4-[(E)-3-hydroxy-8,10-dimethyl-2-(methylamino)dodec-6-enyl]phenol	$C_{21}H_{35}NO_2$	351.2939	Amphetaminesand derivatives
8	7.518	Baquiloprim	$C_{17}H_{20}N_6$	309.1836	Aminoquinolinesderivatives
9	7.619	(5R)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one NCGC00169702-02!	$C_{17}H_{26}O_4$	277.1762	Gingerols
10	7.67	2-heptadecanone	$C_{17}H_{34}O$	277.2512	Ketones
11	8.276	DOCOSANOL	$C_{22}H_{46}O$	365.3135	Fatty alcohols
12	8.428	Melamine	$C_3H_6N_6$	149.0505	1,3,5-triazines
13	8.832	1-Oleoylglycerophosphocholine	$C_{26}H_{53}NO_7P$	522.4056	1-acyl-sn-glycero-3-
14	8.933	Androsterone	$C_{19}H_{30}O_2$	291.2387	Androgens and derivatives
15	9.034	icosanoic acid	$C_{20}H_{40}O_2$	313.3106	Long-chain fatty acids

Sl. No.	Retention time (min)	Name of the compound	Molecular formula	Molecular Ion (m/z [M-H]⁻)	Nature of the compound
16	9.286	5-en-3-yl[2-(dimethylamino) ethyl]carbamate 3-beta-Cholest-5-en	$C_{32}H_{56}N_2O_2$	501.4342	Cholesterols and derivatives
17	9.488	Trihydroxytetrahydro 3,4,5-2-carboxylic acid-2H-pyran	$C_{30}H_{48}O_{11}$	607.3091	Steroidglucuronide conjugates
18	9.539	1-heptadecanol	$C_{17}H_{36}O$	279.2723	Long-chain fatty alcohols
19	9.589	Dehydroevodiamine	$C_{19}H_{17}N_3O$	326.3475	Beta carbolines
20	9.64	Gestodene	$C_{21}H_{26}O_2$	311.2022	Estrogens and derivatives
21	9.741	Ala-Ala	$C_6H_{12}N_2O_3$	161.088	Dipeptides
22	9.943	Dihydrobacillaene	$C_{34}H_{50}N_2O_6$	583.3751	Medium-chain fatty acids
23	10.044	pachymic acid	$C_{33}H_{52}O_5$	529.3894	Triterpenoids
24	10.044	Dauricine	$C_{38}H_{44}N_2O_6$	625.3257	Benzylisoquinolines
25	10.145	Trimethyl-2-methylidene 6-hydroxy-5,5,8a-trimethyl	$C_{29}H_{46}O_{11}$	609.2676	Diterpene glycosides
26	10.145	11,22-trihydroxy1,6,11,16,22, 27-hexazacyclodotriacontane-2,5,12,15,23,26-hexone	$C_{26}H_{46}N_6O_9$	609.3231	Macrolactams
27	10.448	2-O-rhamnosyl-swertisin	$C_{28}H_{32}O_{14}$	593.1691	Flavonoid C-glycosides
28	10.499	Phloridzin	$C_{21}H_{24}O_{10}$	437.3963	Flavonoid O-glycosides
29	10.549	17(21)-Hopen-6-one	$C_{30}H_{48}O$	425.3836	Hopanoids
30	11.155	(1, 3beta, 9xi, 11alpha, and 14xi)5,20(22) ,25-trien-27-ylbeta-D-glucopyranoside-1,3,11-trihydroxyfurosta	$C_{33}H_{50}O_{10}$	607.3494	Steroidal saponins
31	11.307	4-dihydroxy-4-(hydroxymethyl) oxolan-2-yl]oxy-4,5-dihydroxy	$C_{26}H_{30}O_{13}$	551.1813	Flavonoid O-glycosides
32	11.862	Neohesperidin dihydrochalcone	$C_{28}H_{36}O_{15}$	613.5388	Flavonoid O-glycosides
33	15.905	25S, 9xi, 14xi, 5beta, and 3betaGlucopyranoside beta-D-spirostan-3-yl (1->4) O-6-deoxy-alpha-L-mannopyran osylBeta-D-glucopyranosyl Oxygen	$C_{45}H_{74}O_{17}$	887.5028	Steroidal saponins
34	17.016	6-methyltetrahydro-2H-pyran-3,4,5-triol	$C_{45}H_{74}O_{16}$	871.5034	Steroidal saponins
35	17.016	TAG(50:1)	$C_{53}H_{100}O_6$	871.7088	Triacylglycerols

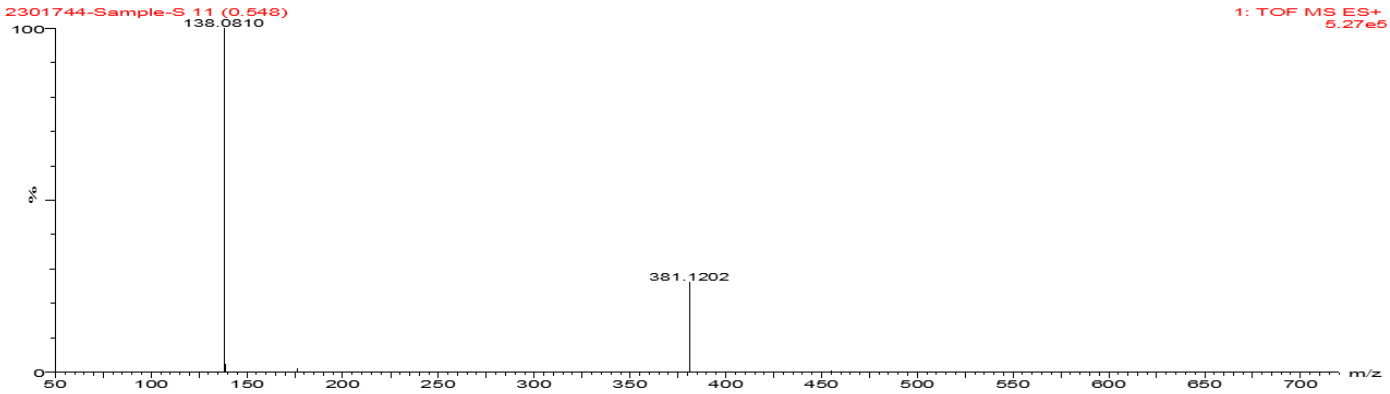
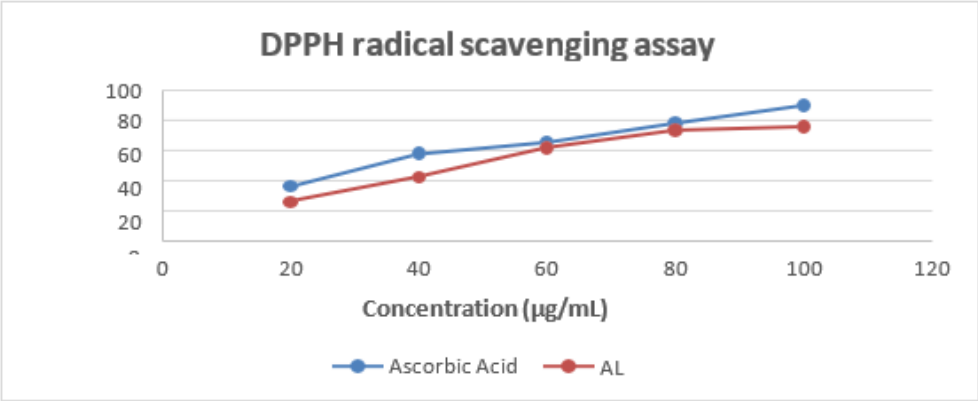
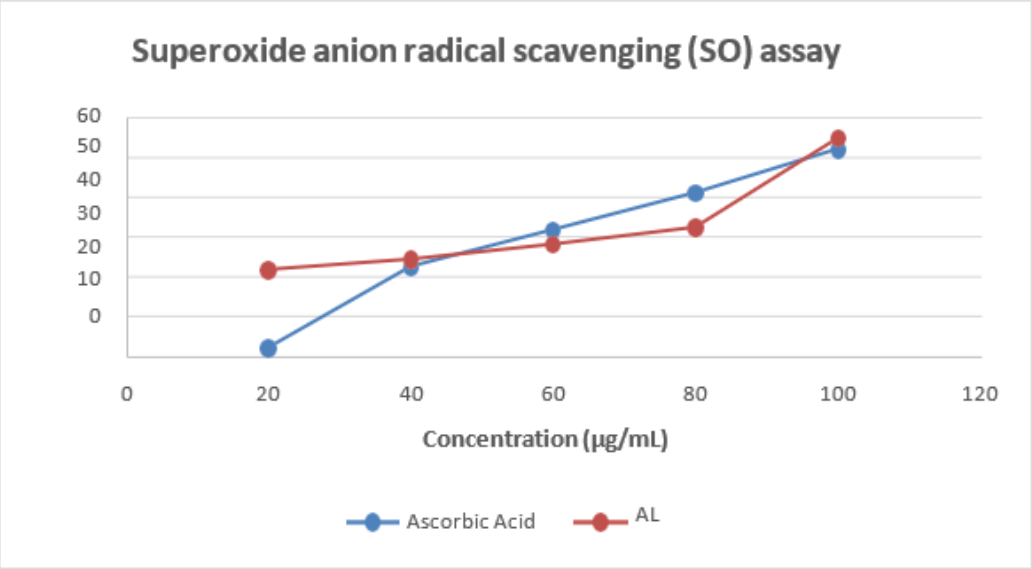


Figure 2: LCMS chromatogram of *ALMN*.





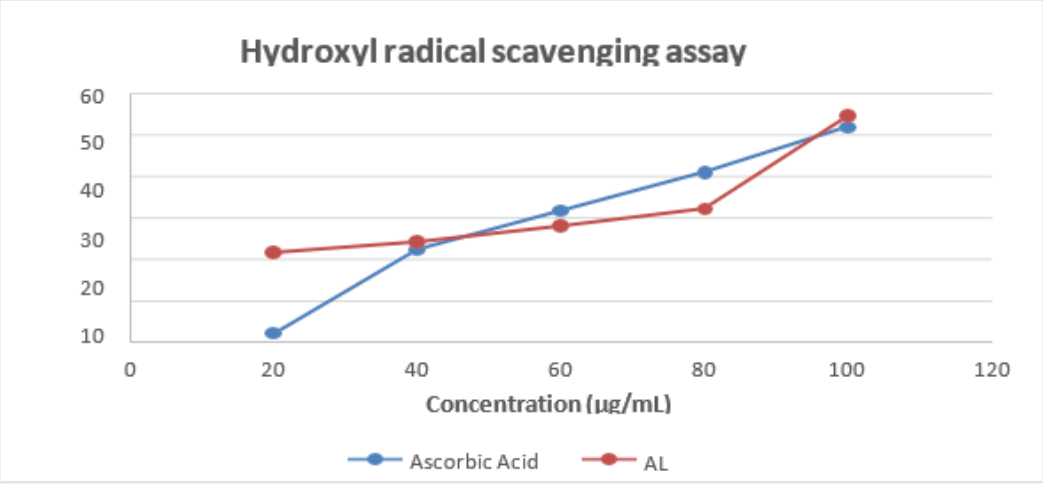


Figure 3: The *in vitro* Antioxidant Activity of ALEN and ALMN. Data are Mean±SEM (Each experiment has triplicate values); AA-Ascorbic acid.

investigated by the outcomes of *in vitro* antioxidant tests. The plant's potential for use in drug discovery will be investigated through additional *in vivo* experiments and other antioxidant mechanisms.

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ABBREVIATIONS

ALEN: *Albizzia lebbeck* Ethanolic extract; **ALMN:** *Albizzia lebbeck* Methanolic extract; **NBT:** Nitroblue tetrazolium; **NADH:** Nicotinamide adenine dinucleotide hydride; **PMS:** Phenazine methosulfate; **OD:** Optical Density; SEM: Standard Error of the Mean; **IC_{50}:** Inhibitory Concentration 50%; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ROS:** Reactive oxygen species; **H_2O_2:** Hydrogen peroxide; **$FeCl_3$:** Ferric chloride; **HCl:** Hydrochloric acid; **H_2SO_4:** Sulfuric acid; **NaOH:** Sodium hydroxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Albizzia lebbeck whole plant ethanolic and methanolic extracts were examined using qualitative investigative techniques to detect phytochemicals and LC-MS to identify bioactive compounds. DPPH, hydroxyl, and superoxide radical scavenging assays were used to evaluate the extracts' *in vitro* antioxidant activities. The phytochemical examination examined whether the ethanolic and methanol extracts contained flavonoids, alkaloids, phenols, steroids, tannins, glycosides, and saponins. Strong radical scavenging abilities were shown by *in vitro* antioxidant assay results, which may be due to the complimentary effects of these bioactive substances.

AUTHOR CONTRIBUTIONS

Regarding the Credit taxonomy, the author's contributions were: Kavitha S K: Conceptualization, visualization, validation, methodology, investigation, data curation, formal analysis, writing-original draft. Ali Mohammad: Supervision, project administration, writing - review and editing.

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