

In-vitro antioxidant analysis of *Aristolochia indica*, *Ipomoea obscura*, *Tylophora indica*, *Glinus oppositifolius* and *Abroma augustum* from Bankura district, West Bengal

Aratrika Bhadra, Manjunath BT*

Department of Life Sciences, Christ (Deemed to be University), Bangalore, Karnataka, India

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Article

ABSTRACT

Five therapeutic plants that have been utilized traditionally across the Sonamukhi Block of Bankura District, West Bengal, were tested for antioxidant activity using three assays: ABTS radical scavenging

activity, FRAP reduction power, and DPPH free radical scavenging. According to the DPPH assay, *Glinus oppositifolius* (68.4%) and *Ipomoea obscura* (23.83%) showed moderate radical-scavenging activity, whereas *Aristolochia indica* (73.07%), *Abroma augustum* (52.87%), and *Tylophora indica* (25%) demonstrated the highest levels. While *Glinus oppositifolius* (0.685) and *Ipomoea obscura* (0.401) showed moderate activity in the FRAP assay, *Abroma augustum* (0.459), *Tylophora indica* (0.637), and *Aristolochia indica* (0.545) demonstrated significant reducing power. According to the ABTS assay, *Aristolochia indica* (90.37%) and *Glinus oppositifolius* (98.7%) had the highest levels of radical scavenging activity. These findings support the traditional medical usage of these plants, especially *Glinus oppositifolius* and *Aristolochia indica*, which showed the most antioxidant qualities. The results highlight the importance of these plants in traditional medicine, shed light on their therapeutic potential, and lay the groundwork for further research on natural antioxidant treatments.

Keywords: Antioxidant, DPPH, FRAP, ABTS, Traditional medicine, Medicinal Plants

INTRODUCTION

The significance of medicinal plants and herbal medicine extends across geographical boundaries and cultural variations. Traditional healers, typically esteemed members of their communities, have played an essential role in maintaining and disseminating this knowledge. Their competence in discovering, producing, and providing plant-based treatments has been critical in meeting healthcare requirements, especially in areas where access to modern medical facilities is restricted.¹

The vast biodiversity of West Bengal is a treasure trove of medicinal plants used by traditional tribes for generations. Traditional healers in the Sonamukhi Block of Bankura District have relied on their extensive knowledge of indigenous flora to treat diseases and improve overall well-being.^{2,3} However, as industrialization and urbanization encroach on traditional traditions, there is an urgent need to objectively assess the therapeutic potential of these plants to ensure their conservation and sustainable usage.⁴

There has been an increasing interest in studying the antioxidant properties of medicinal plants in recent years due to their possible health advantages. Antioxidants from medicinal plants have received attention for treating various ailments.^{5,6} The FRAP test analyzes antioxidants' ability to decrease ferric ions, which indicates their electron-donating capability. In contrast, the DPPH and ABTS tests measure antioxidant scavenging activity against stable free radicals, providing information on their radical-quenching capacity.⁷ **DPPH** (2,2-Diphenyl-1-picrylhydrazyl),

*Corresponding Author: Manjunath BT, Christ (Deemed to be University), Bangalore, Karnataka, India
Tel: 91+ 9731903899; Email: manjunath.bt@christuniversity.in

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ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), and **FRAP** (Ferric Reducing Antioxidant Power). These experiments shed light on plant extracts' potential to lessen oxidative stress and scavenge free radicals.

The DPPH assay analyzes antioxidants' capacity to contribute hydrogen atoms to stabilize the DPPH radical,⁸ whereas the ABTS assay evaluates antioxidants' scavenging effectiveness against the ABTS radical cation. On the other hand, the FRAP test assesses antioxidants' reducing capacity by evaluating their ability to convert ferric ions to ferrous ions.⁹

The ABTS test 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) is a chemical substance used to study the reaction kinetics of certain enzymes. It is extensively used in ELISA to detect the binding of molecules to one another. It has a high reduction potential, therefore it may efficiently function as an electron donor for the reduction of Oxo-species such as O₂/H₂O₂, particularly in biological catalysis that operates at less severe pH levels. This feature of ABTS is utilized to test the antioxidant capabilities of certain foods in the food industry, agriculture, and ethnobotany. The principle behind ABTS is that it was first transformed into a radical cation by adding sodium persulfate. The ABTS radical cation is a blue chromophore with an absorption maximum of 734 nm. The resulting chromophore is reactive to a variety of antioxidants, including phenolics, thiols, and vitamin C.¹⁰ Because the antioxidants were added to the pre-formed ABTS radical action, the antioxidants found in plant extracts actually reduced the ABTS depending on the activity and concentration of the specific antioxidant.

The findings of these tests not only aid in identifying plants with high antioxidant activity but also give helpful information for the creation of antioxidant-rich supplements and pharmaceutical formulations also, understanding medicinal plants' antioxidant qualities makes it easier to include them in preventative and treatment efforts for oxidative stress-related disorders.¹¹ As a result, assessing antioxidant activities with DPPH, ABTS, and FRAP tests is essential in unlocking medicinal plants' therapeutic potential and boosting human health and well-being.⁸ This study aims to bridge the gap between traditional knowledge and current scientific confirmation by thoroughly evaluating medicinal plants' antioxidant activity and phytochemical profile from the Sonamukhi Block. This project aims to conserve indigenous wisdom while fostering evidence-based healthcare approaches utilizing nature's curative power.

MATERIALS AND METHODS

Medicinal Plants Identification

Plants were identified using internet resources such as Plant.id and PlantNet, as well as field identification tools like iNaturalist and PlantSnap. Standard botanical procedures were used to construct herbarium specimens, which included information such as native names, growth patterns, and therapeutic applications. Taxonomic identification and validation were carried out at the Foundation for Revitalization of Local Health Traditions (FRLHT) in Bengaluru, with authenticity confirmation supplied by the FRLHT, TDU.

The five medicinal plants, along with their authentication no. are given below—*Tylophora indica* (68218), *Ipomoea obscura* (127131), *Abroma augustum* (127123), *Glinus oppositifolius* (127122), and *Aristolochia indica* (127110)—they were chosen because of their widespread usage in traditional treatments and reputed usefulness in treating a variety of diseases. *Tylophora indica*, a member of the Apocynaceae family, was used to treat respiratory ailments using its leaves and roots.¹² *Ipomoea obscura*, a member of the Convolvulaceae family, has its leaves and stems used to treat joint discomfort and skin diseases.³ The bark and leaves of *Abroma augustum*, a Malvaceae plant, were used to treat diabetes and menstrual irregularities.¹³ *Glinus oppositifolius*, a member of the Molluginaceae family, employed the entire plant for liver and gastrointestinal disorders, while *Aristolochia indica*, from the Aristolochiaceae family, used the roots and stems for fevers and snake bites.³

Selection of Traditional Medicinal Plants

A selection of traditional plants from the Sonamukhi block in the Bankura district was made after questioning the traditional healers about their usage of herbal drugs. The plants with the maximum usage by the healers were taken into consideration, and these were then ethnomedically collected from the region.

The therapeutic plants taken from forests as well as villages in and around the Sonamukhi Block of Bankura District in October, a non-flowering season, were mostly devoid of blooms. Fully grown, mature plants, mostly composed of roots, stems, and leaves, were dried and extracted for antioxidant evaluations.

Plant Extraction

The powdered samples were subjected to extraction of secondary metabolites using methanol. 40 grams of each powdered sample were filled in blotting paper and placed inside a thimble. 200 ml of the solvent was added to the thimble, which was then fitted into a round bottom flask containing 700 ml of the solvent. The Soxhlet apparatus was run for 6-8 hours at a temperature based on the boiling point of the respective solvent. Afterward, the extract was being subjected to distillation for 2-3 hours. The extract was then placed in a hot air oven at 40°C for drying. The dried extracts of *Ipomoea obscura*, *Tylophora indica*, *Abroma augustum*, *Glinus oppositifolius*, and *Aristolochia indica*. thus obtained were being used for various analyses.

DPPH Assay

The plant extracts' capacity to scavenge free radicals was assessed using the DPPH test. 0.004 g of DPPH was dissolved in 100 mL of methanol to create a 0.004% DPPH solution, and a spectrophotometer was used to adjust the absorbance to around 0.9-1.0 at 517 nm. Methanol was used to make plant extracts in a range of concentrations (0.1 to 0.5 mg/mL). For instance, 20 µL of plant extract and 3980 µL of 0.004% DPPH solution were added to a test tube at a 2 mg/ml concentration, guaranteeing a total reaction volume of 4 mL. Using the same volume proportions, this procedure was performed for each of the plant extract's concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL).^{14,15}

For the blank, methanol was added in lieu of the DPPH solution, and the plant extract was added. 0.1 mL of methanol was added to 4 mL of DPPH solution⁷ for the control. The reaction mixtures

were allowed to sit at room temperature in the dark for 15 to 30 minutes.

Gallic acid was chosen as the benchmark antioxidant for comparison because of its potent ability to scavenge radicals and its applicability in research on phenolic compounds.

Following incubation, each sample's absorbance was measured spectrophotometrically at 517 nm, using methanol as the control reference.¹⁶ The percentage inhibition, which indicates the antioxidant activity of the plant extract samples, was determined using the following formula:

$$\text{Percentage inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of the sample}]}{\text{Absorbance of control}} \times 100.$$

This computation allowed for measuring the amount to which the samples blocked the DPPH radical, providing information about their antioxidant capacity.

FRAP Assay

Different amounts of the methanol extract (100-500µg) of samples and varied concentrations of the standard (1-5µg) were placed in separate test tubes, and the capacity in each test tube was filled up to 0.1mL with methanol. In each tube, 2.5 mL of 0.2 M sodium phosphate buffer [Na₂HPO₄•2H₂O] (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution [K₃Fe (CN) 6] were added. The reaction mixture was thoroughly vortexed before incubating for 20 minutes at 50°C using a vortex shaker.¹⁷ After the incubation, 2.5 mL of 10% trichloroacetic acid C₂HCl₃O₂ was added to the mixture and centrifuged at 3,000 rpm for 10 minutes¹⁵.

ABTS Assay

The free radical scavenging ability of extracts from various plant species was calculated using the stable ABTS radical. Samples of varying concentrations (100-500 µg) were placed in test tubes and filled with 0.1 mL of methanol each. All tubes received 3 mL of ABTS solution (with absorbance pre-set to 1), and the samples were incubated in the dark for 30 minutes. Following incubation, the absorbance was measured spectrophotometrically at 734 nm, using methanol as a blank¹⁸ estimated using the formula:

$$\text{Percentage inhibition} = \frac{[\text{Abs of control} - \text{Abs of sample}]}{\text{Abs of control}} \times 100.$$

As the reaction progressed, the blue ABTS radical cation chromophore returned to its colorless neutral state. the antioxidant compounds in the sample solution quenched the color of the ABTS radical cation, resulting in a decoloration of the solution proportionate to their quantity. So, the endpoint here is stable and reveals the measurement of the antioxidant efficiency.

Statistical Analysis

A one-way ANOVA was employed in the statistical analysis to ascertain the significance of variations in each measured variable. All measurements were performed in triplicate. A significance level of p<0.05 was applied while conducting Duncan's multiple range test (DMRT).^{13,19} This post hoc analysis makes the detection of specific differences between means easier. The results demonstrate that every medicinal plant strongly responded to each assay. The analysis determined the level of statistical significance.

RESULTS

Table 1 provides the absorbance readings at 517 nm and the percentage inhibition of the compounds when evaluated against Gallic Acid as the standard. Sample values ranged from 1 to 5 µg. As the sample concentration grew, the absorbance at 517 nm decreased, suggesting enhanced antioxidant activity. Furthermore, the percentage inhibition values increased with more significant sample concentrations, indicating a remarkable ability to scavenge the DPPH radicals. These findings emphasize the samples' dose-dependent antioxidant capabilities, with higher concentrations and stronger radical scavenging activity.

Five samples of medicinal plants—*Ipomoea obscura*, *Tylophora indica*, *Abroma augustum*, *Glinus oppositifolius*, and *Aristolochia indica*—have their absorbance values at 517 nm displayed in Figure 2. The specimens were examined within a concentration span of 100 µg to 500 µg. As the concentration of each plant sample rose, a consistent pattern of lowering absorbance values was seen, suggesting a dose-dependent decrease in absorbance and an increase in antioxidant activity as a result. This trend implies that increased radical scavenging capacities correlate with larger plant extract concentrations.

Error bar graphs were created to visually depict the results and show the differences and statistical significance of the antioxidant activity across the plant samples at different concentrations. The aforementioned studies offer a comprehension of the antioxidant characteristics of the examined plants and emphasize the significance of concentration in ascertaining their effectiveness.

(Figure 1- Figure 5). Radical Scavenging Activity using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH) in *Ipomoea obscura* (Io), *Tylophora indica* (Ti), *Abroma augustum* (Aa), *Glinus oppositifolius* (Go), *Aristolochia indica* (Ai). The data set corresponds to the mean values + SE of three replicates, with every analysis performed three times. Means with different letters are significantly different at P<0.05, according to Duncan's multiple range test (DMRT).

Table 2 shows absorbance values at 700 nm for sample extracts and the Gallic Acid standard, which rises with concentration. Concentration vs. absorbance at 700 nm, demonstrating increased antioxidant activity of sample extracts compared to the Gallic Acid standard using the FRAP test.

The absorbance values at 700 nm for the medicinal plant extracts *Glinus oppositifolius*, *Aristolochia indica*, *Abroma augustum*, *Tylophora indica*, and *Ipomoea obscura* were displayed in Figure 4 for a range of dosages from 100 µg to 500 µg. Higher quantities of the plant extracts continuously raised the absorbance measurements, showing a dose-dependent increase in antioxidant activity. This pattern showed that plant extracts at higher concentrations were more able to remove ferric ions, indicating increased antioxidant capability.

(Figure 6-Figure 10) Ferric Reducing Antioxidant Power Assay (FRAP) in *Ipomoea obscura* (Io), *Tylophora indica* (Ti), *Abroma augustum* (Aa), *Glinus oppositifolius* (Go), *Aristolochia indica* (Ai). The data set corresponds to the mean values + SE of three replicates, with every analysis performed three times. Means with

different letters are significantly different at $P < 0.05$, according to Duncan's multiple range test (DMRT).

The ABTS Radical scavenging activity percentage was determined for five different plant extract concentrations (100, 200, 300, 400, and 500 $\mu\text{g/ml}$) (Figure 11-Figure 15). Table 3 shows absorbance values at 700 nm for sample extracts and the Gallic Acid standard, which rises with concentration. Concentration vs. absorbance at 700 nm. The scavenging activity for *Ipomoea indica* (Io) arose gradually, peaking at 88.67% at 400 $\mu\text{g/ml}$ with a minor decline to 75.7% at 500 $\mu\text{g/ml}$. The activity climbed progressively from 32.77% at 100 $\mu\text{g/ml}$. *Tinospora indica* (Ti) exhibited a robust scavenging activity, peaking at 87.47% at 500 $\mu\text{g/ml}$ after beginning at 28.93% at 100 $\mu\text{g/ml}$. There was a significant increase at 300 $\mu\text{g/ml}$ (79.63%). At all concentrations, *Abroma augustum* (Aa) exhibited a steady rise in activity, starting at 28.23% and reaching a high of 74.73% at 500 $\mu\text{g/ml}$. *Glinus oppositifolius* (Go) showed a significant and persistent rise in activity, peaking at 400 $\mu\text{g/ml}$ at 98.7% after beginning at 37.93% and declining slightly to 90% at that concentration. Lastly, at 500 $\mu\text{g/ml}$, *Aristolochia indica* (Ai) showed the greatest beginning activity at 43.57% and continued to climb steadily, reaching 89.33%. These findings suggest that the plant extracts have differing levels of antioxidant activity, with *Aristolochia indica* and *Glinus oppositifolius* exhibiting very high effectiveness at greater doses.

Table 1: Sample Extracts' Antioxidant Activity in Comparison to the Gallic Acid Standard: Absorbance and Percentage Inhibition Analysis (DPPH)

| Concentration of sample in μg | Gallic Acid (DPPH) | |
|--|----------------------|-----------------------|
| | Absorbance at 517 nm | Percentage Inhibition |
| 1 | 0.859 | 14.1 |
| 2 | 0.766 | 23.4 |
| 3 | 0.636 | 36.4 |
| 4 | 0.534 | 46.6 |
| 5 | 0.419 | 58.1 |

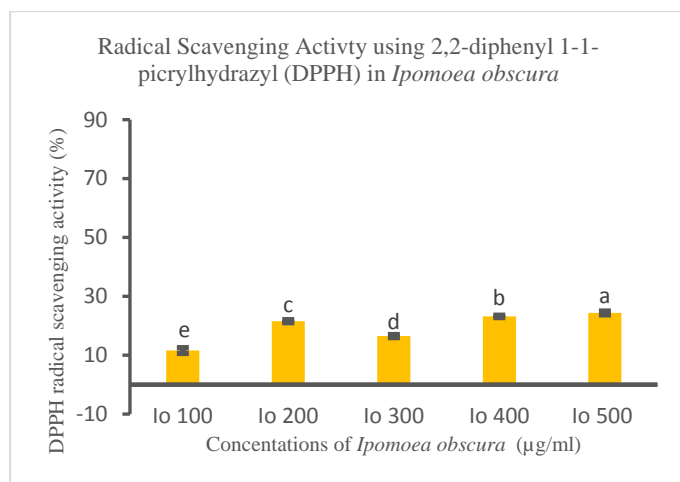


Figure 1. Radical Scavenging Activity using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH) in *Ipomoea obscura* (Io). Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT).

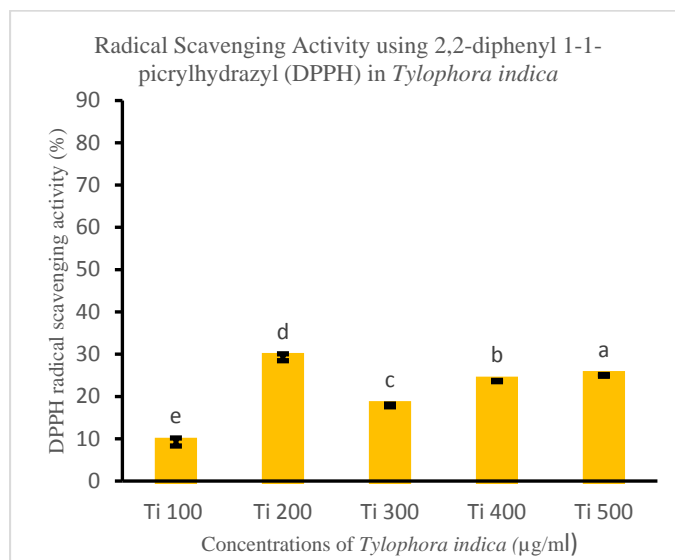


Figure 2. Radical Scavenging Activity using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH) in *Tylophora indica* (Ti). Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

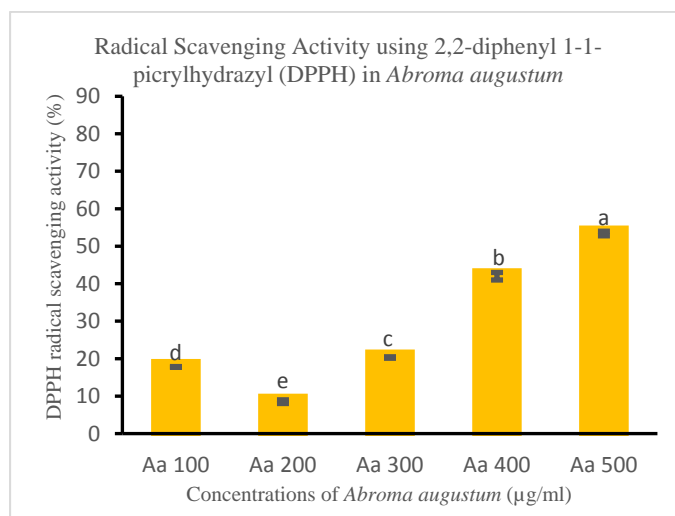


Figure 3. Radical Scavenging Activity using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH) in *Abroma augustum*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT).

Table 2: Sample Extracts' Antioxidant Activity in Comparison to the Gallic Acid Standard: Absorbance and Percentage Inhibition Analysis (FRAP)

| Conc of Sample in μg | Gallic Acid (FRAP) |
|---------------------------------|----------------------|
| | Absorbance at 700 nm |
| 100 | 0.494 |
| 200 | 0.557 |
| 300 | 0.612 |
| 400 | 0.662 |
| 500 | 0.734 |

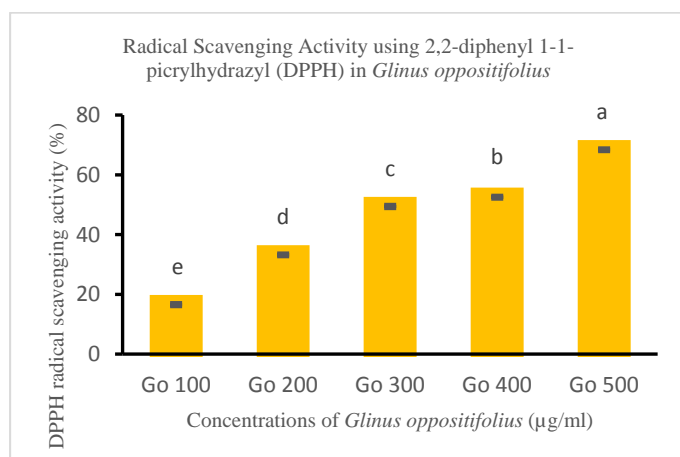


Figure 4. Radical Scavenging Activity using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH) in *Glinus oppositifolius* (Go). Data represent mean values \pm SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

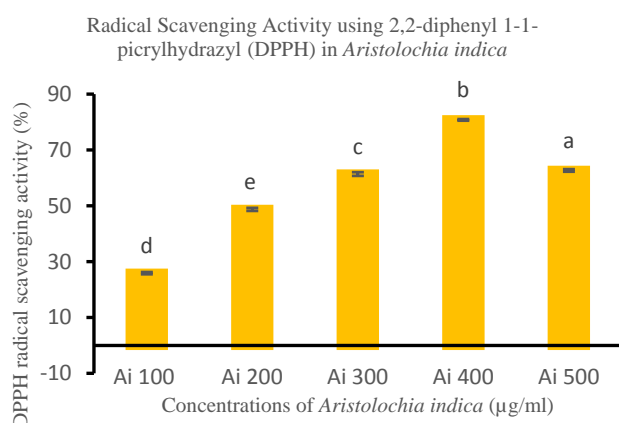


Figure 5. Radical Scavenging Activity using 2,2-diphenyl 1-1-picrylhydrazyl (DPPH) in *Aristolochia indica* (Ai). Data represent mean values \pm SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

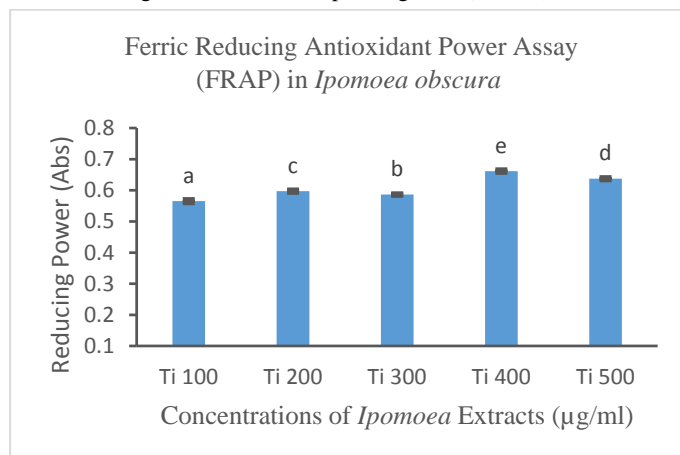


Figure 6. Reducing Power Assay (FRAP) in *Ipomoea obscura*. Data represent mean values \pm SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

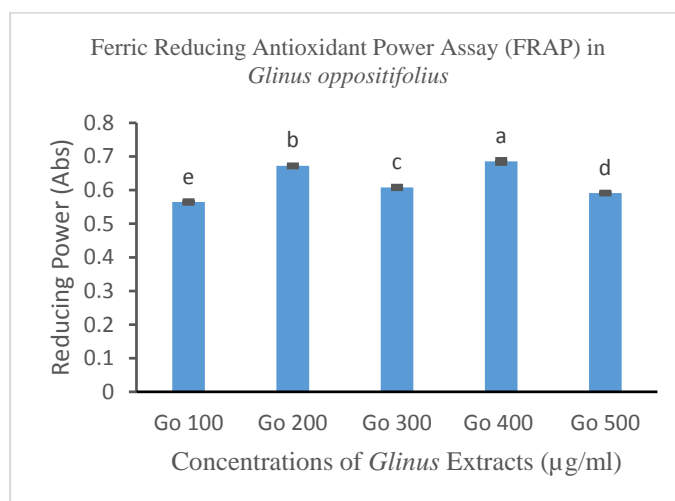


Figure 7. Reducing Power Assay (FRAP) in *Glinus oppositifolius*. Data represent mean values \pm SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

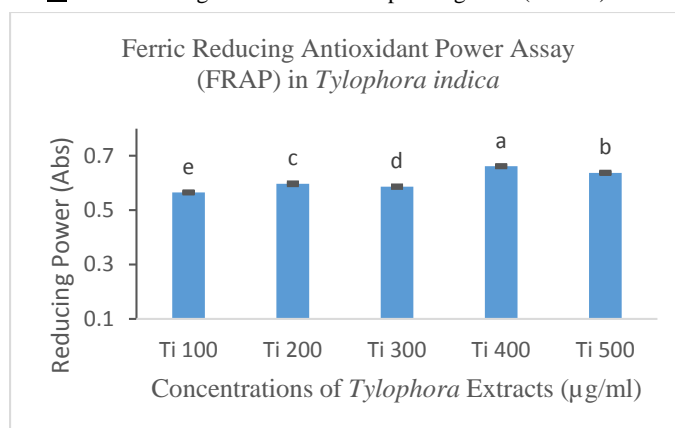


Figure 8. Reducing Power Assay (FRAP) in *Tylophora indica*. Data represent mean values \pm SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

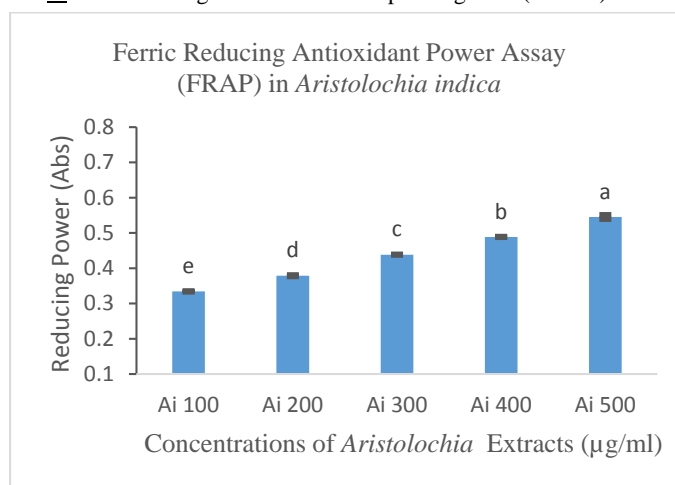


Figure 9. Reducing Power Assay (FRAP) in *Aristolochia indica*. Data represent mean values \pm SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P \leq 0.05$ according to Duncan's multiple range test (DMRT)

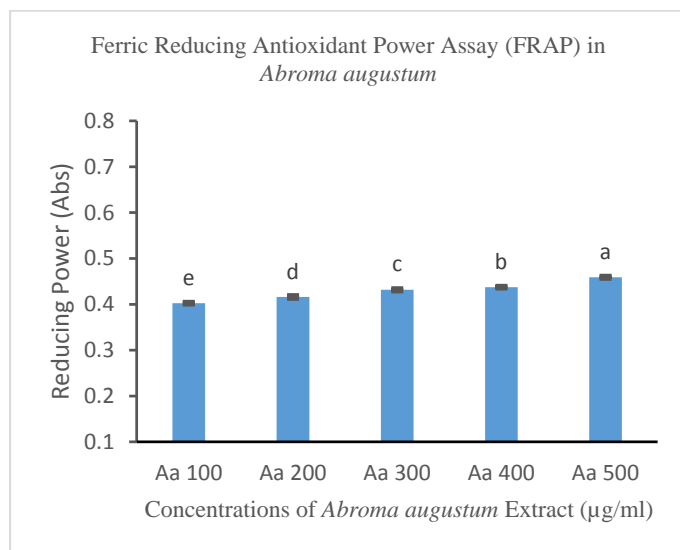


Figure 10. Reducing Power Assay (FRAP) in *Abroma augustum*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

Table 3: Sample Extracts' Antioxidant Activity in Comparison to the Gallic Acid Standard: Absorbance (at 734 nm) (ABTS)

| Conc of Standard in µg | Gallic Acid | |
|------------------------|----------------------|-----------------------|
| | Absorbance at 734 nm | Percentage Inhibition |
| 1 | 0.821 | 17.9 |
| 2 | 0.658 | 34.2 |
| 3 | 0.502 | 49.8 |
| 4 | 0.348 | 65.2 |
| 5 | 0.215 | 78.5 |

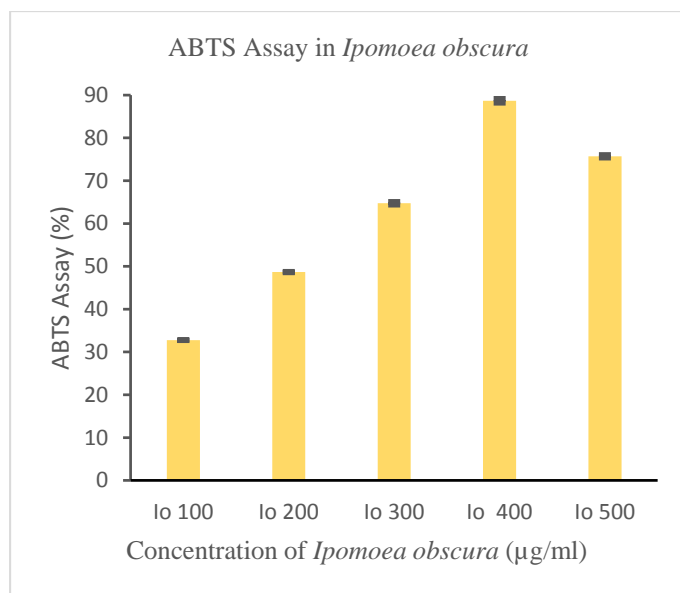


Figure 11. ABTS assay in *Ipomoea obscura*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

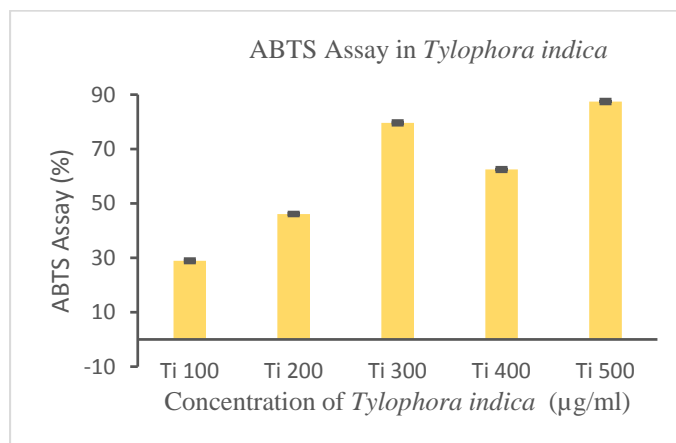


Figure 12. ABTS assay in *Tylophora indica*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

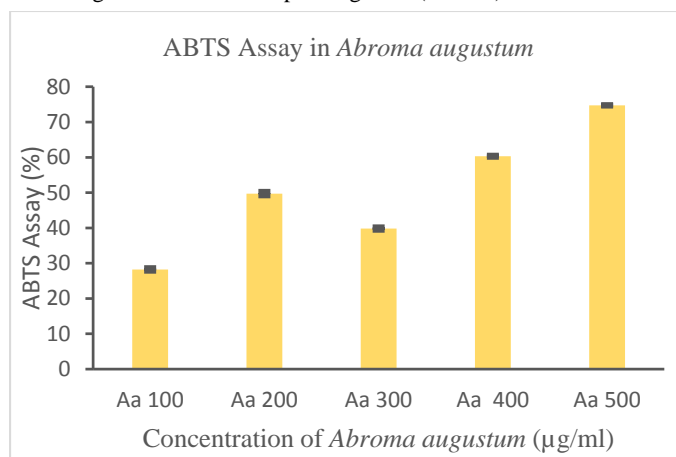


Figure 13. ABTS assay in *Abroma augustum*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

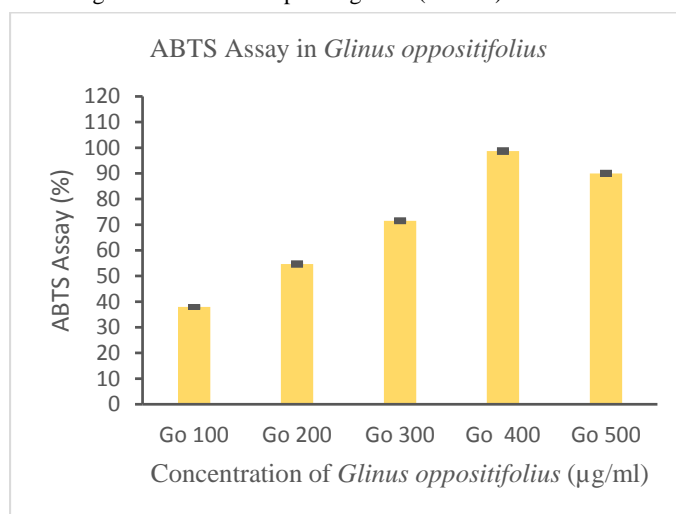


Figure 14. ABTS assay in *Glinus oppositifolius*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

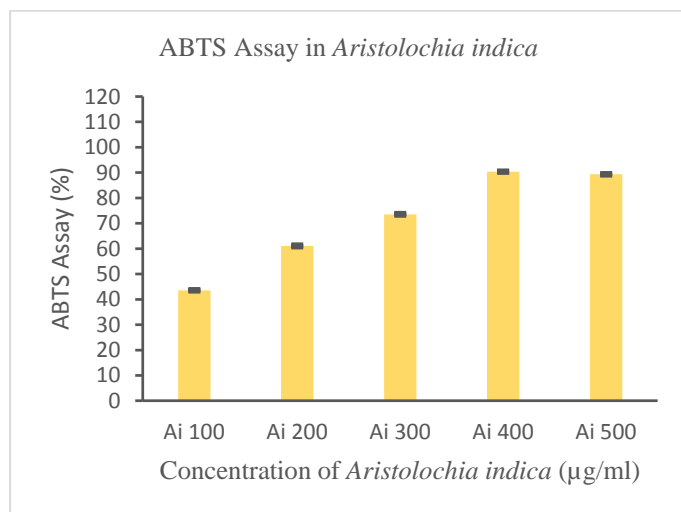


Figure 15. ABTS assay in *Aristolochia indica*. Data represent mean values + SE of 3 replicates; each experiment was repeated thrice. Means with different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test (DMRT)

The mean absorbance values were computed for every dosage with standard deviations and standard errors. One-way ANOVA was used in the statistical analysis to assess the variations in the estimated values' means. The significance test was carried out. The values of Duncan's Multiple Range Test (DMRT) were noted to assess the significance of the mean differences. These studies verified the statistically significant differences in antioxidant activity across the plant extracts at various doses (Figure 1- Figure 15). Statistical data were provided as mean \pm SD. Gallic acid's linear regression coefficient (R^2) was determined using both tests. P-values < 0.05 were deemed significant.

DISCUSSION

Antioxidants operate by impeding enzymes or chelating trace elements involved in free radical production, scavenging reactive species, and increasing the activity of antioxidant enzymes.²⁰

Dose-Dependent Activity: In the FRAP and DPPH tests, all plant extracts showed enhanced concentration-dependent antioxidant activity, as shown by increased absorbance at 700 nm and lowered absorbance at 517 nm, respectively. This pattern is consistent with past research showing that greater doses improve the effects of radical scavenging.¹³ According to Oyaizu M. (1986),²¹ DPPH is a proton-free radical with maximal absorption at 517 nm. When exposed to proton radical scavengers, DPPH's purple hue disappears quickly. Antioxidants can reduce absorbance by donating electrons or giving a hydrogen atom to quench DPPH· free radicals, resulting in a colorless bleached product. A drop in the absorbance band was seen when GOEE concentrations increased, confirming antioxidant action.²¹

Comparative Analysis: *Aristolochia indica* showed the most activity in both tests out of all the plants tested, indicating that it has stronger antioxidant qualities than the other plants examined. *Aristolochia indica* extract showed the highest radical scavenging activity at 500 µg/mL with a radical scavenging activity of 73.06%. *Aristolochia*'s robust ability to scavenge radicals was also noted in

earlier research. Comparable outcomes were noted in *Aristolochia longa*, where the methanolic and aqueous fractions showed higher DPPH radical scavenging activity of 74.66% and 77.17%, respectively, at 416 µg/mL concentration. Furthermore, at all doses, the aqueous extract exhibited reduced activity. These findings demonstrated that *A. longa* has a significant concentration of chemicals capable of donating protons and scavenging radicals.²² After *A. indica*, *Glinus oppositifolius* extract demonstrated superior radical scavenging efficacy (68.4% at 500 µg/mL). These findings are consistent with the results compared. The extract also demonstrated mild DPPH radical scavenging activity and moderate NO radical scavenging activity, which may help to arrest the chain of reactions initiated by excess NO generation that are harmful to human health.²¹ *Abroma augustum* comes in third place, with its 52.8% radical scavenging activity measured at 500 µg/mL; nevertheless, other findings were seen in Since the experiment was conducted using a variety of standards and techniques, the dia resin adsorbed fraction showed the highest DPPH radical scavenging activity, measuring 82.88 µg/mL at a concentration of 200 µg/mL.²³ *T. indica* displayed significant antioxidant activity by blocking DPPH free radicals, indicating that the roots and leaves extract is a very rich source of natural antioxidant agent, which backs up the reading.¹² *Ipomoea obscura* shows the lowest % scavenging activity at 500 µg/mL, 23.83%, but still very good results. Dissimilar results were found in different species of stem and leaf extract Nigerian *Ipomoea*, about 86.64% scavenging activity was observed in the stem extract of *Ipomoea aquatica* of Nigeria.²⁴ *Tylophora indica* had the maximum absorbance in ferric-reducing antioxidant potential (FRAP) tests (0.637333) at 500 µg/mL. SD- 0.00305505. Increased absorbance of the reaction mixture indicated a higher reducing power. The FRAP test for antioxidants is simple, repeatable, and concentration-dependent.²⁵ All of the extracts from the tested plants demonstrated concentration-dependent reduction power, similar to their radical scavenging activity. *Aristolochia indica* had the strongest reducing antioxidant capacity, followed by *Glinus oppositifolius*, *Abroma augustum*, *Tylophora indica* (with the highest ferric reducing absorbance value), and *Ipomoea obscura* when compared to the standard. At a concentration of 181 µg/mL, the aqueous and methanolic fractions of *A. longa* showed 90.89% and 82.58% ABTS scavenging activity respectively.²² This is consistent with the results obtained.

CONCLUSION

The antioxidant capabilities of selected medicinal plant extracts were assessed using DPPH and FRAP tests, which gave helpful information about their prospective therapeutic uses. The results indicated considerable differences in antioxidant activity across the studied plant samples, with some displaying strong free radical scavenging capacities. *Aristolochia indica* was the most promising medicinal plant, with the highest antioxidant activity across all experiments, followed by *Glinus oppositifolius*. *Tylophora indica*, *Ipomoea obscura*, and *Abroma augustum* have much lesser antioxidant potential. These findings highlight the significance of doing more studies to identify the bioactive molecules responsible for the observed antioxidant activity and their mechanisms of action. Understanding the radical scavenging and ferric-reducing

properties might help to clarify their possible health advantages and pharmacological qualities, emphasizing their importance in traditional medicine and drug discovery activities.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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