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Research article

Phytochemical analysis and antioxidant activity of the leaf extract of *Sesbania grandiflora* (L.) Poiret

Arjun Pandian[1]*, Azhagu Madhavan Sivalingam[2], Govindan Lakshmanan[3], Ruchi Badoni Semwal[4], J. Selvakumari[5] and Deepak Kumar Semwal[6]

[1]Department Research and Innovation, Institute of Biotechnology, Saveetha School of Engineering (SSE), Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai 602105, Tamil Nadu, India.
[2]Department of Community Medicine, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai 602105, Tamil Nadu, India.
[3]Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India.
[4]Department of Chemistry, VSKC Government Postgraduate College, Dakpathar, Dehradun, Uttarakhand, India.
[5]Integrated Disease Surveillance Program, Puducherry 605001, India.
[6]Department of Phytochemistry, Faculty of Biomedical Sciences, Uttarakhand Ayurved University, Harrawala, Dehradun-248001, India.

*Corresponding author. E-mail: arjungri@gmail.com

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ABSTRACT

Sesbania grandiflora (L.) Poiret, which is a member of the Fabaceae family and is also known as Vegetable hummingbird, Agati, Katurai, or West Indian pea, has a variety of medicinal uses. As a result, the current study concentrated on phytochemical analysis and antioxidant assays (DPPH, ABTS, superoxide, and OH) of methanol, ethanol, and water extracts of the leaf. The present findings revealed that the highest total phenolic content was found in methanol extract (62.06±1.02 mg GAE/g) followed by ethanol (60.89±0.13 mg GAE/g) and water (61.87±1.96 mg GAE/g). The maximum flavonoid content was recorded in the methanol extract (74.32±1.02 mg catechin equivalents (CE)/g) followed by ethanol (72.05±1.02 mg CE/g) and water (60.44±1.05 mg CE/g). Similarly, the methanol extract (522.21±1.06 mg oleanolic acid equivalents (OAE)/g) showed the highest saponin content followed by ethanol (517.34±1.21 mg OAE/g) and water (450.21±0.75 mg OAE/g). Among four antioxidant assays used in the present study, the highest DPPH free radical scavenging was found in methanol leaf extract (91.22±1.45%) followed by ethanol (85.55±1.11%) and water extract (77.85±1.25%). The ABTS assay also showed maximum scavenging in methanol extract (88.45±1.32%) followed by ethanol extract (79.45±1.25%). The superoxide anion radical and hydroxyl radical scavenging assays also showed the highest activity in the methanol extract (90.12±1.12%) and the lowest (20.21±1.33%), followed by ethanol (85.55±1.26%) and lowest (15.56±1.34%), OH method main amount in methanol (88.95±1.21%) and lowest (20.12±1.46%), followed by ethanol (78.60±1.25%) and lowest (16.32±1.45%). Using ascorbic acid and quercetin as standards and calculated IC_{50} values, our research found that *S. grandiflora* leaf has potential medicinal qualities, and targeted chemicals can use in pharmaceutical and pharmacological aspects.

INTRODUCTION

The plant *Sesbania grandiflora*, which is a member of the Fabaceae family, is used as a folkloric or traditional medicine to cure a variety of illnesses, including diarrhoea, stomatitis, fever, smallpox, sore throat, headache, etc. Siddha and Ayurveda, two traditional medical systems in India, also employ this herb to treat a variety of acute and chronic illnesses (Yasotha et al., 2022). The dried leaves, which are frequently used to make tea, are thought to have beneficial contraceptive, antibacterial, and antihelmintic properties. In the folkloric method, a poultice made from leaf juice works well to cure injuries. The leaf is frequently utilised during the manacle detoxification procedure (Vinothini et al., 2017).

The *S. grandiflora* leaves have been shown to contain considerable levels of proteins, lipids, carbohydrates, fibres, and minerals including iron, calcium, and phosphorus. Young leaves may be eaten and are frequently added to meals (Arjun et al., 2017). Additionally, the herb has been noted to be an effective remedy for ailments brought on by tobacco and smoking. Numerous papers indicate sterol, saponin, and tannin separation from plant leaves, flowers, and aerial parts. These bioactive

components are expected to have significant biological effects, including hepatoprotective, antibacterial, antifungal, antioxidant, antiurolithiatic, anticonvulsant, and anxiolytic properties (Saranya et al., 2023). The locals in Bangladesh's Tangail region utilise a sweetened concentrated juice made from the leaves as a treatment for diarrhoea. *S. grandiflora* crude ethanolic extracts used orally for shortening defecation episodes' frequency and length (Sundaram et al., 2021).

Extract from *S. grandiflora* leaves is said to have significant antimicrobial, antioxidant, antiurolithiatic, anticonvulsant, anxiolytic, and hepatoprotective activities (Ashokkumar et al., 2022). In oxidation, which can harm proteins, lipids, or DNA in tissues and lead to chronic degenerative illnesses including cancer, diabetes, and cardiovascular ailments, free radicals play a crucial role. By providing free radicals electrons and disguising them as innocuous particles, antioxidants are chemical neutralizers that reduce oxidative damage to biological processes. Free radicals and oxidative stress are often linked. Free radicals are produced when oxygen interacts with particular molecules, and once they are, they pose a hazard because of the potential harm they may do when they interact with crucial cellular components including DNA and proteins as well as the cell membrane (Siddartha et al., 2020). By interacting with free radicals and neutralising them, antioxidants might potentially halt damage before it even occurs. There are many different types of antioxidants among the secondary metabolites created by plants (Moon and Shibamoto, 2009).

Aiming to evaluate the total phenol, flavonoid, and saponin contents as well as the various types of antioxidant activity i.e., DPPH, ABTS, Superoxide, and Hydroxyl potential, the current study focused on the methanol, ethanol, and water leaf extract of *S. grandiflora* plant.

MATERIALS AND METHODS

Plant collection and extraction

The fresh *S. grandiflora* (50 g) leaves were collected, and the collected leaves were powdered and used to make ethanol, methanol, and water extracts. The liquid was then condensed using a rotary evaporator, and the crude extract was kept chilled for use in further studies (Arjun, 2011; Azhagu Madhavan, 2021).

Quantitative phytochemical analysis

The total saponin content (TSC), total flavonoid content (TFC), and total phenolic content (TPC) were calculated using conventional methods as described previously (Vinothini et al. 2017).

In vitro antioxidant activities

DPPH assay

The extracts were added to 1.0 mL of a DPPH solution (0.1 mM) in MeOH in varied doses (200-1000 mg/mL). After around 30 minutes of incubation, the absorbance at 517 nm was calculated. The difference in absorbance on DPPH was used to assess the percentage of inhibition, a plot of the percent of inhibition vs. the concentration of the sample solutions was used to estimate the IC_{50} values, or the concentration required to scavenge 50% of the free radical (Nagarajan et al., 2012). For comparison, ascorbic acid was utilised as a reference. The formula, inhibition (%) = [(A0 A)/A0] × 100 was used for calculation.

ABTS radical ion decolourization assay

The reaction is stoichiometric and produces a colour which is called 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) that lasts for more than one hour. To make $ABTS^{\bullet+}$, which was subsequently multiplied by five using phosphate buffer pH 7.0 (0.02 mM), 5.0 mL of ABTS solution (1.8 mM) was combined with 1.25 mL of potassium persulfate (2.0 mM). After that, samples in methanol at varying concentrations (200-1000 mg/mL) were combined with 3.6 mL of ABTS solution, and the combination was kept in the dark for 10 minutes. At 734 nm, the absorbance was measured. The ABTS scavenging impact was calculated as a percentage of ABTS scavenging using the equation, inhibition% = [(OD of control - OD of sample)/OD of control] ×100 (Arjun, 2011).

Superoxide anion radical scavenging activity

In order to create superoxide radicals, the samples (200-1000 mg/mL) were added to a reaction mixture containing EDTA (100 L; 30 mM; pH 7.4), hypoxanthine (10 L; 30 mM) in NaOH (50 mM), and nitroblue tetrazolium (NBT) (200 L; 1.42 mM). After the solution had been incubated at room temperature for 20 minutes, the absorbance was measured at 560 nm. The sample (A2) was added to the reaction mixture that scavenged $O_2^{\bullet-}$ to prevent NBT reduction and utilise quercetin as a standard. When absorbance was measured, the ratio of the sample (A2) to the control (A1) showed a decrease in $O_2^{\bullet-}$ levels. The superoxide anion ($O_2^{\bullet-}$) radical scavenging activity was calculated using the formula: SRSA% = (A2/A1/A1)/100 (Arjun, 2011).

Hydroxyl radical scavenging activity

Modest alteration to the hydroxyl radical ($OH^{\bullet}$) (200-1000 mg/mL), the deoxyribose assay was performed in phosphate buffer (10 mM; pH 7.4) with deoxyribose (2.5 mM), H_2O_2 (1.5 mM), $FeCl_3$ (100 M), EDTA (104 M), and the test sample (0.5 mg/mL). The reaction was initiated by adding ascorbic acid up to a final concentration of 100 M. Using ascorbic acid as a reference, the reaction mixture was heated for 30 minutes at 80°C before being incubated for an hour at 37°C in a water bath to produce the colour. After the incubation time, thiobarbituric acid (0.5%), trichloroacetic acid (2.8%), and NaOH (25 mM) were added. During the control process (A1), the sample (A2) was refrigerated on ice, and the absorbance was measured at 532 nm. HRSA% is equal to (A1/A2/A1)/100 (Azhagu Madhavan, 2021).

Statistical analysis

Data were submitted to an analysis of variance (ANOVA) using a split plot. The Tukey's honestly

significant difference (HSD) test was performed to compare means using the R programming language (p 0.05) and the agricolae package. The antioxidant activity of total saponins, flavonoids, and phenolic content was evaluated using the corrplot tool in R programming (Ali et al., 2021).

RESULTS

We measured the concentrations of phenol, flavonoids, and saponins in methanol, ethanol, and water. The amount of phenolic content in methanol was greatest (62.06±1.02 mg GAE/g), followed by ethanol (60.89±0.13 mg GAE/g), the amount of flavonoid content in methanol was next (74.32±1.02 mg CE/g), then ethanol (72.05±1.02 mg CE/g), and the amount of saponin content in methanol was highest (526.76±2.21 mg OAE/g), then ethanol (520.12±1.43 mg OAE/g) (Table 1).

Table 1. *S. grandiflora* quantitative analysis of different leaf extracts

S.No.	Phytochemicals	Methanol	Ethanol	Water
1	Saponins (mg OAE/g)	522.21 ±1.06	517.34 ±1.21	450.21 ±0.75
2	Flavonoids (mg CE/g)	74.32 ±1.02	72.05 ±1.02	60.44 ±1.05
3	Phenols (mg GAE/g)	62.06 ±1.02	60.89 ±0.13	61.87 ±1.96

DPPH radical scavenging activity

The DPPH converted the unpaired electrons to paired electrons by producing hydrazine because of the extract's ability to donate hydrogen. Leaf extracts were utilised in ethanol, methanol, and water at different doses (200–1000 mg/mL) for the free radical scavenging experiment. Methanol (M) extract demonstrated the highest radical scavenging (91.22±1.11%) and the lowest (18.55±1.21%) followed by ethanol (E) extract uppermost radical scavenging (85.55±1.12%) and lowly (12.32±1.03%), and water (W) radical scavenging (77.85±1.31%), lowest (11.21±1.12%), IC_{50} values were calculated (M – 243 mg/mL; E - 278 mg/mL; W - 280 mg/mL; As - 43 mg/mL). When ethanol, water, and methanol leaf extracts were compared; ascorbic acid (Aa) was employed as the reference standard (Fig. 1), and methanol leaf extract showed the highest overall scavenging activity.

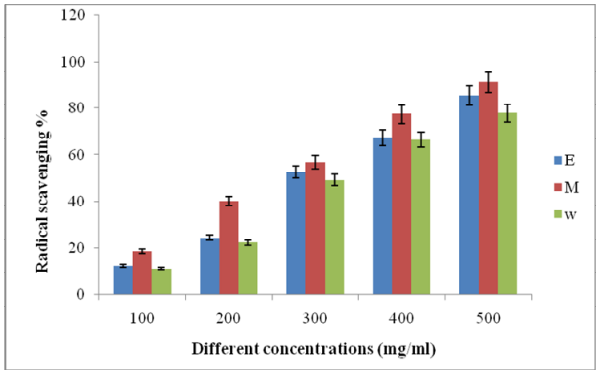

Fig. 1. DPPH antioxidant activities of *S. grandiflora* leaf extracts. E - Ethanol, M - Methanol, W - Water

ABTS radical cation decolourization assay

The $ABTS^{+}$ radical scavenging ability is a crucial method for evaluating antioxidant capability. In ABTS scavenging tests, ABTS, E, M, and W extracts were utilised at varying doses (200–1000 mg/mL) (Fig. 2). When ethanol, water, and other comparisons were made, M extract demonstrated the highest radical scavenging of leaf extract (88.45±1.15%), followed by E extract which established the utmost radical scavenging (79.45±1.31%) and the lowest (11.21±1.32%), W extract which showed the highest radical scavenging (76.54±1.12%) and the lowest (10.21±1.72%), IC_{50} values were calculated (M - 268 mg/mL; E - 328 mg/mL; W - 317 mg/mL; Q - 41 mg/mL) and M extract demonstrated the highest radical scavenging activity.

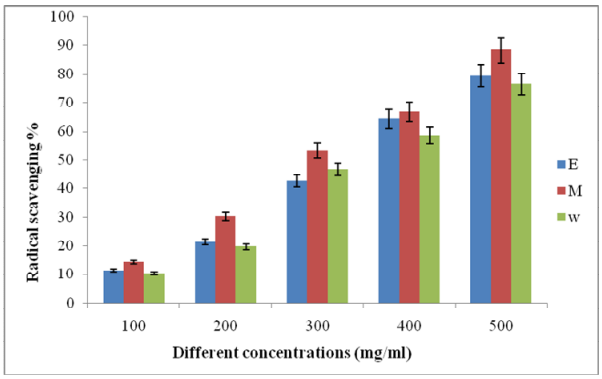

Fig. 2. ABTS antioxidant activities of *S. grandiflora* leaf extracts. E - Ethanol, M - Methanol, W - Water

Superoxide anion radical scavenging activity

Superoxide anion ($O_2^{\bullet-}$) can be created from oxygen (O_2) by a variety of mechanisms, such as oxidation by NADPH oxidase, xanthine or hypoxanthine oxidase. The free radical scavenging experiment was conducted using leaf extracts at various concentrations of ethanol, methanol, and water (200–1000 mg/mL). The highest radical scavenging (90.12±1.21%) and the lowest radical scavenging (20.21±1.05%) were confirmed by the M extract, whereas the highest radical scavenging (85.55±1.15%) and the lowest radical scavenging (15.56±1.32%) were revealed by the E extract, and the highest radical scavenging (80.95±1.12%) and lowest radical scavenging (12.34±1.21%) were discovered in the W extract. IC_{50} values were calculated (M - 227 mg/mL; E - 310 mg/mL; W - 328 mg/mL; Q - 48 mg/mL) and found that M leaf extract had the strongest scavenging effects, with quercetin serving as the reference standard (Fig. 3).

OH radical scavenging activity

The strongest and most frequent reactive oxygen species (ROS) that cause *in vivo* cell damage are hydroxyl radicals, which are created by the body during aerobic metabolism. Leaf extracts with varying amounts of E, M, and W were employed for the free radical scavenging experiment. M leaf extract displayed the highest (90.45±1.42%) and lowest (21.05±1.31%) levels of radical scavenging, followed by E extract, which had the highest

(86.32±2.13%) and the lowest levels (18.56±1.73%). W extract had the highest levels (82.65±1.83%) and the lowest levels (15.21±1.34%), and M leaf extract was used as the primary scavenging activity for comparisons between E and W. Calculated IC_{50} values include (M – 229 mg/mL; E - 287 mg/mL; W - 295 mg/mL; and Q - 40 mg/mL), the reference standard was ascorbic acid (Fig. 4).

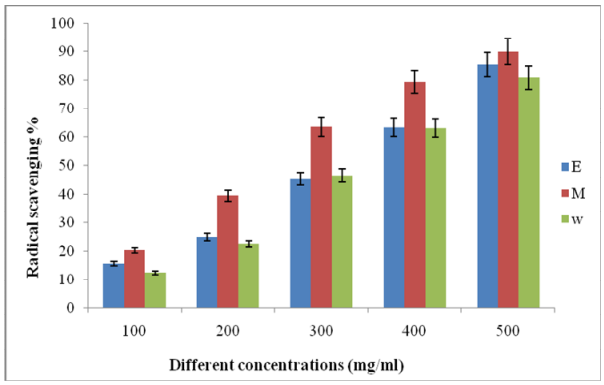

Fig. 3. Superoixde antioxidant activities of *S. grandiflora* extracts. E - Ethanol, M - Methanol, W - Water

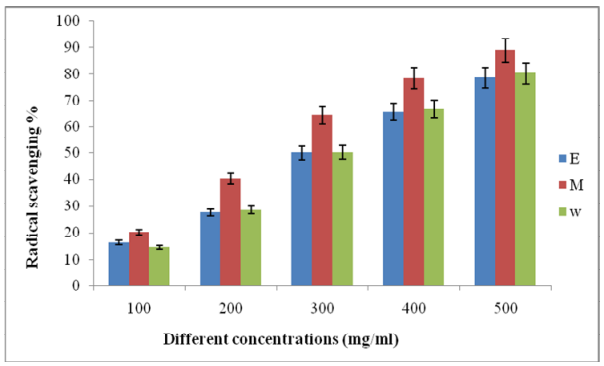

Fig. 4. OH antioxidant activities of *S. grandiflora* leaf extracts. E - Ethanol, M - Methanol, W - Water

DISCUSSION

Free radicals are produced in large quantities during respiration and other activities. These free radicals may injure the organism in numerous ways, leading to functional loss and occasionally even death (Khlebnikov et al., 2007). Utilising antioxidants, which are substances that can prevent other molecules from oxidising, might lessen damage brought on by ROS. Antioxidants are helpful in reducing and avoiding future damage through free-radical reactions because they can supply electrons that may neutralise radical formation (Halliwell, 2012). Several plants produce secondary metabolites that act as antioxidants and are essential to several biological processes, including polyphenols and flavonoids (Nichols and Katiyar, 2010).

By providing free radicals electrons and disguising them as innocuous particles, antioxidants are chemical neutralizers that reduce oxidative damage to biological processes. Free radicals and oxidative stress are often linked. Free radicals are produced when oxygen interacts with particular molecules, and once they are, they pose a hazard because of the potential harm they may do when they interact with crucial cellular components including DNA and proteins as well as the cell membrane (Archana et al., 2020; Semwal et al., 2020). By interacting with free radicals and neutralising them, antioxidants might potentially halt damage before it even occurs, there are many different types of antioxidants among the secondary metabolites formed by plants (Moon and Shibamoto, 2009).

The maximum UV-visible absorbance of the DPPH free radical, which interacts with hydrogen compounds, is 515 nm. The method depends on antioxidants scavenging DPPH, which after a reduction step decolorizes the solution of DPPH methanol. In this assay, the antioxidant's ability to lower the DPPH radical is determined. The TEAC test also assesses total radical scavenging capacity. The test measures how much of an ABTS radical (2,20-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) is scavenged in comparison to Trolox over a predetermined period of time (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The overall radical scavenging capacity of the specimen may be calculated by comparing the reduction across absorbency to Trolox absorbance at 734 nm (Amrulloh et al., 2021). The flaw in this method is that it inhibits oxidation rather than allowing test materials to react with ABTS radicals, which is what the value of TEAC reflects. The ability of a plant extract to reduce Fe^{3+}-tripyridyltriazine into Fe^{2+}-tripyridyltriazine is assessed using the FRAP (ferric-reducing antioxidant properties) technique (Kurniawan et al., 2021).

Testing is done on electron-transfer procedures using the ferric salt potassium ferricyanide as an oxidant. The reaction process is the oxidation of ferric 2,4,6-tripyridyl-s-triazine to the vivid ferrous state. Defects in oxidative phosphorylation can lead to an increase in ROS production, and ROS-mediated biomolecule degradation can actually affect the parts of the electron transport chain (Kedare and Singh, 2011).

CONCLUSION

Antioxidants help to neutralise free radicals, which are the main source of inflammatory disorders and may thus work to prevent illnesses brought on by free radicals. *S. grandiflora* can be utilised for many medical ailments and may be helpful in inflammatory diseases. To determine the active components of *S. grandiflora* for use in medicine, additional investigation will be required. With the use of several preclinical types of research, activity-guided extraction of different phytochemicals aims to ascertain their antioxidant capacity as well as other disease-curing properties.

CONFLICTS OF INTEREST

The author(s) declare(s) no conflicts of interest.

DECLARATION

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