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Phytochemical characterization and evaluation of antioxidant and antimicrobial properties of *Sesbania grandiflora* leaf extracts

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Abstract

Sesbania grandiflora (L.) Pers. is a medicinal plant widely used in traditional healthcare systems owing to its diverse therapeutic properties. The present study aimed to evaluate the phytochemical composition, antioxidant potential, and antibacterial activity of petroleum ether and methanolic leaf extracts of S. grandiflora. Preliminary phytochemical screening revealed the presence of alkaloids and coumarins in the petroleum ether extract, whereas the methanolic extract contained phenolics, tannins, flavonoids, cardiac glycosides, saponins, and coumarins, indicating a richer phytochemical profile. Antioxidant activity was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay at concentrations ranging from 20-100 µg mL $^{-1}$. The methanolic extract exhibited superior antioxidant activity, with inhibition increasing from 32.57% to 78.11%, compared with 13.50% to 60.40% for the petroleum ether extract, approaching the activity of the standard ascorbic acid (49.09-80.34%). Antibacterial activity was evaluated against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus using the disc diffusion method. The methanolic extract showed stronger antibacterial activity, producing maximum inhibition zones of 17, 13, and 18 mm against E. coli, P. aeruginosa, and S. aureus, respectively, at 10 mg disc $^{-1}$, whereas the petroleum ether extract exhibited corresponding inhibition zones of 11, 9, and 10 mm. The enhanced antioxidant and antibacterial activities of the methanolic extract were attributed to its higher content of phenolic and flavonoid compounds. The findings demonstrate that S. grandiflora leaves are a promising source of natural bioactive compounds with significant antioxidant and antimicrobial potential, supporting their traditional medicinal use and warranting further phytochemical characterization and pharmacological investigations.

Keywords: *Sesbania grandiflora; phytochemical screening; FRAP assay; antioxidant activity; antibacterial activity; medicinal plants.*

INTRODUCTION

Medicinal plants have been used by humankind since ancient times for the prevention and treatment of various diseases and remain an indispensable source of therapeutic agents in modern healthcare. Among the approximately 250,000 known plant species worldwide, more than 80,000 are utilized for medicinal purposes. India, recognized as one of the world's major biodiversity centers, possesses nearly 45,000 plant species, of which about 15,000–20,000 are reported to have medicinal value (Joy et al., 1998; Dewick, 2002). The growing concern over antimicrobial resistance, adverse effects of synthetic drugs, and the increasing incidence of oxidative stress-related disorders has stimulated renewed interest in plant-derived bioactive compounds as safer and sustainable alternatives.

Sesbania grandiflora (L.) Pers., commonly known as Agathi, Hummingbird tree, Sesban, or Swamp pea, is a fast-growing small tree belonging to the family Fabaceae (Leguminosae). The species is believed to have originated in India or Southeast Asia and is widely distributed throughout tropical and subtropical regions including India, Indonesia, Malaysia, Myanmar, Australia, and the Philippines. The plant thrives under hot and humid climatic conditions and exhibits remarkable adaptability to waterlogged, saline, and alkaline soils, making it an important multipurpose species in tropical agriculture (Gutteridge, 1994; Bhattacharjee, 1998).

Leaves are particularly recognized as excellent sources of vitamin C and calcium, while flowers contain significant quantities of calcium, iron, and B-complex vitamins. Seeds also provide valuable proteins, carbohydrates, oils, and minerals. Because of its superior nutritional profile, *S. grandiflora* contributes significantly to food security and livestock nutrition, with studies reporting improved milk production and rumen microbial activity in cattle supplemented with its foliage (Govindan and Shanmugasundaran,

1987; Vijayakumar et al., 2000a,b). Bark preparations are valued for their astringent, febrifuge, and antiulcer properties, while roots are commonly applied to inflammatory conditions and rheumatic swellings. Several pharmacological investigations have further validated these traditional claims by demonstrating antiulcer, antiurolithiatic, anxiolytic, anticonvulsant, anticancer, anti-inflammatory, and hepatoprotective activities of the plant (Kasture et al., 2002; Doddola et al., 2008; Laladhas, 2009).

Several bioactive compounds including kaempferol derivatives, oleanolic acid methyl esters, leucocyanidin, and cyanidin have been isolated from different plant parts. These phytochemicals are recognized for their ability to modulate numerous biological pathways and contribute significantly to the therapeutic properties of the plant (Kalyanagurunathan et al., 1985; Andal and Sulochana, 1986). Extracts obtained from leaves, flowers, and other plant parts have exhibited significant free-radical scavenging activity, reducing power, lipid peroxidation inhibition, and cytoprotective effects, highlighting the plant as a promising source of natural antioxidants (Gowri and Vasantha, 2010; Sarkar et al., 2012; Panda et al., 2013).

In addition to antioxidant activity, *S. grandiflora* has emerged as a potential source of plant-derived antimicrobial agents. The increasing prevalence of multidrug-resistant pathogens and the limitations associated with conventional antibiotics have created an urgent need for alternative antimicrobial compounds. Various extracts of *S. grandiflora* have shown inhibitory activity against several Gram-positive and Gram-negative bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. The antimicrobial activity is believed to be associated with the presence of flavonoids, polyphenols, tannins, and related secondary metabolites capable of interfering with microbial growth and metabolism (Subramanian and Varghese, 2003; Anantaworasakul et al., 2011; Ouattara, 2011).

Comprehensive investigations integrating phytochemical characterization with antioxidant and antibacterial evaluations of leaf extracts prepared using different solvents remain relatively limited. Furthermore, information correlating the occurrence of specific phytochemical groups with biological activities is insufficient. Such integrated studies are necessary for identifying the most effective extraction systems and establishing scientific evidence for the medicinal efficacy of the species. Therefore, the present investigation was undertaken to evaluate the phytochemical constituents of *S. grandiflora* leaf extracts and to assess their antioxidant and antibacterial activities. By establishing the relationship between phytochemical composition and biological efficacy, the study aims to provide scientific validation for the traditional use of *S. grandiflora* and contribute to the development of natural antioxidant and antimicrobial agents for pharmaceutical, nutraceutical, and healthcare applications.

MATERIALS AND METHODS

Plant material collection and preparation

Fresh, healthy leaves of *Sesbania grandiflora* (L.) Pers. were collected from Trivandrum district, Kerala, India. Diseased, infected, or mechanically damaged leaves were excluded from the study to ensure sample uniformity. The collected leaves were thoroughly cleaned and shade-dried at room temperature for two weeks. The dried material was pulverized into a fine powder using a mechanical grinder and stored in airtight containers until further analysis.

Preparation of extracts

Powdered leaf material was subjected to successive solvent extraction using petroleum ether and methanol in increasing order of polarity. Extraction was carried out using a Soxhlet apparatus at 30-35°C for 24 h with each solvent. The concentrated extracts were stored in airtight containers and reconstituted with their respective solvents prior to analysis. All chemicals and reagents used throughout the study were of analytical grade.

Phytochemical screening

Qualitative phytochemical analysis of petroleum ether and methanolic extracts was carried out following standard procedures described by Kokate et al. (2009), Trease and Evans (1994), and Siddiqui and Ali (1997).

- **Detection of Alkaloids by Dragendorff's Test, Hager's Test and Wagner's Test**

a) Dragendorff's Test: A few drops of Dragendorff's reagent (potassium bismuth iodide)were added to the extract. An orange or orange-red precipitate indicates alkaloids.

b) Hager's Test: The extracts were treated with Hager's reagent (saturated picric acid solution). A yellow precipitate/crystal indicates alkaloids.
c) Wagner's Test: Addition of Wagner's reagent (iodine in potassium iodide) resulting in a reddish-brown precipitate indicates alkaloids.

- **Detection of Steroids and Triterpenoids by Liebermann–Burchard Test, Salkowski Test.**

a) Liebermann–Burchard Test: Extracts were treated with acetic anhydride and a few drops of concentrated H_2SO_4. A colour change from pink to purple, blue, and finally deep green indicates steroids.
b) Salkowski Test: Extracts were mixed with with concentrated H_2SO_4. A red to reddish-brown colour in the chloroform (lower) layer indicates the presence of steroids or triterpenoids.

- **Detection of Tannins and Phenolic Compounds by Ferric Chloride Test, Iodine Test, Nitric Acid Test**

a) Ferric Chloride Test: A few drops of 5% ferric chloride solution to the extract. A blue-black or green-black coloration indicates the presence of tannins and phenolics.
b) Iodine Test: Addition of diluted iodine solution produces a transient purple/brown or precipitate formation indicate the presence of tannins.
c) Nitric Acid Test: Treat the extract with concentrated nitric acid. A colour change to red, yellow, or reddish-brown indicates tannins and phenolic compounds.

- **Detection of Flavonoids by Shinoda Test, Alkaline Reagent Test, Sulfuric Acid Test and Lead Acetate Test**

a) Shinoda Test: Add magnesium ribbon fragments and concentrated HCl to the extract in alcohol. The appearance of an orange, pink, red, or magenta colour indicates flavonoids.
b) Alkaline Reagent Test: Addition of a few drops of sodium hydroxide. An intense yellow colour that turns colourless upon adding dilute acid indicates flavonoids.
c) Sulfuric Acid Test: Treat the extract with concentrated H_2SO_4. An orange-red or deep-yellow colour indicates the presence of flavones and flavonoids.
d) Lead Acetate Test: Add a 10% lead acetate solution. The formation of a yellow precipitate indicates flavonoids.

- **Detection of Cardiac Glycosides by Keller–Killiani Test**

Treat the extract with glacial acetic acid, 1 drop of $FeCl_3$, and concentrated H_2SO_4. A brown ring at the interface and a violet/green turning blue colour in the acetic acid layer indicate the presence of deoxysugars characteristic of cardiac glycosides

- **Detection of Saponins by Froth Test**

Dilute the extract with distilled water and shake vigorously for several minutes. The formation of stable, honeycomb-like foam that persists for 10-15 minutes indicates the presence of saponins.

- **Detection of Coumarins by Ferric Chloride Test, Fluorescence Test**

a) Ferric Chloride Test: Add a few drops of $FeCl_3$ solution to the extract. A greenish, brown, or blue-black colour indicates the presence of phenolic coumarins.
b) Fluorescence Test: Observe the extract under UV light. A strong yellow, green, or blue fluorescence often indicates the presence of coumarins.

In Vitro Antioxidant Activity by Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power of petroleum ether and methanolic leaf extracts was determined according to the method of Pulido et al. (2000). The assay is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by antioxidant compounds present in the extracts. The resulting ferrous ions form a Prussian blue complex measurable at 700 nm (Chung et al., 2002).

Briefly, 0.1 mL of extract (20–100 µg mL^{-1}) was mixed with 0.9 mL of 96% ethanol, 5 mL distilled water, 1.5 mL of 1 M HCl, 1.5 mL of 1% potassium ferricyanide, 0.5 mL of 1% sodium dodecyl sulfate (SDS), and 0.2% ferric chloride. The reaction mixture was incubated in a water bath at 50°C for 20 min and subsequently cooled. Absorbance was recorded at 700 nm using a UV–Visible spectrophotometer. Ascorbic acid served as the positive control.

$$Inhibition\ \% = \left(1 - \frac{A}{A1}\right) X\ 100$$

Where, A is the absorbance of the control (blank, without extract) and A_1 is the absorbance of the sample or extract.

The IC_{50} value, defined as the concentration required to achieve 50% inhibition, was calculated from the linear regression equation:

$$y = mx + c$$

where the corresponding value of (x) at (y = 50) was considered the IC_{50} value. Lower IC_{50} values indicate higher antioxidant activity.

In Vitro Antibacterial Activity

Test Microorganisms

The antibacterial activity of petroleum ether and methanolic leaf extracts of *Sesbania grandiflora* was evaluated against three bacterial strains, namely *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The bacterial cultures used in the study were procured from Polyclinic Laboratory Private. Ltd., Thrissur, Kerala, India.

Procedure

For antibacterial evaluation, nutrient agar medium containing beef extract (2.8 g L^{-1}), peptone (5 g L^{-1}), sodium chloride (5 g L^{-1}), and agar (15 g L^{-1}) was prepared in distilled water and adjusted to pH 7.0. The medium was sterilized and poured into sterile Petri dishes to a uniform depth of approximately 4 mm. All glassware was sterilized in a hot-air oven at 180°C for 3 h, while forceps, inoculating loops, and other instruments were sterilized by dipping in 70% ethanol followed by flaming. Twenty-four-hour-old cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were inoculated into sterile nutrient broth and incubated at 37°C for 6 h until visible turbidity developed. The bacterial suspensions were uniformly spread onto nutrient agar plates using sterile cotton swabs, and the inoculated plates were allowed to stand for 15–20 min to facilitate absorption of excess inoculum. Antibacterial activity was determined using the disc diffusion method described by Barry and Thornsberry (1991). Sterile paper discs (3 mm diameter) were impregnated with 50 µL of petroleum ether and methanolic extract solutions at concentrations of 50, 100, and 200 mg mL^{-1}, corresponding to 2.5, 5.0, and 10.0 mg extract per disc, respectively. Ciprofloxacin (10 µg $disc^{-1}$) was used as the positive control, while sterile blank discs served as the negative control. The impregnated discs were placed on the inoculated agar plates and incubated at 35°C. After incubation, antibacterial activity was assessed by measuring the diameter of the inhibition zones around each disc and the results were recorded in millimeters. The inhibition zone diameter, including the diameter of the disc, was measured in millimeters. All experiments were conducted in triplicate, and results were expressed as mean values.

Inhibition annula for antimicrobial activity by disc diffusion assay was calculated using the following formula,

$$Inhibition\ annula\ (mm^2) = \pi\ (R + r)(R - r)$$

Where, π = 3.14, r = radius of the disc, R = radius of the disc + radius of inhibition zone.

Activity index (Rajasekaran and Murugesan, 2001) is a measure of the efficiency of the extract in comparison with a standard antibiotic.

The formula used to calculate the activity index is as follows.

$$Activity\ index = \frac{inhibition\ zone\ diameter\ of\ the\ test\ substance}{Inhibition\ zone\ diameter\ of\ the\ standard}$$

RESULTS

The qualitative phytochemical screening of petroleum ether and methanolic leaf extracts of *Sesbania grandiflora* revealed considerable variation in the distribution of bioactive constituents between the two solvent systems (Table 1). The petroleum ether extract tested positive only for alkaloids, as confirmed by Dragendorff's, Hager's, and Wagner's tests, and for coumarins, while all other phytochemical groups were absent. In contrast, the methanolic extract exhibited a richer phytochemical profile and showed the presence of tannins and phenolic compounds, flavonoids, cardiac glycosides, saponins, and coumarins. The presence of phenolic compounds was confirmed by ferric chloride, iodine, and nitric acid tests, whereas flavonoids were detected through positive responses in Shinoda, alkaline reagent, sulfuric acid, and lead acetate tests. Cardiac glycosides and saponins were also detected exclusively in the methanolic extract. Steroids and triterpenoids were absent in both extracts as evidenced by negative Liebermann–Burchard and Salkowski tests. Coumarins were the only phytochemical group detected in both petroleum ether and methanolic extracts. Overall, the results indicate that methanol was more effective than petroleum ether in extracting polar bioactive constituents from *S. grandiflora* leaves, suggesting its

suitability for recovering phytochemicals associated with biological activities such as antioxidant and antimicrobial properties.

Table 1. Qualitative phytochemical constituents detected in petroleum ether and methanolic leaf extracts of *Sesbania grandiflora*.

Sl. No	Phytochemical test	Petroleum ether extract	Methanol extract
1.	**Tests for alkaloids**		
(a)	Dragendroff's test	+	-
(b)	Hager's test	+	-
(c)	Wagner's test	+	-
2.	**Tests for Steroids and Triterpenoids**		
(a)	Libermann-Burchard test	-	-
(b)	Salkowski's test	-	-
3.	**Tests for tannins and phenolic compounds**		
(a)	Ferric chloride test	-	+
(b)	lodine test	-	+
(c)	Nitric acid test	-	+
4.	**Tests for Flavonoids**		
(a)	Shinoda test	-	+
(b)	Alkaline reagent test	-	+
(c)	H_2SO_4 test	-	+
(d)	Lead acetate test	-	+
5.	**Test for cardiac glycosides**	-	+
6.	**Test for Saponins**	-	+
7.	**Tests for Coumarins**	+	+

('+' sign denotes for presence and '-' for absence)

The ferric reducing antioxidant power (FRAP) assay demonstrated a concentration-dependent increase in antioxidant activity for both petroleum ether and methanolic leaf extracts of *Sesbania grandiflora* (Table 2). Among the tested samples, ascorbic acid exhibited the highest reducing activity, with percentage inhibition increasing from 49.09% at 20 µg mL^{-1} to 80.34% at 100 µg mL^{-1}. The methanolic extract showed significantly greater ferric reducing activity than the petroleum ether extract at all tested concentrations, indicating the presence of higher levels of antioxidant constituents. The methanolic extract exhibited percentage inhibition values ranging from 32.57% at 20 µg mL^{-1} to 78.11% at 100 µg mL^{-1}, approaching the activity of the standard ascorbic acid at higher concentrations. In contrast, the petroleum ether extract showed comparatively lower antioxidant activity, with inhibition values increasing from 13.50% at 20 µg mL^{-1} to 60.40% at 100 µg mL^{-1}. The results suggest that antioxidant activity increased proportionally with extract concentration and that the methanolic extract possessed superior reducing power compared to the petroleum ether extract. This enhanced activity may be attributed to the presence of phenolic compounds, flavonoids, and other polar phytochemicals detected in the methanolic extract during phytochemical screening. Overall, the findings indicate that methanol is a more effective solvent for extracting antioxidant constituents from *S. grandiflora* leaves and highlight the potential of the methanolic extract as a natural source of antioxidant compounds.

A consolidated comparison of the antibacterial activity results shows that the methanolic extract exhibited substantially higher antibacterial efficacy than the petroleum ether extract against all tested bacterial strains (Table 3&4). The methanolic extract produced larger inhibition zones, higher inhibition annula values, and greater activity indices at all tested concentrations, indicating superior antimicrobial potency. Escherichia coli was the most susceptible organism, showing the highest inhibition zone of 17 mm and activity index of 0.94 at 10 mg/disc in the methanolic extract, compared with 11 mm and 0.61 in the petroleum ether extract. Similarly, Pseudomonas aeruginosa exhibited moderate susceptibility, with the methanolic extract producing an inhibition zone of 13 mm and activity index of 0.68 at 10 mg/disc, whereas the petroleum ether extract showed only 9 mm and 0.47, respectively. Against Staphylococcus

aureus, the methanolic extract demonstrated the strongest antibacterial effect with an inhibition zone of 18 mm, inhibition annulus of 339.12 mm^2, and activity index of 0.90 at 10 mg/disc, while the petroleum ether extract produced a maximum inhibition zone of 10 mm and activity index of 0.50. In both extracts, antibacterial activity increased with increasing concentration, confirming a dose-dependent response. The superior antibacterial performance of the methanolic extract may be attributed to the presence of phenolics, flavonoids, cardiac glycosides, and saponins identified during phytochemical screening, whereas the petroleum ether extract contained only a limited range of phytoconstituents. Overall, the results demonstrate that the methanolic extract of Sesbania grandiflora leaves possesses significantly greater antibacterial activity than the petroleum ether extract and shows promising potential as a natural antimicrobial agent.

DISCUSSION

Medicinal plants continue to serve as valuable sources of bioactive compounds for the prevention and treatment of various diseases. It is estimated that nearly 25% of modern pharmaceuticals are derived directly or indirectly from plant sources, highlighting the importance of phytochemical investigations in drug discovery (Cragg et al., 1997; De Smet, 1997; Shu, 1998). The therapeutic properties of medicinal plants are largely attributed to their secondary metabolites, which exhibit diverse biological activities including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Mitscher et al., 1980). In the present study, the phytochemical profile, antioxidant potential, and antibacterial activity of petroleum ether and methanolic leaf extracts of *Sesbania grandiflora* were evaluated to provide scientific evidence supporting its traditional medicinal use.

The qualitative phytochemical screening revealed considerable differences in the extraction efficiency of petroleum ether and methanol. Methanolic extracts contained phenolics, tannins, flavonoids, cardiac glycosides, saponins, and coumarins, whereas petroleum ether extracts were positive only for alkaloids and coumarins. These findings indicate that methanol is more effective in extracting polar phytochemicals from *S. grandiflora* leaves. Similar observations have been reported by Reji and Alphonse (2013), Dethe et al. (2013), and Malviya et al. (2013), who documented the presence of flavonoids, phenolics, tannins, saponins, and glycosides in various parts of *S. grandiflora*. The greater abundance of phytochemicals in methanolic extracts is consistent with the polarity principle of extraction, whereby polar solvents dissolve and recover a wider spectrum of biologically active compounds than non-polar solvents (Moure et al., 2001).

The presence of phenolic compounds and flavonoids is particularly significant because these metabolites are among the most potent naturally occurring antioxidants. Epidemiological and experimental studies have demonstrated that phenolic compounds contribute to the prevention of oxidative stress-related diseases through their ability to donate hydrogen atoms, chelate metal ions, and scavenge reactive oxygen species (Ness & Powles, 1997; Riboli & Norat, 2003). Flavonoids are known to possess anti-inflammatory, anti-allergic, cardioprotective, and anticancer properties and can inhibit enzymes involved in free radical generation such as lipoxygenase, cyclooxygenase, xanthine oxidase, and NADH oxidase (Das & Pereira, 1990; Younes, 1981; Lattanzio et al., 2009). Likewise, tannins contribute to antimicrobial and antioxidant functions, while saponins exhibit membrane-modulating, anti-inflammatory, and immunomodulatory activities (Shi et al., 2004; Chung et al., 1998). The occurrence of these phytochemicals in *S. grandiflora* leaves therefore supports its traditional use as a medicinal plant and explains many of its reported pharmacological activities.

The antioxidant activity assessed by the FRAP assay demonstrated a concentration-dependent increase in ferric reducing power for both extracts. However, the methanolic extract consistently exhibited higher antioxidant activity than the petroleum ether extract and approached the activity of the standard antioxidant, ascorbic acid, at higher concentrations. The superior antioxidant activity of the methanolic extract can be attributed to its higher content of phenolics and flavonoids, which were absent in the petroleum ether extract. Similar findings have been reported by Panda et al. (2013), Sarkar et al. (2012), and Gowri and Vasantha (2010), who observed significant antioxidant activity in *S. grandiflora* extracts and correlated this activity with phenolic and flavonoid content.

The FRAP assay is widely recognized as a reliable method for evaluating antioxidant capacity because it measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in the formation of a colored complex measurable spectrophotometrically (Pulido et al., 2000). Higher FRAP

values indicate greater reducing power and consequently higher antioxidant potential. The increase in antioxidant activity observed with increasing extract concentration in the present study suggests the concentration-dependent action of antioxidant constituents. Similar concentration-dependent antioxidant responses have been reported in numerous medicinal plants rich in polyphenols (Adedapo et al., 2008; Zheng & Wang, 2001). According to Frei and Higdon (2003), the antioxidant effectiveness of phenolics and flavonoids is primarily due to their redox properties, which enable them to neutralize free radicals, quench singlet oxygen, and inhibit lipid peroxidation. Therefore, the high ferric reducing activity exhibited by the methanolic extract may be directly linked to its rich phytochemical composition. Oxidative stress is implicated in the pathogenesis of numerous chronic disorders including cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative disorders, and premature aging. Natural antioxidants derived from plants are increasingly preferred over synthetic antioxidants because of their lower toxicity and greater safety (Asgarirad et al., 2010). The antioxidant activity demonstrated by *S. grandiflora* leaves in the present study suggests that this plant may serve as a valuable natural source of antioxidant compounds capable of mitigating oxidative damage and associated degenerative diseases.

Table 2. Ferric reducing antioxidant power (FRAP) activity of petroleum ether and methanolic leaf extracts of *Sesbania grandiflora*

Concentration (g/ml)	**% Inhibition**		
	Ascorbic Acid	**Petroleum ether Extract**	**Methanol Extract**
20	49.09	13.5	32.57
40	63.88	22.60	40.70
60	65.5	36.12	57.55
80	71.78	48.03	62.77
100	8034	60.40	78.11

The antibacterial evaluation revealed that both petroleum ether and methanolic extracts inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. However, the methanolic extract showed substantially higher antibacterial activity, as evidenced by larger inhibition zones, higher inhibition annula values, and greater activity indices. The antibacterial activity increased with increasing extract concentration, indicating a dose-dependent response. Among the tested organisms, *Staphylococcus aureus* and *Escherichia coli* were more susceptible to the methanolic extract than *Pseudomonas aeruginosa*. Similar antibacterial activities of *S. grandiflora* have been reported previously by Anantaworasakul et al. (2011), Reji and Alphonse (2013), and Ouattara (2011), who demonstrated inhibitory effects against both Gram-positive and Gram-negative bacterial pathogens.

The stronger antibacterial activity of the methanolic extract can be attributed to the presence of phenolics, flavonoids, tannins, saponins, and glycosides. Phenolic compounds and flavonoids are known to exert antimicrobial effects through disruption of microbial cell membranes, inhibition of nucleic acid synthesis, interference with energy metabolism, and inactivation of essential enzymes (Tsuchiya et al., 1996). Tannins possess the ability to complex with extracellular proteins and bacterial cell wall components, thereby impairing microbial growth and colonization (Chung et al., 1998). Saponins may increase membrane permeability leading to leakage of cellular constituents, while certain alkaloids have also been reported to exhibit antimicrobial activity against a broad range of pathogens (Omulokoli et al., 1997; Alghazeer et al., 2012). The combined action of these phytochemicals likely contributes to the broad-spectrum antibacterial activity observed in the present investigation.

Table 3. Antibacterial activity of petroleum ether leaf extract of *Sesbania grandiflora* against selected bacterial stains

Name of Bacteria	**Diameter of inhibition zone in mm**					**Inhibition annula (mm^2)**	**Activity Index**
	Control		**Extract**				
	Solvent	Ciprofloxacin	2.5	5 mg	10 mg		

			mg							
Escherichia coli	0	18	0	6	11	0	56.52	146.79	0	0.33	0.61
Pseudomonas aeruginosa	0	19	0	5	9	0	105.97	105.97	0	0.26	0.47
Staphylococcus aureus	0	20	5	7	10	43.17	125.6	125.6	0.25	0.35	0.5

Table 4. Antibacterial activity of methanolic leaf extract of *Sesbania grandiflora* against selected bacterial stains

Name of Bacteria	**Diameter of inhibition zone in mm**					**Inhibition annula (mm^2)**			**Activity Index**		
	Control		**Extract**								
	Solvent	Ciprofloxacin	2.5 mg	5 mg	10 mg						
Escherichia coli	0	18	8	12	17	0	169.5	306.93	0.44	0.66	0.94
Pseudomonas aeruginosa	0	19	0	7	13	0	71.43	193.89	0	0.368	0.68
Staphylococcus aureus	0	20	6	11	18	43.17	146.7	339.12	0.3	0.55	0.9

The comparatively lower antibacterial activity of the petroleum ether extract may be due to the absence of several major polar phytochemicals and the presence of only a limited number of secondary metabolites. Similar solvent-dependent variations in antibacterial activity have been documented in medicinal plants, where methanolic and ethanolic extracts generally exhibit stronger antimicrobial activity than non-polar solvent extracts because of their higher extraction efficiency for phenolics and flavonoids (Srinivasan et al., 2001; Moure et al., 2001). Overall, the present findings demonstrate that *Sesbania grandiflora* leaves are a rich source of biologically active secondary metabolites and possess significant antioxidant and antibacterial activities. The superior performance of the methanolic extract in both antioxidant and antimicrobial assays highlights the importance of polar phytochemicals, particularly phenolics and flavonoids, in mediating these biological effects. These results provide scientific validation for the traditional medicinal use of *S. grandiflora* and suggest that its leaves may serve as a promising source of natural antioxidant and antimicrobial agents for future pharmaceutical and nutraceutical applications. Further studies involving quantitative phytochemical analysis, compound isolation, and mechanistic investigations are warranted to identify the specific constituents responsible for the observed bioactivities.

CONCLUSION

The present study provides preliminary scientific validation for the traditional use of *Sesbania grandiflora* leaves as a natural source of antioxidant and antibacterial agents. Phytochemical screening revealed the presence of several bioactive secondary metabolites, including alkaloids, phenolics, flavonoids, tannins, saponins, cardiac glycosides, and coumarins, which are known to contribute to various therapeutic effects. Among the tested extracts, the methanolic extract exhibited superior antioxidant and antibacterial activities, indicating its higher efficiency in extracting biologically active compounds. These findings highlight the medicinal potential of *S. grandiflora* leaves and support their use in promoting health and managing diseases. However, further studies involving extraction, purification, separation, crystallization, and characterization of the active constituents are necessary to identify the compounds responsible for these bioactivities and to explore their pharmaceutical applications.

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Conflict of interest
The authors declares that there is no potential conflict for the study

REFERENCES

- Adedapo, A. A., Jimoh, F. O., Afolayan, A. J., & Masika, P. J. (2008). Antioxidant properties of the methanol extracts of the leaves and stems of *Celtis africana*. *Records of Natural Products, 2*(1), 23-31.
- Alghazeer, R., El-Saltani, H., Saleh, N., Al-Najjar, A., & Hebail, F. (2012). Antioxidant and antimicrobial properties of five medicinal Libyan plants extracts. *Natural Science, 4*(5), 324-335.https://doi.org/10.4236/ns.2012.45046
- Anantaworasakul, P., Chaiyana, W., & Phongpradist, R. (2011). Antibacterial activities of *Sesbania grandiflora* bark extracts. *Journal of Medicinal Plants Research, 5*(22), 5178-5183.
- Anantaworasakul, P., Chongsiriwatana, N. P., Tiyaboonchai, W., & Chaiyana, W. (2011). Antibacterial activities of *Sesbania grandiflora* bark extracts. *Journal of Medicinal Plants Research, 5*(22), 5178-5183.
- Asgarirad, H., Abdollahi, M., & Mohammad, K. (2010). Antioxidant activity and protective effects of medicinal plants. *Iranian Journal of Pharmaceutical Research, 9*(4), 375-382.
- Barry, A. L., & Thornsberry, C. (1991). Susceptibility tests: Diffusion test procedures. In A. Balows, W. J. Hausler Jr., K. L. Herrmann, H. D. Isenberg, & H. J. Shadomy (Eds.), *Manual of clinical microbiology* (5th ed., pp. 1117-1125). American Society for Microbiology.
- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition, 38*(6), 421-464. https://doi.org/10.1080/10408699891274273
- Chung, Y. C., Chang, C. T., Chao, W. W., Lin, C. F., & Chou, S. T. (2002). Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *Journal of Agricultural and Food Chemistry, 50*(8), 2454-2458. https://doi.org/10.1021/jf011369q
- Cragg, G. M., Newman, D. J., & Snader, K. M. (1997). Natural products in drug discovery and development. *Journal of Natural Products, 60*(1), 52-60. https://doi.org/10.1021/np9604893
- Das, N. P., & Pereira, T. A. (1990). Effects of flavonoids on thermal autooxidation of palm oil: Structure–activity relationships. *Journal of the American Oil Chemists' Society, 67*(4), 255-258.
- De Smet, P. A. G. M. (1997). The role of plant-derived drugs and herbal medicines in healthcare. *Drugs, 54*(6), 801-840.
- Dethe, S., Bhaskar, V. H., & Thakurdesai, P. A. (2013). Phytochemical screening of *Sesbania grandiflora* and *Pistia stratiotes*. *International Journal of Pharmaceutical Sciences and Research, 4*(10), 3894-3898.
- Dewick, P. M. (2002). *Medicinal natural products: A biosynthetic approach* (2nd ed.). John Wiley & Sons.
- Frei, B., & Higdon, J. V. (2003). Antioxidant activity of tea polyphenols in vivo: Evidence from animal studies. *Journal of Nutrition, 133*(10), 3275S-3284S. https://doi.org/10.1093/jn/133.10.3275S
- Gowri, S. S., & Vasantha, K. (2010). Free radical scavenging and antioxidant activity of leaves from *Sesbania grandiflora* (L.) Pers. *International Journal of PharmaTech Research, 2*(2), 981-986.
- Joy, P. P., Thomas, J., Mathew, S., & Skaria, B. P. (1998). *Medicinal plants*. Kerala Agricultural University.
- Kalyanagurunathan, S., Radhakrishnan, M., & Natarajan, S. (1985). Flavonoid constituents of *Sesbania grandiflora* flowers. *Phytochemistry, 24*(9), 2171-2172.
- Kashyap, P., & Mishra, A. (2012). Pharmacological importance of *Sesbania grandiflora*: A review. *International Journal of Pharmaceutical Sciences Review and Research, 16*(2), 90-94.
- Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2009). *Pharmacognosy* (42nd ed.). Nirali Prakashan.
- Lattanzio, V., Lattanzio, V. M. T., & Cardinali, A. (2009). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In F. Imperato (Ed.), *Phytochemistry: Advances in research* (pp. 23-67). Research Signpost.
- Malviya, N., Jain, S., & Malviya, S. (2013). Medicinal attributes of *Sesbania grandiflora*: A review. *Asian Journal of Pharmaceutical and Clinical Research, 6*(4), 1-7.
- Mitscher, L. A., Leu, R. P., Bathala, M. S., Wu, W. N., & Beal, J. L. (1980). Antimicrobial agents from higher plants. *Lloydia, 35*(2), 157-166.
- Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., Núñez, M. J., & Parajó, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*(2), 145-171. https://doi.org/10.1016/S0308-8146(00)00223-5
- Ness, A. R., & Powles, J. W. (1997). Fruit and vegetables and cardiovascular disease: A review. *International Journal of Epidemiology, 26*(1), 1-13.
- Omulokoli, E., Khan, B., & Chhabra, S. C. (1997). Antiplasmodial activity of four Kenyan medicinal plants. *Journal of Ethnopharmacology, 56*(2), 133-137.
- Ouattara, M. B. (2011). Antibacterial and antioxidant activities of *Sesbania grandiflora* used in traditional medicine. *African Journal of Pharmacy and Pharmacology, 5*(24), 2713-2718.
- Panda, C., Dash, S. K., & Sahoo, S. (2013). Free radical scavenging activity and phenolic content estimation of *Sesbania grandiflora*. *International Journal of Pharmaceutical Sciences and Research, 4*(6), 2243-2248.
- Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry, 48*(8), 3396-3402. https://doi.org/10.1021/jf9913458

- Rajasekaran, S., & Murugesan, S. (2001). Antibacterial activity of medicinal plant extracts by disc diffusion method. *Indian Journal of Pharmaceutical Sciences, 63*(5), 421-424.
- Reji, A. F., & Alphonse, R. (2013). Preliminary phytochemical evaluation and antimicrobial investigations of *Sesbania grandiflora*. *International Journal of Current Pharmaceutical Research, 5*(3), 19-22.
- Riboli, E., & Norat, T. (2003). Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition, 78*(3), 559S-569S.
- Sarkar, B. K., Chakraborty, D., & Das, A. (2012). Antioxidant activity of *Sesbania grandiflora* flower extracts. *International Journal of Pharmacy and Pharmaceutical Sciences, 4*(3), 298-302.
- Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from edible legumes: Chemistry, processing, and health benefits. *Journal of Medicinal Food, 7*(1), 67-78.
- Shu, Y. Z. (1998). Recent natural products-based drug development: A pharmaceutical industry perspective. *Journal of Natural Products, 61*(8), 1053-1071.
- Siddiqui, A. A., & Ali, M. (1997). *Practical pharmaceutical chemistry* (1st ed.). CBS Publishers & Distributors.
- Srinivasan, D., Nathan, S., Suresh, T., & Lakshmana Perumalsamy, P. (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *Journal of Ethnopharmacology, 74*(3), 217-220.
- Trease, G. E., & Evans, W. C. (2009). *Trease and Evans pharmacognosy* (16th ed.). Saunders Elsevier.
- Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T., & Iinuma, M. (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *Journal of Ethnopharmacology, 50*(1), 27-34.
- Younes, M. (1981). Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. *Planta Medica, 43*(3), 240-245.
- Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*(11), 5165–5170. https://doi.org/10.1021/jf010697n