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Antioxidant Potential of Gynandropsis Pentaphylla Leaves Extracts

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The present study investigates the antioxidant properties of acetone extract due to strong phytochemical profile derived from the leaves of Gynandropsis pentaphylla. The antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays. The acetone extract exhibited significant free radical scavenging activity by showing IC50 value 66.69µg/ml when compared to standard Ascorbic acid IC50 value 5.89µg/ml, whereas ABTS exhibited IC50 value 224.32ug/ml when compared to standard Ascorbic acid IC50 value 9.81ug/ml and ferric reducing antioxidant potential equivalent to standard to exhibit their potential by indicating its natural antioxidant property. The findings suggest that Gynandropsis pentaphylla leaves contain bioactive compounds with strong antioxidant properties, which could be harnessed for therapeutic applications. Further research is recommended to isolate and identify the specific phytochemicals responsible for these activities and study the various biological properties to investigate newer agent for the therapeutic system.
Keywords: Gynandropsis pentaphylla, Antioxidants, Phytochemicals, Free radical, Leaves.

1. Introduction

Medicinal plants have long been recognized for their therapeutic properties, particularly in the treatment of chronic diseases such as diabetes and inflammatory conditions. These plants contain bioactive compounds like polyphenols, flavonoids, and alkaloids, which exhibit significant antidiabetic and anti-inflammatory effects. For instance, polyphenols found in various medicinal plants can improve insulin sensitivity and reduce blood glucose levels, making them effective in managing type 2 diabetes mellitus (T2DM) (1).

Additionally, these compounds possess strong antioxidant properties that help mitigate

oxidative stress and inflammation, which are common underlying factors in both diabetes and inflammatory diseases (2). The use of medicinal plants in traditional medicine systems, such as Ayurveda, highlights their accessibility and affordability compared to conventional pharmaceuticals, making them a valuable resource for primary healthcare, especially in developing countries (3). Antioxidants play a crucial role in neutralizing harmful free radicals and oxidative stress, which are implicated in various chronic diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders. As interest in natural remedies and preventive healthcare grows, understanding the antioxidant capacities of medicinal plants becomes increasingly important (4). Medicinal plants such as paradise tree (Simarouba glauca), thumbai (Leuca aspera), and Rain lilly (Zephyranthes citrina) are renowned for their high antioxidant content. These plants contain bioactive compounds like polyphenols, flavonoids, and vitamins, which contribute to their antioxidant activity and therapeutic effects (5-7). The diverse antioxidant compounds found in these plants help protect cells from oxidative damage, thus promoting health and longevity (8). Gynandropsis pentaphylla also known as the African spider plant, is a medicinal plant with a wide range of therapeutic properties. This plant is rich in bioactive compounds such as flavonoids, alkaloids, terpenoids, and saponins, which contribute to its medicinal benefits. Traditionally, Gynandropsis pentaphylla has been used to treat various ailments, including neuralgia, headaches, coughs, and wounds. It also exhibits significant antimicrobial, antidiabetic, and anti-inflammatory properties (9).

The leaves of this plant are particularly noted for their high vitamin C, iron, and calcium content, making them a nutritious addition to the diet (4). Furthermore, the plant's strong antioxidant activity helps in combating oxidative stress, which is beneficial in preventing chronic diseases (10, 11). In this study, we aim to provide a comprehensive analysis of the antioxidant profile of acetone extract derived from the leaves of Gynandropsis pentaphylla. The focus is on elucidating further pure compounds and know their biological activities and potential pharmacological properties in in vivo models.

2. Material and Methods

The chemicals, reagents, and solvents used in this study were of analytical grade and were procured from Hi-media Laboratories Pvt. Ltd., Mumbai, and Qualigens Fine Chemicals Pvt. Ltd., Mumbai. The leaves of Gynandropsis pentaphylla were collected from agricultural areas in North Karnataka between August and October 2023. The plant material was authenticated by the Department of Botany, following the herbarium accession and flora deposits listed in the Indian Medicinal Plant Directory.

Preparation of Plant Material and Extraction

The collected Gynandropsis pentaphylla leaves were initially cleaned and rinsed with tap water, followed by washing with distilled water and sterilization using 70% ethanol. The leaves were then thoroughly dried in the shade at room temperature for 10 days. Once dried, the leaves were mechanically ground into a fine powder, which was then neatly packed in a glass container and stored in a refrigerator. For the extraction process, the powdered leaves were subjected to successive Soxhlet extraction carried out using acetone solvent for 18 hours.

The extract was subsequently dried using a Buchi rotary vacuum evaporator and stored in a refrigerator for further analysis (2).

Antioxidant Activity of Gynandropsis pentaphylla Leaves Acetone Extract

Antioxidant activity of Acetone extract of Gynandropsis pentaphylla leaves was determined by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay, ABTS (2, 2'-azinobis-{3-ethyl-benzothiazoline-6-sulphonic acid}) assay and FRAP (ferric reducing antioxidant power) assay (13-16).

1. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) Assay

The potential of Gynandropsis pentaphylla leaf extracts to scavenge DPPH radicals was assessed using the Brand-Williams et al (13). method. For this, 25 mg of the extracts were dissolved in 100 ml of methanol. Standard Ascorbic acid (3.0 ml containing 18 µg of Ascorbic acid) and 3.0 ml of serially diluted extracts, ranging from 15.625 µg/ml to 250 µg/ml, were mixed with 3.0 ml of DPPH solution and incubated for 30 minutes at room temperature. A test control was prepared by mixing 3.0 ml of DPPH solution with 3.0 ml of methanol. The absorbance was measured at 517 nm against methanol. The percentage of radical scavenging activity of the plant extracts was calculated using the following formula and expressed as grams of Ascorbic acid equivalent per 100 grams of dry weight.

2. ABTS assay (2,2'–azinobis–{3–ethylbenzothiazoline–6–sulphonic acid})

The original principle of the assay is the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS (2, 2'-azinobis-{3-ethyl-benzothiazoline-6-sulphonic acid}) to produce a ABTS radical cation (ABTS·+), a soluble chromogen, which is green in color and can be determined spectrophotometrically at 734 nm (14). Antioxidant activity of Gynandropsis pentaphylla leaves acetone extract as per ABTS·+ decolorization assay was measured using the method. The working solution of ABTS·+radical cation was prepared by adding ABTS (47.5 ml, 7 mM) to potassium persulfate (1.3 ml, 100 mM), and making up the volume to 50 mL with distilled water. The solution was kept in the dark at room temperature for 18 h, and then diluted with potassium phosphate buffer 1:1ratio (0.1 M, pH 7.4) to an absorbance of 0.70 (± 0.02) at 734 nm. 100 mg of the extracts were dissolved in 100 ml methanol and 15.62 µg to 250 µg of extracts containing 1 mg / ml was placed in different test tube and mixed thoroughly with 2.75 ml of $ABTS^{\cdot+}$ radical cation working solution and volume made upto 3ml with methanol. Absorbance of the resulting mixture was recorded at 734 nm. 15.62 µl of BHT (1µg/ µl in methanol) was used as standard and methanol as a Blank. Controls were prepared containing the same volume without any extract. The percent antioxidant activity of the sample was determined using the following formula and expressed as gm BHT equivalent / 100 gm dry weight.

3. Ferric Reducing Antioxidant Power Assay

Ferric reducing antioxidant potential (FRAP) of the given extracts was measured according to the method proposed by Benzie and Strain (15). FRAP reagent was prepared by mixing in 25 mL sodium acetate buffer (30 mM; pH 3.6), 2.5 mL 10 mM TPTZ solution (312.33 mg/100ml in 40 mM HCl) and 2.5 mL ferric chloride solution (20mM). The mixture was incubated for 15 min at 37 °C before use. The blank contained an equal volume of methanol instead of the plant sample. The results were reported as µg of Ascorbic acid equivalents (AAE) per ml.

Statistical Analysis

All the experiments were performed in triplicates and the results were expressed as mean ± standard error. Statistical analysis of the data was executed using Microsoft Office Excel 2016.

3. Results

Results of Antioxidant Property of Gynandropsis pentaphylla Leaves Extracts

1 DPPH free radical scavenging of the Acetone Extract of Gynandropsis pentaphylla Leaves

The observations in statistical data of DPPH RSA study by spectrophotometer/ELISA reader suggesting us that given tested extracts along with Ascorbic acid and acetone extract showed the DPPH RSA inhibition on dose dependent manner with IC_{50} values of Ascorbic acid was 5.89µg/ml and acetone extract was 66.69µg/ml. Ascorbic acid was used as a std control for the study and observed acetone extract was highly effective in DPPH RSA inhibition (Table 1 and Figure 1).

2 ABTS•+ Decolorization Activity of the Acetone Extract of Gynandropsis pentaphylla Leaves

The observations in statistical data of ABTS•+ Decolorization potential study by spectrophotometer/ELISA reader suggesting us that given test compounds viz Ascorbic acid, acetone, ethyl acetate and ethanol extract showed IC_{50} concentrations of Ascorbic acid was 9.81ug/ml and acetone was 224.32ug/ml. Among given graded concentration 100µg/ml exhibited 96.88% of ABTS inhibition in acetone extract and it was satisfactory ABTS•+ decolorization potential property with the considerable low IC_{50} values compared to the standard drug, Ascorbic acid which exhibited 9.81ug/ml used in the study (Table 1 and Figure 2).

Table 1: Comparative % DPPH inhibition values and % ABTS•+ decolorization potential values of the Ascorbic acid and acetone, ethyl acetate and ethanol extract on dose dependent manner along with IC_{50} concentration.

Concentration (µg/ml)	DPPH RSA activity of Ascorbic acid	DPPH RSA activity of Acetone extract	ABTS•+ decolorization potential of Ascorbic acid	ABTS•+ decolorization potential of Acetone of extract
	% DPPH RSA Inhibition	% DPPH RSA inhibition	% ABTS Inhibition	% ABTS inhibition
DPPH alone	0.00	0.00	0.00	0.00
3.12µg/ml	38.64	7.99	4.16	6.07
6.25µg/ml	50.87	49.34	35.79	20.71
12.5µg/ml	78.35	55.56	64.21	32.24
25µg/ml	96.00	65.94	88.56	54.59
50µg/ml	96.70	74.70	96.27	87.44
100µg/ml	98.07	87.46	100.78	96.88
	IC_{50} conc= 5.89ug/ml	IC_{50} conc= 66.69µg/ml	IC_{50} conc = 9.81ug/ml	IC_{50} conc = 224.22µg/ml

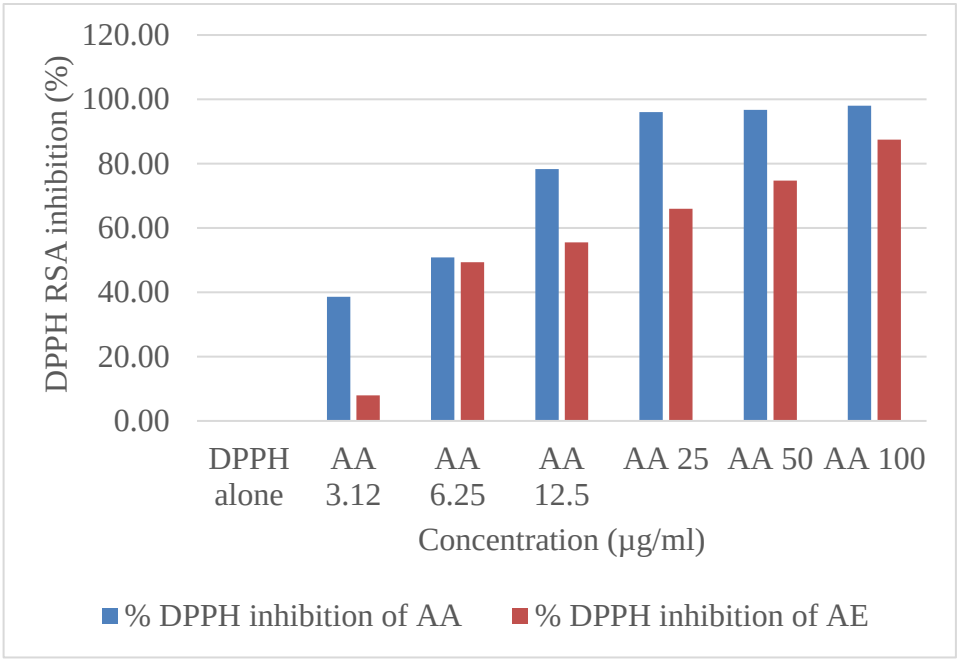

Figure 1: Comparative % DPPH inhibition values potential values of the Ascorbic acid and acetone extract on dose dependent manner

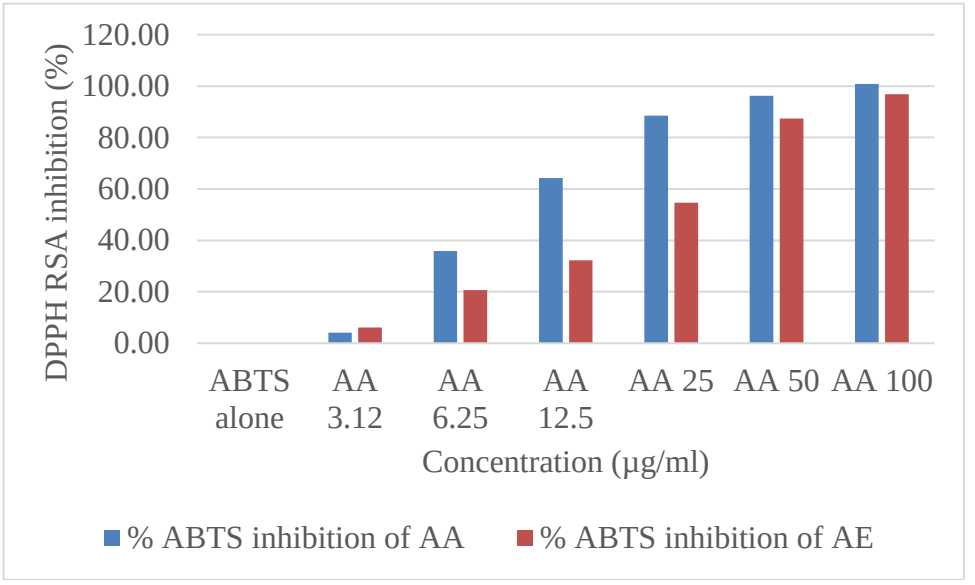

Figure 2: Comparative % ABTS •+ decolorization potential values of the Ascorbic acid and acetone extract on dose dependent manner

3. Ferric Reducing Antioxidant Potential (FRAP) Acetone Extract of Gynandropsis pentaphylla Leaves

The observations in statistical data of FRAP study by spectrophotometer suggesting us that given tested extract such as acetone showed the effective Ferric reducing antioxidant potential (FRAP) similar to the standard drug, Ascorbic acid used for the study. All the concentration found equivalent and inhibition observed 100% in 500 µg/ml of acetone extract compared to standard Ascorbic acid (Table 2).

Table 2: FRAP Antioxidant Potential values of the Ascorbic acid and Acetone Extract on Dose dependent Manner of Gynandropsis pentaphylla Leaves

Concentration (µg/ml)	FRAP of Ascorbic acid	FRAP Acetone extract
	% FRAP inhibition	% FRAP inhibition
FRAP alone	0.00	0
31.25µg/ml	5.65	12.5
62.5µg/ml	25.97	33.75
125µg/ml	48.21	61.75
250µg/ml	68.60	74.25
500µg/ml	92.25	158.25
1000µg/ml	128.78	255.00

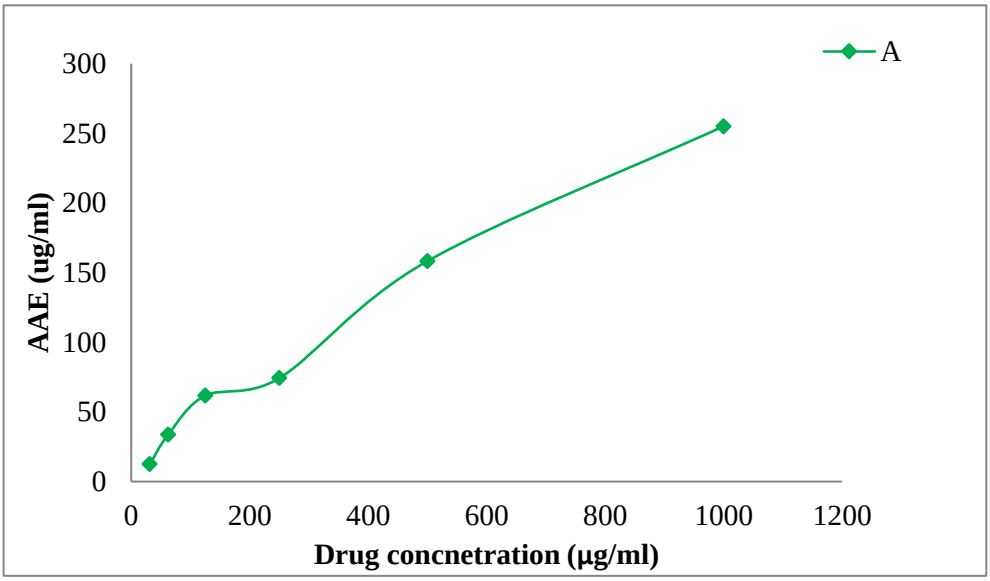

Figure 3. Scattered graph showing the Ferric reducing antioxidant potential (FRAP) values of the petroleum ether extract in terms of Ascorbic acid equivalents (AAE/ml).

4. Discussion

The antioxidant activity of the acetone extract of Gynandropsis pentaphylla leaves was evaluated using three different assays: DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)), and FRAP (ferric reducing antioxidant power).

In the DPPH assay, the acetone extract demonstrated significant free radical scavenging activity. This method measures the ability of antioxidants to donate hydrogen atoms or electrons to neutralize DPPH radicals, resulting in a color change from purple to yellow. The

degree of discoloration indicates the scavenging potential of the extract. The acetone extract of Gynandropsis pentaphylla showed a high percentage of DPPH radical inhibition, suggesting strong antioxidant properties (17).

The acetone extract of G. pentaphylla leaves demonstrated significant free radical scavenging activity using the DPPH assay, showing higher activity compared to the Ascorbic acid control. The increase in scavenging activity was concentration-dependent, likely due to the extract's ability to donate hydrogen during the oxidation reaction (18). However, no single concentration achieved total inhibition of the enzymes in the extract. The dose inhibition curve and IC_{50} value of 66.69µg/ml for the acetone extract indicated maximum free radical scavenging activity, attributed to the crude nature of the extract, suggesting its potential antioxidant properties. These results are consistent with similar findings, where the IC_{50} value of the crude plant extract was higher than that of the standard in biochemical studies.

The ABTS assay further confirmed the antioxidant capacity of the acetone extract. This assay involves the generation of the ABTS radical cation, which is blue-green in color. Antioxidants in the extract reduce the ABTS radical cation to a colorless form, and the extent of decolorization is measured spectrophotometrically. The acetone extract exhibited substantial ABTS radical scavenging activity, indicating its effectiveness in neutralizing free radicals (14).

The ABTS assay is a versatile method used to determine free radical scavenging activity, as well as hydrophilic and lipophilic biochemical reactions. It can be applied to various solvent extracts to compare their antioxidant activities with other assays. The ABTS assay relies on the interaction between antioxidant reactants and the ABTS radical cation, leading to a reduction in decolorization potential (19). In this study, the petroleum ether extract of S. glauca seeds was evaluated for its ABTS free radical scavenging activity. The standard Ascorbic acid exhibited an IC_{50} value of 10.00 µg/ml, while the petroleum ether extract showed a maximum IC_{50} value of 224.22 µg/ml. Similar results were observed with different standards, ranging from stronger to weaker antioxidant activities. The ABTS decolorization potential assay demonstrated the antioxidant capacity of various medicinal plant extracts, both in their crude form and as isolated fractions (20).

The FRAP assay provided additional insights into the antioxidant potential of the acetone extract. This method assesses the ability of antioxidants to reduce ferric (Fe^3+^) to ferrous (Fe^2+^) ions. The reduction is monitored by measuring the change in absorbance at 593 nm. The acetone extract of Gynandropsis pentaphylla demonstrated a significant increase in absorbance, reflecting its strong ferric reducing power and confirming its antioxidant efficacy (21). The biochemical components responsible for the reduction of ferric (Fe^3+^) to ferrous (Fe^2+^) ions form a blue-colored ferrous tripyridyltriazine complex (Fe^2+-TPTZ) at pH 3.6. This change is monitored spectrophotometrically at 593 nm (21). The reduction of the ferric cyanide complex (Fe^3+/(CN^−)^6) to the ferrous cyanide form (Fe^2+/(CN^−)^6) indicates the extract's electron-donating ability (22). The acetone extract of G. pentaphylla leaves exhibited a maximum FRAP value of 92.25 % µg/ml at a concentration of 500 mg/ml, equivalent to Ascorbic acid per gram of extract. This suggests that the extract has significant iron reduction potential, indicating that some phytochemicals act as electron donors, reacting with free ion radicals to terminate the radical chain reaction. Similar

observations were reported by Haleshappa et al. (23) on Simarouba glauca seed extracts, which showed potential as chelating metal agents with scavenging activity. Furthermore, the extract's flavonoid and phenol content suggest that these biochemical reactions may be due to the extract's redox potential, highlighting its potential as a novel antioxidant.

These findings collectively highlight the potent antioxidant activity of the acetone extract of Gynandropsis pentaphylla leaves, making it a promising candidate for further pharmacological studies and potential therapeutic applications.

5. Conclusion

In conclusion, the study of Gynandropsis pentaphylla leaves has revealed significant antioxidant properties, as demonstrated through various assays such as DPPH, ABTS, and FRAP. The acetone extract, in particular, showed strong free radical scavenging activity, indicating its potential as a natural antioxidant. These findings highlight the importance of medicinal plants in providing bioactive compounds that can contribute to health and wellness. Further research into the specific phytochemicals responsible for these activities could lead to the development of new therapeutic agents derived from Gynandropsis pentaphylla.

Conflict of Interest

No conflict of interest

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