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EVALUATION OF ANTIOXIDANT ACTIVITY AND FREE RADICAL SCAVENGING POTENTIAL OF *STROBILANTHES LURIDA* LEAF EXTRACTS

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ABSTRACT
The current study aims to evaluate the antioxidant activity of leaf extracts of *Strobilanthes lurida,* belonging to the family Acanthaceae. The different solvent leaf extracts of *S. lurida* were subjected to DPPH, ABTS, FRAP, Nitric oxide, and reducing power assays. Different solvent extracts exhibited assay-dependent antioxidant activity. Chloroform extract showed the strongest DPPH and ABTS radical scavenging activity, petroleum ether extract demonstrated the highest FRAP and nitric oxide scavenging activity, while ethyl acetate extract exhibited the highest reducing power at a concentration of 100 µg/ml. Chloroform extract showed the strongest DPPH and ABTS radical scavenging activity, petroleum ether extract demonstrated the highest FRAP and nitric oxide scavenging activity, while Different solvent extracts exhibited assay-dependent antioxidant activity. Chloroform extract showed the strongest DPPH and ABTS radical scavenging activity, petroleum ether extract demonstrated the highest FRAP and nitric oxide scavenging activity, while ethyl acetate extract exhibited the highest reducing power at a concentration of 100 µg/ml. This enhanced activity is attributed to their effectiveness in extracting a broad spectrum of phenolic and flavonoid compounds. Aqueous extracts showed moderate antioxidant activity across assays, whereas the chloroform extract exhibited strong radical scavenging potential in DPPH and ABTS assays with their respective IC_{50} values, indicating their limited ability to extract phenolic antioxidants. From the result, it can be concluded that these findings underscore the promising antioxidant potential of *S. lurida* extracts and provide a strong foundation for further phytochemical and pharmacological investigations.

KEYWORDS: *S. lurida,* DPPH, ABTS, FRAP, Nitric oxide, Reducing power, $IC_{50.}$ PBS, NED, and Gallic acid.

1. INTRODUCTION

Medicinal plants have long served as an important source of therapeutic agents and continue to contribute significantly to traditional and modern healthcare systems. In ancient times, people adored plants due to their strong dependence on plant-based medicine guided by experience. The knowledge of the various important medicinal properties of the plants has been transmitted in an empirical way (Balakumar *et al*. 2011). All over the world, medicinal plants have been used traditionally, and they have transferred implausible knowledge from generation to generation. Medicinal plants represent an important reservoir of bioactive compounds with therapeutic potential. Medicinal plants are important for their potential to heal wounds, relieve pain and prevent infections (Sukumaran *et al*. 2014). Plants can synthesise a large variety of chemical compounds that are of physiological importance not only to the plants but also to human beings. Medicinal plants are the richest bio-resource of drugs of both traditional & modern systems of medicine. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases and is responsible for the production of free radicals. Plants are a rich source of antioxidants that help to counteract the negative effects of free radicals, which arise during the oxidation process in cells of our body (Yamagishi & Matsui 2011). Free radicals are chemically reactive species containing one or more unpaired electrons, making them highly unstable and capable of initiating oxidative damage. Due to the insufficient stable number of electrons in the outermost shell, these atoms are constantly searching for other molecules to become stable. In a living system, free radicals are produced during uncontrolled mitochondrial respiration or as by-products of other metabolic processes. Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are molecules containing oxygen and nitrogen molecules, respectively, mainly synthesized in cells through mitochondrial oxidative phosphorylation. These free radicals are molecules that can cause damage to cells and are responsible for various ailments, viz., sicknesses, asthma, inflammatory arthropathies, diabetes, Parkinson's, Alzheimer's, cancers, as well as atherosclerosis (Sharma, 2020). Antioxidants function by interrupting oxidative chain reactions and limiting molecular damage caused by reactive oxygen and nitrogen species. (Yamagishi & Matsui 2011). The main characteristic of an antioxidant is its ability to trap free radicals (Wu *et al*. 2011). Many plants contain antioxidant compounds, and these compounds protect cells against free radicals. *Strobilanthes* is one of the important genera in the family Acanthaceae. About 350 species of this genus are traditionally used for various medicinal purposes because of the presence of many bioactive compounds, viz., alkaloids, flavonoids, terpenoids, phytosterols, etc. *Strobilanthes lurida* is one of the important species in this genus, commonly called Lurid coneflower. It is one of the significant ornamental as well as medicinal plants

endemic to the Southern Western Ghats region of India. Morphologically, it is a shrub with thick, virgate stems that are swollen at the nodes and are hairless. The plant bears compact, bristly spikes of flowers, often from older stems. The flower bracts are large, curved and have a distinctive purple colour with a black tinge (Sankara Rao and Kumar 2025). Previous studies revealed that, so far, there is no study pertaining to the antioxidant activity of *S. lurida*. Hence in the present investigation, it is imperative to evaluate the various anti-radical scavenging assays of crude leaf extracts.

2. MATERIALS AND METHODS Collection of Plant Material

Strobilanthes lurida twigs with inflorescence 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 leaves 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 they 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 (Demilew 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 percentage inhibition (I %) using the following equation (Rajurkar & Hande 2011).

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

Where A control is the absorbance of the 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 percentage inhibition (I %) using the following equation (Rajurkar & Hande 2011).

I% = [(Ao-As)/ Ao] × 100

Where Ao is the absorbance of the 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% $FeCl_3$. 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).

***In vitro* antioxidant**

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). The nitric oxide radical scavenging assay protocol was adapted from previously described methodology using sodium nitroprusside and Griess reagent (Borah et al. 2020).

NO Scavenging (%) = [(A control-A sample)/A control] *100

Where A control is the absorbance of the 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 deionised water and 0.1 ml of 0.1% $FeCl_3$. 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

3.1. DPPH free radical scavenging assay

The DPPH assay measures how effectively an extract can scavenge or neutralise free radicals. Lowering the IC_{50} value indicates more potent antioxidant activity. The IC_{50} values of the leaf extracts are represented in Table 1 in µg/mL against the standard Ascorbic acid.

Sl. No	Sample	IC $_{50}$ value (µg/ml)
1	Ascorbic acid	1.32
2	LDW	49.52
3	LMA	80
4	LEA	68.57
5	LChl	47.61
6	LPE	61.18

Table 1: DPPH radical scavenging capacity of different leaf extracts against the standard Ascorbic acid

All the tested samples exhibited lower or weaker antioxidant potency than the standard (Ascorbic acid, IC50 = 1.32 µg/ml), as expected for crude plant extracts. The chloroform leaf extract exhibited potent antioxidant activity, with an IC_{50} value of 47. 61 µg/ml, followed by the aqueous leaf extract (IC $_{50}$ = 49.52 µg/ml). Graphical representation of the DPPH free radical scavenging potential of leaf extracts and the standard is shown in Figure 1. Moderate free radical scavenging activity was observed in the petroleum ether and ethyl acetate leaf extracts, while the methanol leaf extract showed the weakest antioxidant activity.

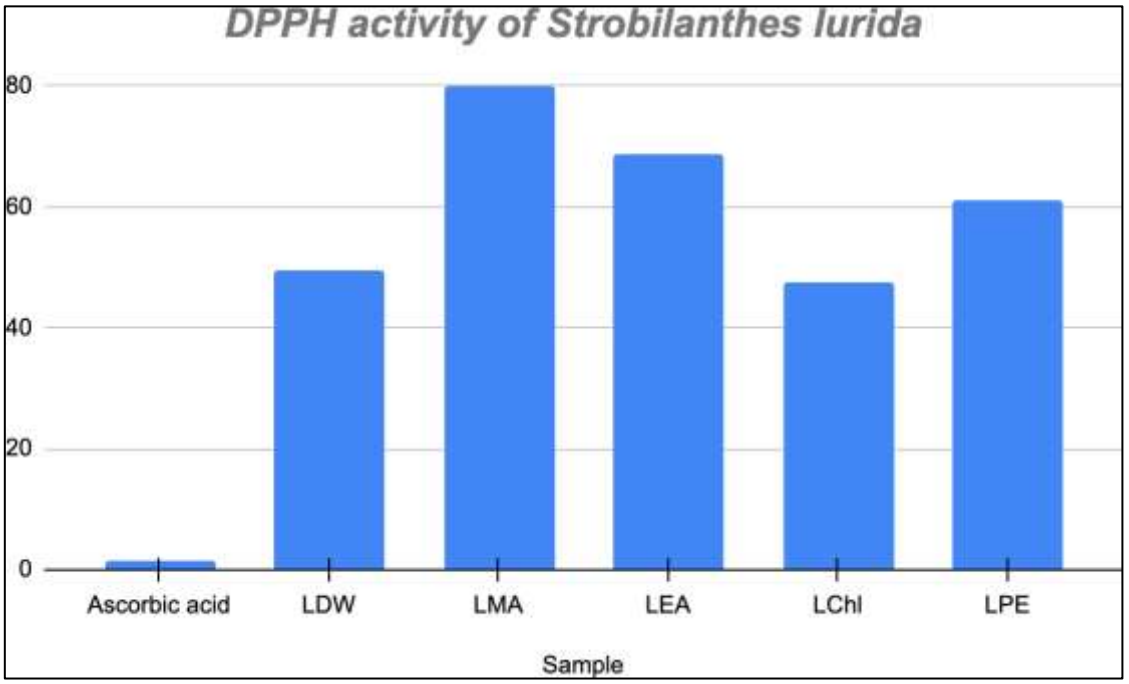

Fig. 1: DPPH free radical scavenging potential of *Strobilanthes lurida* leaf extracts and standard ascorbic acid.

3.2. ABTS free radical scavenging activity assay:

Sl. No	Sample	IC $_{50}$ value (µg/ml)
1	Ascorbic acid	1.71
2	LDW	39.67
3	LMA	72.72
4	LEA	65.29
5	LChl	32.23
6	LPE	61.45

Table 2: ABTS free radical scavenging capacity of different leaf extracts against the standard Ascorbic acid

The ABTS assay measures the ability of an extract to quench the $ABTS^{+}$radical cation. A lower IC_{50}value indicates more potent antioxidant activity. Table 2 presents the IC_{50} values of the tested samples. In the present study, the standard antioxidant ascorbic acid showed an IC_{50} of 1.71 µg/ml. When compared to the standard, all *Strobilanthes lurida* leaf extracts exhibited weaker ABTS-scavenging activity, but with notable differences among solvents. The chloroform extract (LChl, 32.23 µg/ml) showed the most vigorous activity among the extracts, requiring ~19 times the concentration of ascorbic acid to achieve 50% inhibition. This was followed by the water extract (LDW, 39.67 µg/ml), which also demonstrated relatively good activity. Moderate scavenging capacity was observed in the petroleum ether (LPE, 61.45 µg/ml) and ethyl acetate extracts (LEA, 65.29 µg/ml). The methanol extract (LMA, 72.72 µg/ml) showed the weakest ABTS radical-scavenging activity. The IC_{50} values of Strobilanthus lurida leaf extract are represented in the figure. 2.

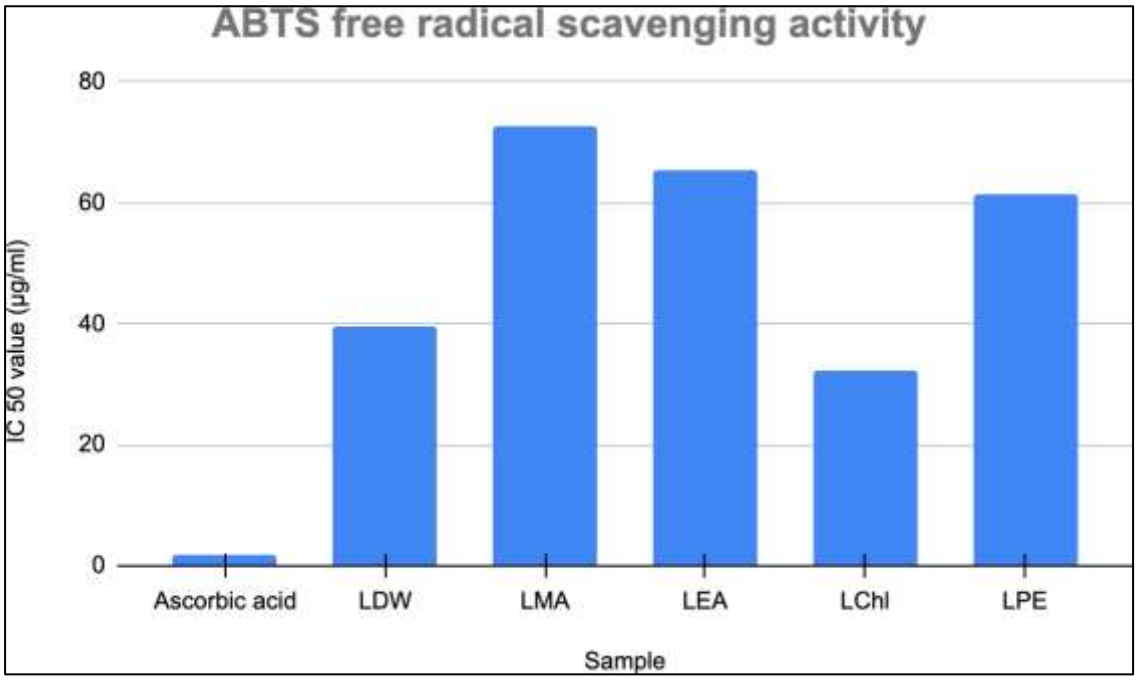

Fig. 2: ABTS free radical scavenging potential of *Strobilanthes lurida* leaf extracts

3.3. Determination of Ferric reducing antioxidant power (FRAP):

The reducing ability of the different leaf extracts of *S. lurida* is expressed in ascorbic acid equivalents (AAE µg/ml) of extract.

Sl. No	Sample	AAE µg/ml
1	LDW	3.56
2	LMA	10.54
3	LEA	9.68
4	LChl	5.8
5	LPE	27.25

Table 3: Ferric reducing antioxidant power (FRAP) of the leaf extracts of *S. lurida* expressed in µg of AAE.

The FRAP assay measures the ability of plant extracts to reduce Fe^{3+} to Fe^{2+}. A higher FRAP value indicates stronger reducing power and better antioxidant potential. The reducing ability of the *Strobilanthes lurida* leaf extracts is expressed in terms of ascorbic acid equivalents (AAE µg/ml), meaning higher values reflect stronger electron-donating ability.

Among the extracts, the petroleum ether extract (LPE) showed the highest reducing power, with a FRAP value of 27.25 µg AAE/mL, indicating a significantly greater capacity to act as an electron donor compared to all other solvents. This suggests that certain non-polar antioxidant constituents are intensely active in this fraction. The methanol (LMA, 10.54 µg AAE/mL) and ethyl acetate extracts (LEA, 9.68 µg AAE/mL) exhibited moderate reducing ability. In contrast, the chloroform extract (LChl, 5.8 µg AAE/ml) and aqueous extract (LDW, 3.56 µg AAE/ml) exhibited the lowest reducing potential (Table 3, Fig. 3).

Overall, the FRAP results clearly indicate that petroleum ether leaf extract is the most powerful reducing agent, showing a markedly higher electron-transfer ability than the other extracts. In contrast, aqueous and chloroform leaf extracts demonstrate the weakest ferric-reducing antioxidant capacity.

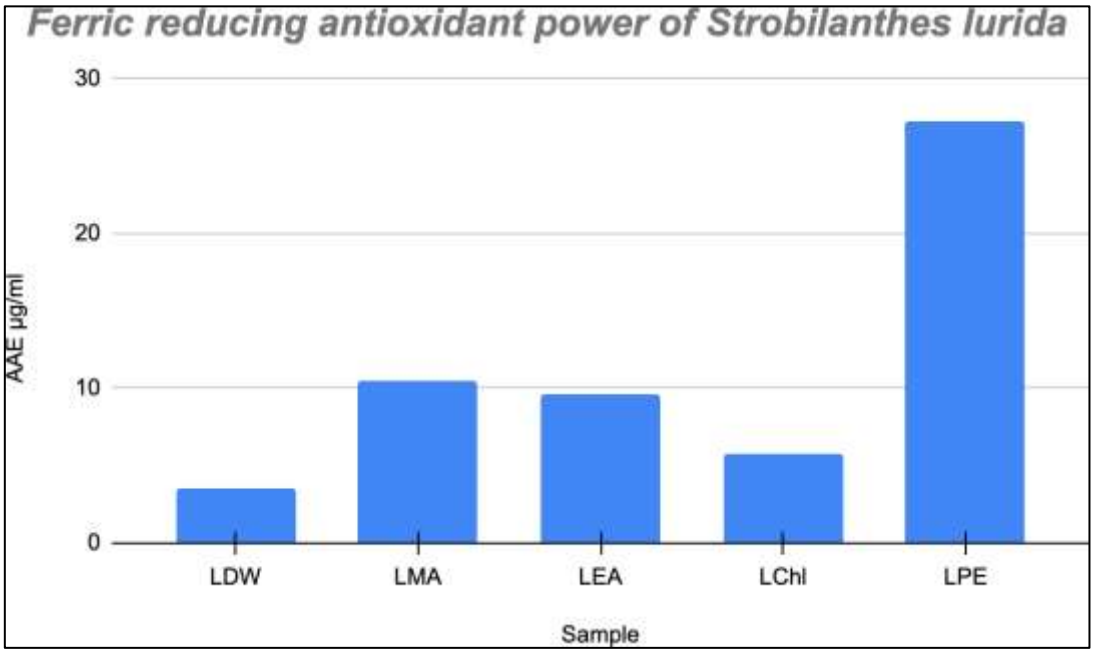

Fig. 3: Ferric reducing antioxidant power (FRAP) of *Strobilanthes lurida* leaf extracts expressed in AAE µg/ml

3.4. Nitric oxide scavenging assay (NOS):

Sl. No	Sample	IC 50 value (µg/ml)
1	Gallic acid	6.70
2	LDW	41.88
3	LMA	72.65
4	LEA	75.21
5	LChl	52.99
6	LPE	37.26

Table 4: Inhibition of nitric oxide free radicals by leaf extracts of *S. lurida*.

The nitric oxide scavenging assay measures the ability of an extract to inhibit nitric oxide free radicals. A lower IC_{50} value indicates more vigorous scavenging activity. In the present study, standard Gallic acid exhibited a very low IC_{50} of 6.70 µg/mL, confirming its high potency as a standard antioxidant. The results are tabulated in Table 4 and graphically represented in Figure 4. When compared with Gallic acid, all *Strobilanthes lurida* leaf extracts displayed weaker NO-scavenging activity, but with apparent variation across solvents. The petroleum ether extract (LPE) exhibited the strongest activity among the extracts with an IC_{50} of 37.26 µg/ml, indicating a relatively high capacity to neutralise nitric oxide radicals, though it remains about 5.5 times less potent than Gallic acid. This was followed by the aqueous extract (LDW, 41.88 µg/ml) and chloroform extract (LChl, 52.99 µg/ml), which showed moderate scavenging effects. The ethyl acetate (LEA, 75.21 µg/mL) and methanol extracts (LMA, 72.65 µg/mL) demonstrated the weakest activity, requiring significantly higher concentrations to achieve 50% inhibition.

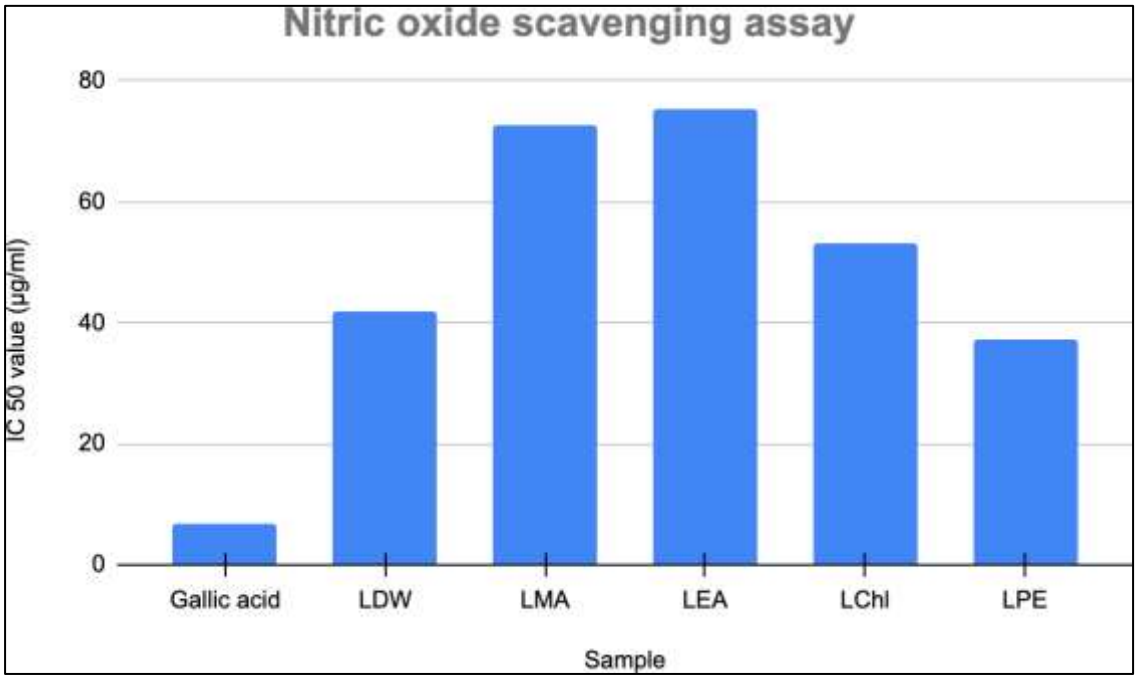

Fig. 4: Nitric oxide free radical scavenging potential of *Strobilanthes lurida* leaf extracts against the standard Gallic acid.

3.5. Reducing power assay:

The reducing power assay measures the ability of the extracts to donate electrons and convert Fe^{3+} to Fe^{2+}. A higher AAE value indicates a stronger reducing (electron-donating) ability and better antioxidant potential.

Sl. No	Sample	AAE µg/ml
1	LDW	6.15
2	LMA	10.8
3	LEA	11.58
4	LChl	5.37
5	LPE	0.47

Table 5: Reducing power assay of the leaf extracts of *S. lurida* expressed in µg of GAE/L.

Among the *Strobilanthes lurida* leaf extracts, the ethyl acetate extract (LEA) exhibited the highest reducing power with a value of 11.58 µg AAE/ml, indicating the strongest electron-donating ability among the tested samples. This was closely followed by the methanol extract (LMA, 10.8 µg AAE/ml) and the aqueous extract (LDW, 6.15 µg AAE/ml), both of which demonstrated moderate reducing activity. The chloroform extract (LChl) exhibited a comparatively lower reducing ability, with 5.37 µg AAE/ml. The petroleum ether extract (LPE) showed the weakest reducing power, with a value of only 0.47 µg AAE/mL, indicating a minimal electron-transfer capacity in this non-polar fraction (Table 5, Fig. 5).

Overall, the results clearly indicate that more polar solvents (ethyl acetate > methanol > water) extract compounds with stronger reducing potential, while the petroleum ether fraction contains minimal reducing constituents. This highlights the influence of solvent polarity on the antioxidant profile of *S. lurida* leaf extracts.

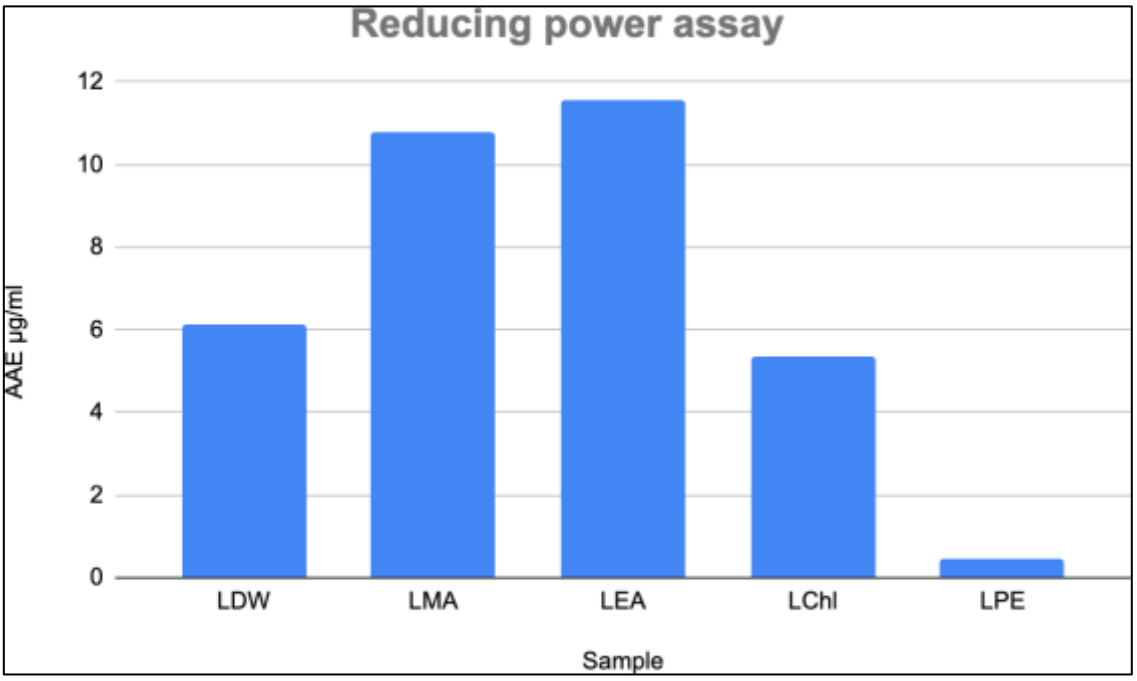

Fig. 5: Reducing power of *Strobilanthes lurida* leaf extracts expressed in AAE µg/ml.

The antioxidant activity of *Strobilanthes lurida* leaf extracts exhibited apparent solvent-dependent variation across all assays. In the DPPH and ABTS radical-scavenging assays, the chloroform (LChl) and aqueous (LDW) extracts showed the highest activity among the fractions. This trend aligns with the mechanisms described by Yamauchi *et al*. (2024), who demonstrated that phenolic hydroxyl groups and substitution patterns strongly govern DPPH scavenging efficiency. Similarly, Kotha *et al*. (2022) explained that ABTS is sensitive to both hydrophilic and lipophilic antioxidant constituents, which helps account for the relatively strong performance of LChl and LDW in the present study. These results collectively suggest that these fractions contain accessible phenolic and semi-polar compounds capable of effective electron and hydrogen donation. Similar free radical scavenging behaviour associated with antioxidant protein fractions has also been described by Mao et al. (2011).

In contrast, the FRAP assay identified petroleum ether extract (LPE) as the most substantial ferric-reducing fraction, exhibiting a markedly higher AAE value than the other extracts. This observation is consistent with the findings of Ilie *et al*. (2024), who reported that FRAP is exceptionally responsive to potent electron-donor compounds, including certain non-polar reductants that may not necessarily display high DPPH or ABTS activity. The high FRAP value of LPE, therefore, suggests the presence of lipophilic compounds capable of robust Fe^{3+} to Fe^{2+}reduction despite their lower radical-scavenging performance.

The nitric oxide scavenging results further support the unique antioxidant profile of LPE, which exhibited the most vigorous NO-inhibitory activity among the extracts. As demonstrated by Pradubyat *et al*. (2024), plant extracts rich in anti-inflammatory phytochemicals often exhibit significant NO-scavenging potential, since many bioactive constituents can neutralise reactive nitrogen species. This correspondence highlights the possibility that the LPE fraction contains lipophilic compounds with potential anti-inflammatory relevance. The reducing power (RP) assay presented a different pattern: ethyl acetate (LEA) and methanol (LMA) extracts showed the highest general reducing capacity, while LPE displayed minimal activity. This divergence is expected and reflects methodological differences between RP and FRAP assays. As explained by Kotha *et al*. (2022), RP and FRAP rely on distinct reaction chemistries and pH conditions, meaning fractions rich in mid-polarity phenolic may score highly in RP even when they are not the strongest in FRAP. The contrasting behaviours of LPE, LEA and LMA in the current study align well with this principle.

Taken together, these findings demonstrate that *S. lurida* leaves contain a diverse array of antioxidant constituents distributed across various solvent fractions. The literature consistently supports the multi-assay approach adopted here, as emphasised by Yamauchi *et al.* (2024) and Kotha *et al*. (2022). The present results demonstrate that each assay highlights different mechanistic aspects of antioxidant behaviours. Overall, LChl and LDW appear to be strong radical scavengers. LPE exhibits potent ferric-reducing and nitric oxide-inhibitory activity, and LEA/LMA demonstrate superior reducing power, reflecting the complex phytochemical diversity of the species.

CONCLUSION

Overall, the antioxidant evaluation of *Strobilanthes lurida* leaf extracts using DPPH, ABTS, FRAP, nitric oxide scavenging, and reducing power assays revealed clear solvent-dependent differences in activity. In both DPPH and ABTS radical-scavenging assays, all extracts showed lower potency than their respective standards (ascorbic acid), yet the chloroform (LChl) and aqueous (LDW) extracts consistently exhibited comparatively stronger scavenging abilities, while the methanol extract (LMA) remained the weakest. In the nitric oxide assay, although none approached the activity of Gallic acid, the petroleum ether extract (LPE) demonstrated the highest NO-scavenging potential among the extracts. FRAP analysis revealed a contrasting trend, with LPE exhibiting the

strongest ferric-reducing ability, suggesting the presence of potent electron-donating constituents in this non-polar fraction. However, in the general reducing power assay, the ethyl acetate and methanol extracts showed superior reducing capacity, whereas LPE exhibited minimal activity. Collectively, the results indicate that *S. lurida* leaves possess significant antioxidant properties, with different solvent fractions exhibiting distinct strengths across assays, reflecting the diverse phytochemical composition and polarity-dependent extraction of antioxidant compounds.

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.
All authors read and approved the final manuscript.

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