Research Article

Evaluation Of Antioxidant Activity And Free Radical Scavenging Potential Of Strobilanthes Lurida Stem Extracts

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ABSTRACT

The antioxidant potential of stem extracts obtained using four solvents of varying polarity — distilled water (SDW), methanol (SMA), ethyl acetate (SEA), and chloroform (SChl) — was systematically evaluated through a battery of complementary in vitro assays, comprising DPPH radical scavenging, ABTS radical cation scavenging, ferric reducing antioxidant power (FRAP), nitric oxide scavenging, and reducing power assays. Marked and statistically significant variations in antioxidant activity were recorded across the four extracts, underscoring the well-established influence of solvent polarity on the selective recovery of bioactive phytochemicals from plant tissues. Among the extracts, the methanolic fraction (SMA) demonstrated the strongest DPPH radical scavenging activity (61.91%), reflecting its capacity to effectively neutralise free radicals in a hydrogen-transfer-dependent mechanism. The ethyl acetate extract (SEA), however, outperformed all other fractions across the remaining assays, recording the highest ABTS scavenging activity (67.77%), FRAP value (11.15 mg/mL GAE), nitric oxide scavenging activity (75.21%), and reducing power (12.01 mg/mL GAE). The distilled water extract (SDW) consistently registered the lowest antioxidant performance across all five assays, suggesting that the predominant antioxidant constituents of this stem material are not readily recovered by highly polar aqueous extraction. The superior performance of the methanolic and ethyl acetate fractions is attributed to their preferential extraction of phenolic compounds and other redox-active secondary metabolites, which are known to contribute to multiple mechanisms of free-radical neutralisation. Collectively, these findings demonstrate that moderately polar solvents offer a more effective strategy for recovering antioxidant-rich fractions from stem tissues and position these extracts as promising candidates for further characterisation and application in food preservation, nutraceutical formulation, and pharmaceutical development.

Keywords: S. lurida, DPPH, ABTS, FRAP, Nitric oxide, Reducing power, IC50. PBS, NED,and Gallic acid.

1. INTRODUCTION

Medicinal plants have served as an essential source of healthcare since ancient times. Long before the development of modern medicine, people relied on plants for treating diseases and maintaining health. Knowledge about the medicinal properties of plants was passed down through generations based on practical experience and traditional practices (Balakumar et al., 2011). Across the world, medicinal plants continue to play a significant role in traditional healthcare systems and are considered nature's valuable reservoirs of therapeutic compounds. Medicinal plants possess numerous healing properties, including wound healing, pain relief, and infection prevention (Sukumaran et al., 2014). They synthesize a wide range of bioactive chemical compounds that are important not only for plant survival but also for human health. As a result, plants remain one of the richest sources of drugs used in both traditional and modern medicine. One of the major causes of several chronic diseases is oxidative stress, which results from the excessive production of free radicals. Free radicals are unstable molecules containing unpaired electrons, making them highly reactive. In living organisms, these molecules are generated during normal metabolic activities such as mitochondrial respiration. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are common forms of free radicals produced within cells. Excessive accumulation of these reactive molecules can damage cellular components and contribute to the development of diseases such as diabetes, cancer, asthma, atherosclerosis, Parkinson's disease, and Alzheimer's disease (Sharma, 2020). Antioxidants are compounds capable of neutralizing free radicals and preventing oxidative damage. Their primary function is to scavenge free radicals, thereby protecting cells from oxidative stress (Yamagishi & Matsui, 2011; Wu et al., 2011). Many medicinal plants are rich in natural antioxidants, making them important candidates for the development of therapeutic agents. The genus Strobilanthes belongs to the family Acanthaceae and comprises approximately 350 species. Many species of

this genus are traditionally used in medicine due to the presence of various bioactive compounds such as alkaloids, flavonoids, terpenoids, and phytosterols. Among them, Strobilanthes lurida (commonly known as Lurid Coneflower) is an important ornamental and medicinal shrub endemic to the Southern Western Ghats of India. The plant is characterized by thick, virgate stems swollen at the nodes, compact bristly flower spikes, and large purple bracts with a distinctive black tinge (Sankara & Kumar, 2025). Despite the medicinal significance of the genus, there is limited scientific information regarding the antioxidant potential of Strobilanthes lurida. Available literature indicates that no detailed study has been conducted on its antioxidant activity. Therefore, the present study aims to evaluate the free radical scavenging and antioxidant activities of crude stem extracts of S. lurida using various in vitro antioxidant assays.

2. Materials and Methods

Collection of Plant Material

Strobilanthes lurida twigs with inflorescences were collected from the Tadiandamol mountain range, Kodagu district, Karnataka (Latitude: 12° 13' 2" N and Longitude: 75° 36' 30" E), reaching an elevation of 1,748meters. The Botanical Survey of India (BSI) authenticated the plant with Voucher number - BSI/SRC/5/23/2023-24/Tech - 478. Voucher specimen (twig with inflorescence) was deposited in the Herbaria, Department of Botany, Bangalore University.

Preparation of Extract by Decoction Method

Fresh and healthy stems of S. lurida were collected and thoroughly washed with running tap water to remove dust and other debris. Excess water was removed by decanting and wiping the plant materials with dry cotton cloth, and were also allowed to dry in the shade. Then the dried leaves were ground into powder separately using an electric mixer grinder to obtain fine powder, which was stored in an air-tight container. 15g powder was weighed and extracted using 150 ml of Distilled water, methanol, chloroform, ethyl acetate, and petroleum ether solvents for 4 hrs. in a water bath at 50°C by decoction method. The contents were filtered through the Whatman No.1 filter paper. The extracts thus obtained were allowed to evaporate the solvent in a hot air oven at 60°C for 3-4 days. The condensed extracts were stored in microcentrifuge vials at 4°C for the in vitro antioxidant assay (Adinew et al., 2018).

In vitro antioxidant activity

DPPH Radical scavenging assay

The total free radical scavenging capacity of the extracts was estimated using the DPPH assay. A radical solution was prepared by dissolving 2.4 mg of DPPH in 100 ml of methanol. To this, 5 µl of the test sample (100 µg/ml) was added to 3.995 mL of the methanolic DPPH solution. The mixture was shaken vigorously and incubated at room temperature in the dark for 30 minutes. The absorbance of the reaction mixture was then measured spectrophotometrically at 515 nm. A blank containing the DPPH solution without any antioxidant was also measured. The percentage scavenging activity of each extract was calculated as the percent inhibition (I %) using the following equation (Rajurkar & Hande, 2011).

% inhibition = [(A control-A sample)/A control] *100

Where, A control is the absorbance of control and A sample is the absorbance of the tested extract solution.

ABTS Radical scavenging assay

The ABTS stock solution was prepared by reacting equal volumes of ABTS (7 mM; Sigma Aldrich, India) aqueous solution with potassium persulfate (2.45 mM; Merck, India) aqueous solution. The mixture was allowed to stand in the dark at room temperature for 12–16 hours before use. The working ABTS solution was obtained by diluting the stock solution with methanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Subsequently, 2.0 mL of the ABTS working solution was mixed with 1.0 ml of the aqueous extracts (100 µg/ml). The mixture was incubated in the dark at room temperature for exactly 10 minutes. A control solution was prepared by mixing 2.0 ml of the ABTS solution with 1.0 mL of double-distilled water. Absorbance was measured at 734 nm using a spectrophotometer, with BHT (Merck, India) serving as the standard. The percentage scavenging activity of each extract was calculated as the percent inhibition (I %) using the following equation (Rajurkar & Hande, 2011).

I% = [(Ao-As)/ Ao] × 100
Where, Ao is the absorbance of control and As is the absorbance of the tested extract solution.

FRAP Assay
The ferric-reducing power of the extract was determined using gallic acid as the standard. Standard solutions of gallic acid at different concentrations (1 ml each) and plant extracts (100 µg/ml) were taken in 10 ml volumetric flasks and mixed with 2.5 ml of potassium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added to stop the reaction. Subsequently, 2.5 ml of the resulting solution was mixed with 2.5 ml of distilled water, followed by the addition of 0.5 ml of 0.1% FeCl. The mixture was allowed to stand for 30 minutes, and absorbance was measured at 593nm. The ferric-reducing power was expressed as milligrams of gallic acid equivalent per gram of dry material (GAE/g), calculated using the gallic acid standard curve (Rajurkar & Hande, 2011).

Nitric oxide scavenging assay
The nitric oxide (NO) scavenging activity of the plant extracts was evaluated using sodium nitroprusside as the NO donor, and the nitrite generated was quantified using the Griess reagent. Sodium nitroprusside was dissolved in phosphate-buffered saline (PBS, pH 7.4) to obtain a final concentration of 10 mm. The Griess reagent was prepared using two solutions: solution A containing 1% sulfanilamide in 5% phosphoric acid, and solution B containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NED) in water. These two solutions were mixed in a 1:1 ratio immediately before use. For the assay, plant extracts at a concentration of 100 µg/ml were prepared. A volume of 0.5 ml of each plant extract was mixed with 2 mL of sodium nitroprusside solution, while 0.5 ml of solvent without extract served as the control. The mixtures were incubated at 25°C for 150 minutes under light to allow the generation of nitric oxide. After incubation, 1 ml of the reaction mixture was combined with 1 ml of freshly prepared Griess reagent, and the mixture was allowed to stand at room temperature for 10 minutes. The absorbance of the resulting pink azo dye was measured at 540 nm using a spectrophotometer. Ascorbic acid or another known antioxidant was used as the positive control to generate the standard curve (Patel et al., 2010).

NO Scavenging (%) = [(A control-A sample)/A control] *100
Where, A control is the absorbance of control and A sample is the absorbance of the tested extract solution.

Reducing Power Assay
The reducing power assay was performed according to the method of Hue et al. (2012) with slight modifications. Plant extracts (0.050 ml) at a concentration of 100 µg/ml were mixed with 0.2 mL of 0.2 M phosphate buffer (pH 6.6) and 0.2 ml of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 minutes. After incubation, 0.25 ml of trichloroacetic acid was added, and the mixture was centrifuged at 1000 rpm for 10 minutes at room temperature. The resulting supernatant (0.5 ml) was combined with 0.5 ml of deionized water and 0.1 ml of 0.1% FeCl. The absorbance was measured at 700 nm. The reducing power was expressed as mg GAE/L, and the gallic acid standard curve obtained from the previous assay was used for the calculation.

3. Results and Discussions

Antioxidant activity of stem extracts
The antioxidant potential of stem extracts differed considerably depending on the extraction solvent. Among the four extracts investigated, the methanolic (SMA) and ethyl acetate (SEA) fractions exhibited substantially greater antioxidant activity than the distilled water (SDW) and chloroform (SChl) extracts across most assays. Table:1 presents the comparative antioxidant activities of the different stem extracts evaluated using DPPH, ABTS, FRAP, nitric oxide scavenging, and reducing power assays.
The free radical scavenging potential of different stem extracts shows considerable antioxidant efficacy against different scavenging assays. Figure 1 & 2 shows the comparative antioxidant profile of stem extracts.

Table 1: Comparative antioxidant potential of stem extracts (SDW, SMA, SEA and SChl) evaluated using multiple in vitro antioxidant assays.

Extract	DPPH (%)	ABTS (%)	FRAP (mg/ml GAE)	Nitric-Oxide (%)	Reducing-Power (mg/ml GAE)
SDW	20.95	28.09	2.61	29.91	5.72
SMA	61.91	62.81	10.11	66.67	10.72
SEA	35.24	67.77	11.15	75.21	12.01
SChl	30.48	33.06	6.92	37.61	7.27

In the DPPH radical scavenging assay, SMA demonstrated the strongest free radical quenching capacity (61.91%), followed by SEA (35.24%), SChl (30.48%), and SDW (20.95%). The superior activity of the methanolic extract suggests that methanol effectively extracted hydrogen- and electron-donating phytochemicals, particularly phenolics and flavonoids, which are known to neutralise DPPH radicals through proton transfer mechanisms. Similar observations have been reported for medicinal plants where methanolic extracts exhibited significantly higher antioxidant activities due to their enhanced ability to solubilise phenolic compounds of varying polarity (Kumar et al., 2024). Furthermore, polyphenols are widely recognised as major contributors to free radical scavenging activity because of their ability to donate hydrogen atoms and stabilise reactive oxygen species (Khan et al., 2024).

A slightly different trend was observed in the ABTS assay. SEA recorded the highest scavenging activity (67.77%), followed closely by SMA (62.81%). Since the ABTS radical cation can react with both hydrophilic and lipophilic antioxidants, the high activity of SEA may indicate the presence of moderately polar antioxidant compounds that were preferentially extracted into ethyl acetate. According to Lang et al. (2025), the ABTS assay is particularly useful for detecting a broad spectrum of antioxidant molecules and often identifies compounds that may not be fully represented in DPPH-based evaluations due to differences in reaction mechanisms and solvent compatibility.

The ferric reducing antioxidant power (FRAP) assay further confirmed the superiority of the organic solvent extracts. SEA showed the highest reducing capacity (11.15 mg/mL GAE), closely followed by SMA (10.11 mg/mL GAE), whereas SDW exhibited the lowest value (2.61 mg/mL GAE). FRAP reflects the ability of antioxidants to donate electrons and reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Therefore, the elevated FRAP values suggest a higher concentration of redox-active phytochemicals in the ethyl acetate and methanolic fractions. Recent reviews have demonstrated a strong positive relationship between FRAP values and phenolic content, indicating that phenolic compounds are among the principal contributors to ferric reducing activity (Lisjak et al., 2026; Khan et al., 2024).

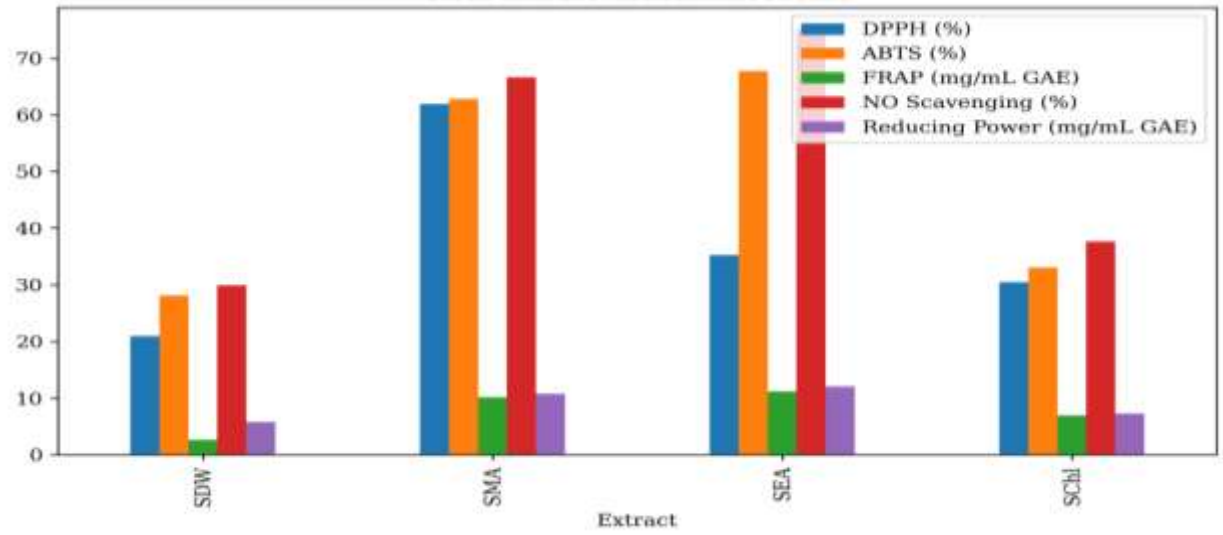

Figure 1. Comparative antioxidant profile of stem distilled water extract (SDW), stem methanol extract (SMA), stem ethyl acetate extract (SEA), and stem chloroform extract (SChl). Antioxidant activity was evaluated using DPPH radical scavenging, ABTS radical cation scavenging, ferric reducing antioxidant power (FRAP), nitric oxide scavenging, and reducing power assays.

The nitric oxide scavenging assay revealed the highest activity for SEA (75.21%), followed by SMA (66.67%). Excess nitric oxide and its reactive derivatives contribute significantly to oxidative stress and inflammatory damage in biological systems. Therefore, the marked inhibition exhibited by SEA suggests the presence of compounds capable of suppressing reactive nitrogen species. The ability of ethyl acetate extracts to concentrate flavonoids, phenolic acids, and other moderately polar antioxidants may explain this enhanced activity. Similar relationships between solvent polarity and nitric oxide scavenging efficiency have been documented in studies investigating antioxidant-rich medicinal plant extracts (Khan et al., 2024).

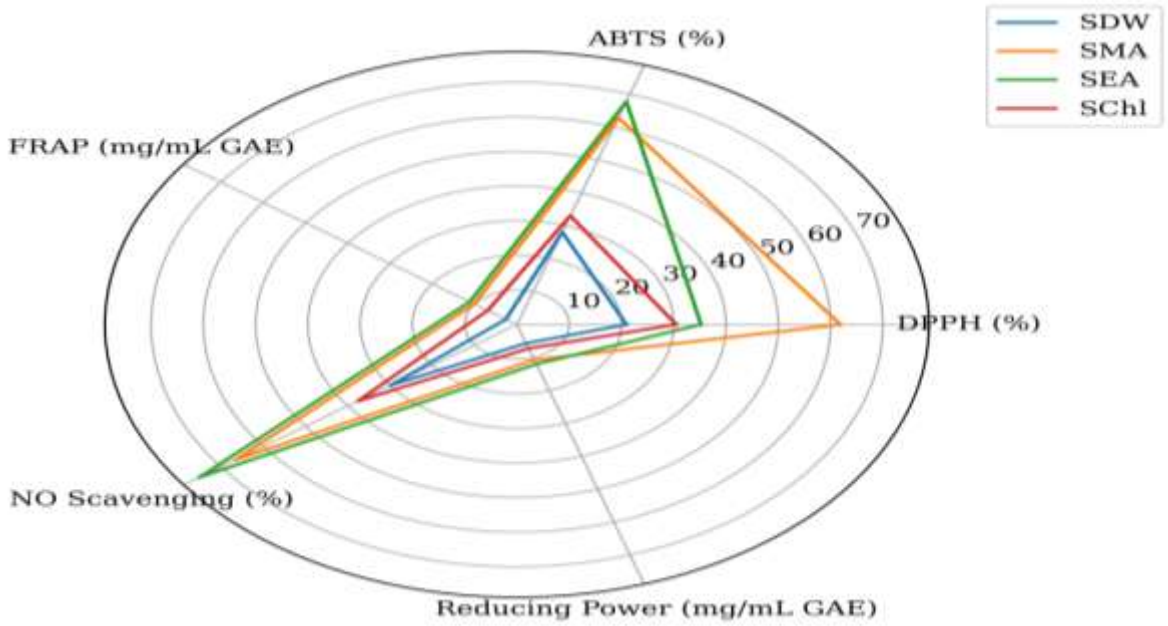

Figure 2: Radar plot illustrating the relative antioxidant performance of SDW, SMA, SEA and SChl across DPPH, ABTS, FRAP, nitric oxide scavenging and reducing power assays.

The reducing power assay produced results consistent with the FRAP analysis. SEA exhibited the strongest reducing capacity (12.01 mg/mL GAE), followed by SMA (10.72 mg/mL GAE), SChl (7.27 mg/mL GAE), and SDW (5.72 mg/mL GAE). Strong reducing power is generally associated with the presence of reductones and phenolic hydroxyl groups that donate electrons to stabilise reactive species. The close agreement between FRAP and reducing power values suggests that electron-transfer mechanisms constitute a major component of the antioxidant activity observed in these stem extracts. Similar findings have been reported by Lang et al. (2025), who emphasised that antioxidant assays based on electron-transfer reactions frequently exhibit strong correlations because they evaluate related mechanisms of antioxidant action.

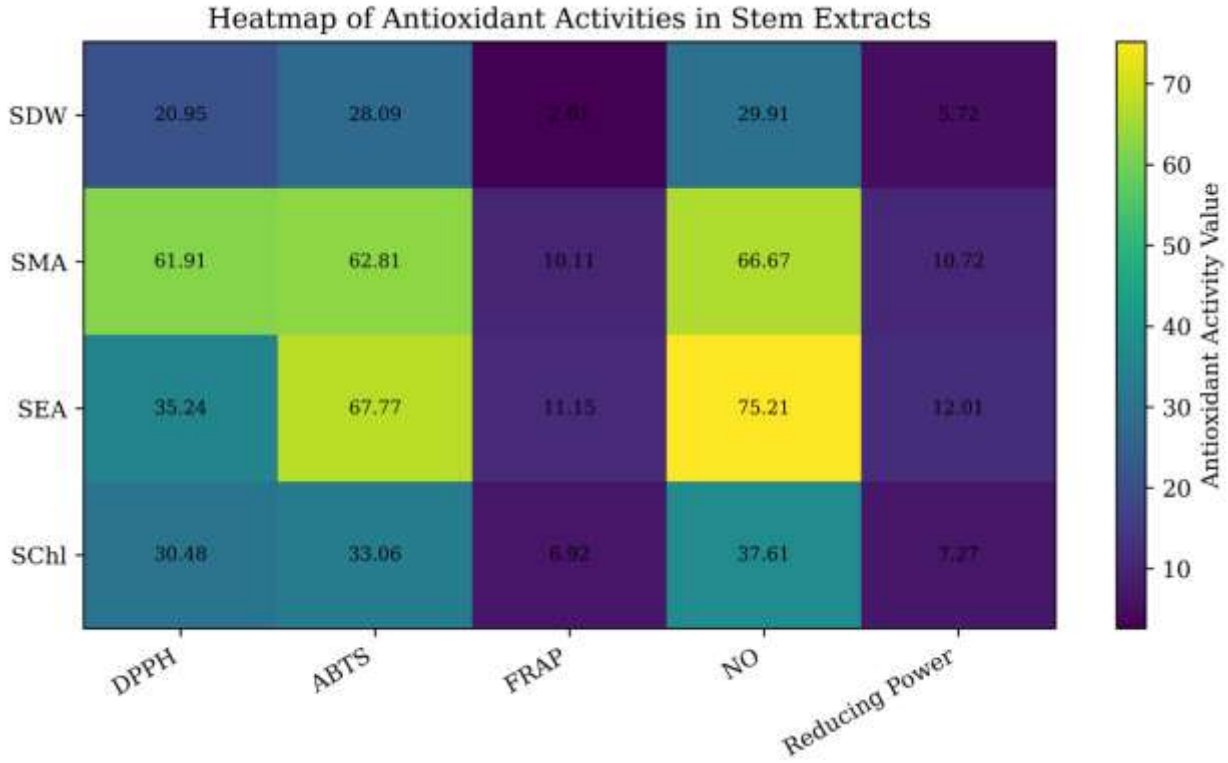

Figure 3. Heatmap visualisation of antioxidant activities measured by DPPH, ABTS, FRAP, nitric oxide scavenging and reducing power assays. Colour intensity represents the relative magnitude of antioxidant activity among the stem extracts.

Figure 3 provides a heatmap visualisation of the antioxidant activities of the different stem extracts. Collectively, the results indicate that antioxidant constituents are more efficiently extracted by methanol and ethyl acetate than by water or chloroform. While methanol displayed the highest DPPH scavenging activity, ethyl acetate showed superior performance in ABTS, FRAP, nitric oxide scavenging, and reducing power assays. This pattern suggests that stem tissues contain a heterogeneous mixture of antioxidant compounds with varying polarity. The relatively poor performance of SDW may reflect the limited extraction of less polar phenolic compounds, whereas the lower activity of SChl suggests that highly non-polar fractions contain comparatively fewer antioxidant metabolites. These findings are consistent with reports indicating that solvents of intermediate polarity generally provide the highest recovery of antioxidant phytochemicals and polyphenolic compounds from plant matrices (Kumar et al., 2024; Khan et al., 2024).

Conclusion

The present study demonstrated that the antioxidant potential of stem extracts was strongly influenced by the extraction solvent. Among the four extracts evaluated, the ethyl acetate extract (SEA) exhibited the highest overall antioxidant activity, recording superior ABTS radical scavenging activity, ferric reducing antioxidant power (FRAP), nitric oxide scavenging capacity, and reducing power. In contrast, the methanolic extract (SMA) showed the

strongest DPPH radical scavenging activity, highlighting its excellent ability to neutralise free radicals through hydrogen- or electron-donating mechanisms. The distilled water extract (SDW) consistently displayed the lowest antioxidant activity across all assays, indicating its limited efficiency in extracting bioactive antioxidant compounds from the stem material. The close agreement observed among the ABTS, FRAP, nitric oxide scavenging, and reducing power assays suggests that electron-transfer reactions play a major role in the antioxidant mechanisms of the stem extracts. Overall, the findings indicate that moderately polar solvents, particularly methanol and ethyl acetate, are more effective than water and chloroform in recovering antioxidant phytochemicals from stem tissues. These results highlight the potential of the methanolic and ethyl acetate stem extracts as promising sources of natural antioxidants and provide a basis for further phytochemical characterisation and identification of the bioactive compounds responsible for their antioxidant activity.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

B. G. Satvik: Conceptualisation, methodology, investigation, data analysis, and manuscript drafting.

C. Maya: Supervision, validation, and manuscript review and editing.

Authors have read and approved the final manuscript.

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