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Research Article

Phytochemical screening and evaluation of *in vitro* antioxidant activity of different fractions of bark extracts of *Stereospermum suaveolens*

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ABSTRACT

Purpose: This study aimed at evaluating the antioxidant capacity of methanolic bark extracts and fractions obtained from silica gel column chromatography of the methanolic bark extracts of *Stereospermum suaveolens*.

Methods: Air-dried powdered *S. suaveolens* bark was extracted with methanol and concentrated to obtain crude extracts. The crude bark extracts and the fractions obtained from silica gel column chromatography were screened for the presence of different groups of phytochemicals and for the determination of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power assays. Statistical significance was evaluated using an independent sample t-test, and $p < 0.05$ was considered statistically significant. The half-maximal inhibitory concentration (IC_{50}) values for the DPPH assay were calculated using GraphPad Prism 9 software.

Results: The presence of flavonoids, saponins, and phenolic compounds was detected in the bark extracts. The fractionation of the bark extract resulted in seven bark fractions (F1B to F7B), with the highest antioxidant activity detected in the 3rd fraction F3B (82.51%). The ferric reducing antioxidant power assays of both *S. suaveolens* bark extracts and L-ascorbic acid increased with concentration, and a statistically significant difference ($p < 0.05$) was observed between the bark extracts of *S. suaveolens* and L-ascorbic acid, except at a concentration of 15.62 µg/mL. The IC_{50} values determined using the DPPH method for the methanolic bark extracts of *S. suaveolens* and L-ascorbic acid were found to be 5.46 ± 0.56 µg/mL and 2.82 ± 0.23 µg/mL, respectively, indicating that the antioxidant activity of L-ascorbic acid was greater than that of the bark extracts ($p = 0.0147$).

Conclusion: The bark extracts and the fractions obtained from column chromatography exhibited antioxidant activity, indicating that the bark of *S. suaveolens* has the potential to serve as an antioxidant.

Keywords: antioxidant, bark extracts, fractions, L-ascorbic acid, *Stereospermum suaveolens*

INTRODUCTION

The widespread use of herbal remedies and healthcare preparations has been traced to the occurrence of natural medicines possessing therapeutic and pharmacological effects. (1) Therefore, the World Health Organization is now encouraging the use of herbal medicines, as they have been traditionally used for centuries, with a growing emphasis on scientific justification for their use. (2)

The human body is constantly exposed to oxidants and free radicals generated during physiological processes. These free radicals also act as signalling molecules within animal cells, playing important roles in apoptosis, gene expression, and ion transportation. (3) The body has an effective antioxidant defence system to balance free radical production and oxidative stress, employing both enzymatic and non-enzymatic antioxidants. However, when this system malfunctions, excessive free radical production can occur, leading to damage of biomolecules such as DNA, proteins, and lipids. This damage can disrupt redox-sensitive metabolic and signalling pathways, contributing to various pathological conditions. (4)

Antioxidants are defined as substances that neutralise free radicals. They enhance the body's antioxidant defences or can be supplemented externally, making them a promising approach to mitigating diseases associated with oxidative stress (5) Literature suggests that medicinal plants could serve as valuable sources of effective antioxidants. Various plant constituents, such as phenolic acids, flavonoids, terpenes, tocopherols, vitamin C (ascorbic acid), and carotenoids (including beta-carotene), are believed to contribute to their antioxidant capabilities. (6)

Stereospermum suaveolens, a member of the family Bignoniaceae, is a medicinal plant widely used in traditional Ayurvedic medicine. Its bark is utilised as an analgesic, antidyspeptic, liver stimulant, treatment for asthma, remedy for semen debility, and for wound healing (7,8). Additionally, the plant is widespread across the moist regions of Bangladesh, India, Sri Lanka, and Myanmar. However, existing literature indicates a lack of scientific records on the antioxidant properties of this plant. (9,10) Therefore, the present study was undertaken to evaluate the antioxidant potential of the bark extracts of *S. suaveolens* and its fractions obtained from silica gel column chromatography.

METHODS

Collection of raw material

The fresh stem bark of *S. suaveolens* was collected and authenticated at the Government Herbal Garden, Navakkiri, Jaffna, Sri Lanka. The bark was then washed thoroughly with water and shade-dried at room temperature until a constant weight was obtained. Subsequently, it was mechanically crushed and ground into a fine powder. The dried powder was stored in an airtight container for future use.

Preparation of the crude extract

The extraction was performed in small scale using 50 g of dried bark powder, which was extracted with 200 mL of methanol and subjected to sonication (ultrasound-assisted extraction) at 30°C for four 60-minute periods (4 hours). The extracts were filtered through Whatman grade 1 filter paper under a vacuum using a Büchner funnel. The filtrate was then concentrated using a rotary evaporator under reduced pressure at 40°C. The concentrated extract was dried in a freeze dryer, weighed, and stored at 4°C in an airtight container until further analysis.

Preparation of fractions using silica gel column chromatography

Silica gel 60 (230–400 mesh) was used as the stationary phase in normal-phase column chromatography. The residue (800 mg) was loaded into a silica column (height: 20 cm, internal diameter: 20 mm) using the dry loading method. A gradient solvent system (Table 1) was used as the mobile phase, yielding seven bark fractions (F1B to F7B) through column chromatographic separation.

Thin-layer chromatography analysis

All fractions were subjected to thin-layer chromatography (TLC) analysis. Detection was performed using a UV-visible detector at short wavelength (254 nm) and long wavelength (366 nm). The bands were then marked with a pencil. All fractions were concentrated under reduced pressure and tested for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay at a concentration of 200 µg/mL.

DPPH Radical-Scavenging Assay

A fresh DPPH stock solution was prepared at a concentration of 0.1 mM. Stock solutions of bark extracts and L-ascorbic acid were prepared at a concentration of 500 µg/mL. From these stock solutions, different concentrations (7.81–250 µg/mL) of sample solutions and L-ascorbic acid solutions were prepared. Additionally, 200 µg/mL fraction solutions were prepared from stock solutions with a concentration of 2000 µg/mL. A 1 mL volume of 0.1 mM DPPH solution was added to 3 mL of the control (without the test compound but containing an equivalent amount of methanol) and 3 mL of test solutions at different concentrations (7.81–250 µg/mL) and fractions (200 µg/mL) in separate test tubes. The reaction mixture was agitated using a vortex mixer and incubated in a dark room for thirty minutes. After incubation, the resulting-colored solutions were measured for absorbance at 517 nm. L-ascorbic acid was used as positive control.

The concentration of extract at which the maximum percentage inhibition of DPPH radical occurred, and the half-maximal inhibitory concentration (IC_{50}) values were determined using a standard calibration curve. The percentage of DPPH radical scavenging activity was determined by comparing the absorbance of the sample to that of the control and expressing the reduction in absorbance as a percentage of the control. (11)

Ferric reducing antioxidant power assay

A stock solution of 500 µg/mL bark extract was prepared by dissolving the crude residue in methanol. A total of 10 mg of L-ascorbic acid was dissolved in methanol µg/mL) in separate test tubes. The reaction and diluted to 20 mL to obtain a stock concentration of 500 µg/mL.

Stock solution of 500 µg/mL concentration of bark extracts was prepared by dissolving the crude residue in methanol. A weight of 10 mg L-Ascorbic acid was dissolved in methanol and made up to 20 mL to prepare a stock concentration of 500 µg/mL. From the stock solutions, different concentration (7.81-250 µg/mL) of sample solutions and L-Ascorbic acid solutions were prepared. Here, the L-Ascorbic acid was used as standard. Different concentrations of 1 mL methanolic bark extract of *S. suaveolens* were added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was mixed thoroughly using a vortex mixture and incubated at 50°C for 20 minutes in a water bath. At the end of the incubation period, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 minutes. The supernatant (2.5 mL) was mixed with 2.5 mL of

Table 1. Gradient solvent system used for column chromatography

Solvent system	Solvent ratio	Total volume added (mL)
Hexane	100	20
Hexane: Ethyl acetate	90:10	20
Hexane: Ethyl acetate	80:20	25
Hexane: Ethyl acetate	70:30	20
Hexane: Ethyl acetate	60:40	25
Hexane: Ethyl acetate	50:50	30
Hexane: Ethyl acetate	40:60	25
Hexane: Ethyl acetate	30:70	20
Hexane: Ethyl acetate	20:80	25
Hexane: Ethyl acetate	10:90	20
Ethyl acetate	100	20
Methanol	100	30

deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance of the resulting-coloured solutions was measured at 700 nm against the blank using a UV spectrophotometer. (12)

Determination of antioxidant activity by TLC-bioautography

A total of 12.5 mg of DPPH was weighed using an analytical balance, dissolved in methanol, and diluted to 25 mL in a volumetric flask. The volumetric flask was then covered with aluminium foil to prevent light exposure. A small amount of bark extract was spotted onto a TLC plate using a capillary tube, and the plate was dipped into a 0.05% w/v DPPH solution in methanol. It was then kept at room temperature. The appearance of a yellow spot on a purple background indicated antioxidant activity.

Phytochemical screening

A small portion of the crude bark extract was used for phytochemical screening to detect the presence of various compounds, including alkaloids, proteins, glycosides, phenolic compounds, flavonoids, tannins, saponins, terpenoids, and steroids. These tests were conducted according to their respective methods: Wagner's test (alkaloids), Ninhydrin test (proteins), Legal's test (glycosides), Lead acetate test (phenolic compounds and flavonoids), Gelatin test (tannins), Foam test (saponins), Salkowski test (terpenoids), and Liebermann-Burchard test (steroids). (13)

Statistical analysis

All experiments for each sample were conducted in triplicate, and the results were expressed as the mean value ± standard deviation (n=3). Test samples were statistically compared with the control using an independent samples t-test in SPSS version 25.0, with $p < 0.05$ considered statistically significant. The IC_{50} values for the DPPH assay were calculated using GraphPad Prism 9 software.

RESULTS

Yield of the extracts and fractions

A total of 2.62 g of crude *S. suaveolens* bark extract was obtained after extraction. The weights of the seven different bark fractions and are presented in Table 2.

Table 2. Yields of crude bark extracts and bark fractions of *S. suaveolens*

Bark Fractions	Obtained weight (g)
F_1B	0.09
F_2B	0.12
F_3B	0.15
F_4B	0.08
F_5B	0.09
F_6B	0.18

DPPH radical-scavenging assay of bark extracts of *S. suaveolens*

Figure 1 depicts that the DPPH radical scavenging activity of *S. suaveolens* bark extracts and L-ascorbic acid increased with concentration, reaching 95.98% and 87.34%, respectively, at 250.0 µg/mL. The IC_{50} of methanolic extracts of bark of *S. suaveolens* was 5.46 ± 0.56 µg/mL, while the IC_{50} of L-Ascorbic acid (standard) was 2.82 ± 0.23 µg/mL. A lower IC_{50} value indicates higher antioxidant activity. L-ascorbic acid exhibited significantly greater antioxidant activity compared to the methanolic extract of *S. suaveolens* bark, as evidenced by its lower IC_{50} value. Statistical analysis confirmed that this difference was significant, with a p value of 0.0147 (confidence interval: 1.2489 to 4.0311).

DPPH radical-scavenging assay for the bark fractions of *S. suaveolens*

The fractions from the methanolic bark extracts were separated using TLC and column chromatography. This process led to the isolation of seven bark fractions (F1B to F7B). To further analyse these fractions, TLC was performed. Detection was carried out using a UV-visible detector at short (254 nm) and long (366 nm) wavelengths. The retention factor (Rf) values were calculated for each fraction, and based on these values, the same seven bark fractions were confirmed. Table 3 shows that the third (F3B) and fourth (F4B) fractions demonstrated significantly higher antioxidant potential compared to the other bark fractions ($p < 0.05$).

Table 3. Absorbance and percentage of inhibition of different bark fractions of *S. suaveolens*

Bark Fractions	% DPPH radical scavenging activity
F_1B	17 %
F_2B	35 %
F_3B	83 %
F_4B	56 %
F_5B	21 %
F_6B	25 %
F_7B	13 %

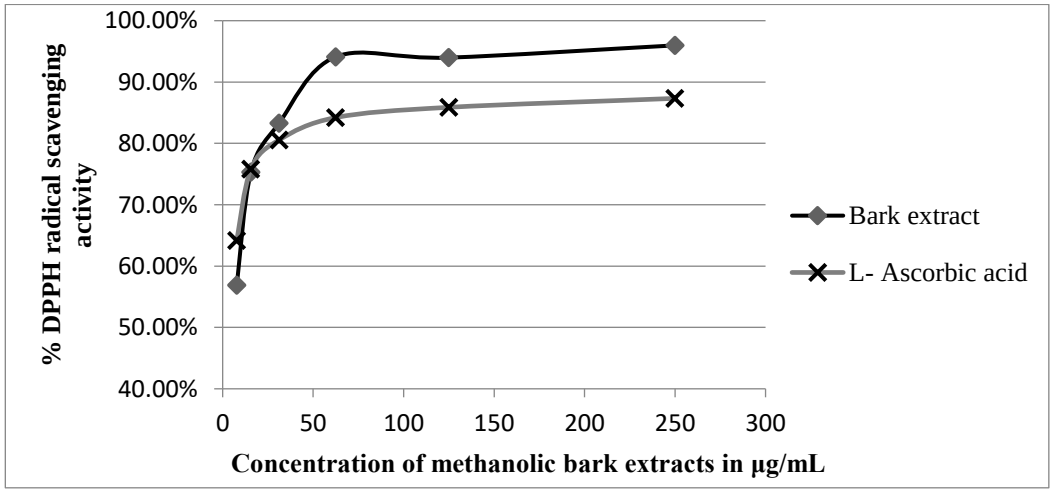

Figure 1. Percentage of DPPH radical scavenging activity of *S. suaveolens* bark extracts and L-ascorbic acid at different concentrations

Overall, the fractions obtained from the fractionation of *S. suaveolens* bark extracts exhibited antioxidant activity, with inhibition ranging from 13.22% to 82.51%. However, L-ascorbic acid (standard) demonstrated the highest antioxidant activity (92.83%) compared to the bark fractions.

Ferric reducing antioxidant power assay of bark extracts of *S. suaveolens*

Methanolic bark extracts of *S. suaveolens* and L-ascorbic acid (standard) yielded various absorbance values in the ferric reducing antioxidant power assay. According to the mean absorbance values for each sample shown in Table 4, *S. suaveolens* bark extracts and L-ascorbic acid demonstrated statistically significant differences in ferric reducing power ($p < 0.05$), except at a concentration of 15.62 µg/mL. An increase in absorbance of the reaction mixture indicated enhanced reducing power. (14) Table 4 shows that the reducing power of *S. suaveolens* bark extracts and L- ascorbic acid increased with concentration.

Determination of antioxidant activity by TLC-bioautography

The bark extracts were sprayed with 0.05% DPPH in methanol to identify compounds with radical scavenging activity. The plates were then observed under visible light, where yellow to pink coloration against a purple background confirmed the presence of antioxidant molecules indicating that the bark extracts of *S. suaveolens* exhibit antioxidant activity. (15)

Phytochemical screening of bark extracts of *S. suaveolens*

Phytochemical analysis was carried out for the bark extracts of *S. suaveolens*. The findings indicate the presence of flavonoids, saponins and phenolic compounds in the bark extracts (Table 5).

Table 4. *In vitro* ferric reducing antioxidant power of L- Ascorbic acid and bark extracts of *S. suaveolens*

Concentration of L-Ascorbic acid/ Bark extracts (µg/mL)	Absorbance of L-Ascorbic acid (Mean±SD)	Absorbance of bark extracts (Mean±SD)	p-value	Confidence interval	
				Lower	Upper
7.81	0.070 ± 0.00	0.082 ± 0.00	0.010*	0.00650	0.01684
15.62	0.124 ± 0.01	0.114 ± 0.01	0.082	- 0.02314	0.00314
31.25	0.416 ± 0.02	0.073 ± 0.00	< 0.001*	- 0.34554	- 0.33980
62.50	0.567 ± 0.01	0.133 ± 0.00	< 0.001*	- 0.43510	- 0.43223
125.00	0.741 ± 0.00	0.330 ± 0.01	< 0.001*	- 0.42378	- 0.38889
250.00	0.643 ± 0.01	0.451 ± 0.02	< 0.001*	- 0.20829	- 0.17571

*p<0.05; SD= Standard deviation

Table 5. Phytochemical components found in the barks of *S. suaveolens*

Name of the test	Phytoconstituents	Result in bark extract
Wagner's test	Alkaloids	-
Ninhydrin test	Proteins	-
Legal's test	Glycosides	-
Lead Acetate test	Phenolic Compounds	+
Lead Acetate test	Flavonoids	+
Foam test	Saponins	+
Libermann-Burchard test	Steroids	-
Gelatin test	Tannins	-
Salkowski test	Terpenoids	-

(+ indicates a positive result and - indicates a negative result)

DISCUSSION

The pharmacological properties of medicinal plants are increasingly being reported across various countries. In the pharmaceutical industry, these plants are highly valued for their bioactive compounds, including polyphenols, flavonoids, glycosides, alkaloids, and tannins, which can be utilized in drug synthesis. (16) In this study, phytochemical analysis of the bark extracts of *S. suaveolens* confirmed the presence of flavonoids, saponins, and phenolic compounds. Among these, phenolic and flavonoid compounds are particularly important due to their diverse pharmacological effects, including antioxidant activity. (16,17)

Phenolic compounds possess an ideal chemical structure for free radical scavenging, as they contain phenolic hydroxyl groups capable of donating a hydrogen atom or electron to neutralize free radicals. Additionally, their extended conjugated aromatic system facilitates electron delocalization, stabilizing the unpaired electron. (17)

For flavonoids, the major structural features that influence radical-scavenging activity include the ortho-dihydroxy structure on the B ring, a 2,3-double bond with a 4-oxo function in the C ring, and hydroxyl groups at positions 3 and 5 in the A and C rings—with the 3-hydroxyl group playing a critical role in antioxidant potential. (17) These findings are consistent with a previous study that identified alkaloids, phenolic compounds, saponins, flavonoids, and tannins in the bark extracts of *S. suaveolens.* (18)

In this study, the antioxidant potential of *S. suaveolens* bark extracts was assessed using the DPPH and ferric reducing antioxidant power assay. The DPPH assay evaluates the ability of antioxidant compounds to donate hydrogen and stabilize free radicals. The DPPH radical, which exhibits maximum absorption at 517 nm, undergoes a color change from deep purple to light yellow upon interaction with radical scavengers in the test sample. (11, 15) Based on this mechanism, the bark extracts of *S. suaveolens* exhibited 95.98% radical scavenging activity at 250 µg/mL. A similar study conducted in India reported that *S. suaveolens* bark extracts exhibited a maximum of 91.44% DPPH radical scavenging activity at 125 µg/mL, further confirming its potent antioxidant properties. (18,19)

The ferric reducing antioxidant power assay measures the antioxidant capacity of plant extracts by evaluating their ability to reduce Fe^{3+}-tripyridyltriazine to Fe^{2+}-tripyridyltriazine. This assay is based on electron transfer reactions, utilizing potassium ferricyanide as an oxidant. The reduction process results in the formation of a colored ferrous complex, which can be quantified by its absorption at 593 nm. (12,20) Our results indicate that the reducing power of *S. suaveolens* bark extracts increased with concentration. A previous study evaluating the antioxidant activity of *S. chelonoides* using the ferric reducing antioxidant power assay also demonstrated a concentration-dependent increase in reducing power. (21)

Furthermore, this study demonstrates that the antioxidant potential of *S. suaveolens* bark extracts is lower than that of L-ascorbic acid, as reflected by IC_{50} values from the DPPH assay. These results align with a previous study comparing the antioxidant activities of the leaves and bark of *S. chelonoides*, a plant from the same Bignoniaceae family. The study reported that L-ascorbic acid exhibited greater antioxidant activity than both the leaf and bark extracts. (21)

Column chromatography has been widely employed to fractionate plant extracts, as seen in previous studies on water lettuce (*Pistia stratiotes*), *Passiflora foetida*, and *Garcinia hombroniana* (22–24). However, this is the first study investigating the antioxidant activity of *S. suaveolens* bark fractions. The results revealed that the antioxidant activity of the seven bark fractions varied from 13.22% to 82.51%. Previous studies have also demonstrated variations in antioxidant activity among different fractions of *G. hombroniana* and *P. stratiotes* methanolic extracts. (22,24)

CONCLUSION

The findings of this study demonstrate that the bark of *S. suaveolens* possesses significant antioxidant potential. Additionally, these results may support its traditional medicinal use for various diseases and highlight its potential as a natural antioxidant source for pharmaceutical and industrial applications. Hence, this plant may serve as a promising candidate for the development of potent antioxidant drugs in future drug discovery. However, further extensive research, including the isolation and characterization of bioactive compounds, is necessary to fully confirm its therapeutic potential.

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AUTHOR'S DECLARATION:

The authors declare that all persons listed as authors have read and given approval for the submission of this manuscript.

CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interests.

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