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Original Research Article

Phytochemical Investigation and Antioxidant Activity Evaluation of *Pyrenacantha staudtii* (Icacinaceae) Leaf

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ABSTRACT

Pyrenacantha staudtii has been used in traditional medicine for various therapeutic applications, including the treatment of oxidative stress-related diseases. This study aimed to evaluate the phytochemical composition of *P. staudtii* leaf extract and the antioxidant activity of its fractions. The leaves were extracted with methanol and fractionated using different solvents. The crude methanol extract was subjected to qualitative phytochemical screening following standard procedures. The antioxidant activity of the fractions was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays, and ferric reducing antioxidant power (FRAP) assay. The extract was found to contain alkaloids, saponins, tannins, phenols, terpenoids, carbohydrates, and flavonoids. The ethyl acetate fraction exhibited the highest antioxidant activity, with a FRAP value of 189.58 mM Fe^{2+}/g, a DPPH IC_{50} of 1.77 µg/mL, and an ABTS IC_{50} of 575.04 µg/mL. This was followed by the dichloromethane (DCM) fraction (FRAP: 187.63 mM Fe^{2+}/g, DPPH IC_{50}: 15.60 µg/mL, ABTS IC_{50}: 922.13 µg/mL) and the aqueous fraction (FRAP: 140.85 mM Fe^{2+}/g, DPPH IC_{50}: 17.75 µg/mL, ABTS IC_{50}: 1108.88 µg/mL). The n-hexane fraction showed the weakest antioxidant activity, with a FRAP value of 114.37 mM Fe^{2+}/g, a DPPH IC_{50} of 348.04 µg/mL, and an ABTS IC_{50} of 5459.09 µg/mL. The findings from this study showed that *P. staudtii* leaf extract contain important phytoconstituents and possess significant antioxidant activity, suggesting their potential therapeutic benefits.

Keywords: *Pyrenacantha staudtii*, Traditional Medicine, Methanol Extract, Antioxidant Activity, Oxidative Stress.

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Introduction

Traditional medicine has been a cornerstone of healthcare for centuries, particularly in regions where access to modern medical facilities is limited.[1] It encompasses the accumulated knowledge, practices, and beliefs developed over generations to prevent, diagnose, and treat ailments.[1] Central to traditional medicine is the use of medicinal plants, which serve as natural repositories of therapeutic compounds. Many modern drugs trace their origins to these plants, as exemplified by morphine from *Papaver somniferum* and quinine from *Cinchona* bark.[2] The inherent value of medicinal plants lies in their affordability, cultural significance, and the diverse range of bioactive compounds they contain. Medicinal plants have been extensively studied for their pharmacological properties, and literature highlights their critical role in both traditional and contemporary therapeutic practices. These plants produce a wide array of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, terpenoids, and glycosides which contribute significantly to their medicinal properties.[3]

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Alkaloids, for instance, are well-known for their potent analgesic and antimalarial effects, while flavonoids are known for their strong antioxidant activity that protects against cellular damage. Similarly, saponins and tannins exhibit antimicrobial and anti-inflammatory properties, underscoring the multifaceted therapeutic potential of plant-derived compounds.[4-6] Within this context, *Pyrenacantha staudtii* (Icacinaceae) has emerged as a plant of considerable interest. Widely distributed across West and Central Africa, this plant has been traditionally used for its broad-spectrum therapeutic applications. Various parts of *P. staudtii*, such as the leaves, roots, and bark, are employed in folk medicine for the treatment of a wide range of conditions, including gastrointestinal disorders, inflammation, and muscle pain.[7] Ethnobotanical surveys have documented its use in local healing practices, and its popularity in traditional medicine is attributed to the presence of bioactive phytochemicals that confer significant therapeutic benefits.[7] The literature emphasizes the importance of phytochemical screening in uncovering the therapeutic potential of medicinal plants. This process involves the systematic identification of chemical constituents in plant extracts, thereby providing insights into their pharmacological actions. Phytochemical analysis does not only validate traditional uses but also guides further research into the isolation and characterization of novel compounds. The structural diversity of these natural compounds offers promising avenues for drug development, especially in the quest to combat diseases associated with oxidative stress and inflammation.[8-9] Oxidative stress, a condition characterized by an imbalance between free radicals and antioxidants in the body, is implicated in the development of many chronic diseases, such as cancer, cardiovascular disorders, and neurodegenerative conditions.[10] Natural antioxidants derived from plants play a crucial role in neutralizing free radicals, and protecting cellular integrity. In this regard, the antioxidant potential of medicinal plants has garnered

significant attention. Several studies have demonstrated that plant extracts rich in phenolic compounds and flavonoids can effectively scavenge free radicals, thereby mitigating oxidative damage.[10] This body of research supports the notion that natural antioxidants from medicinal plants could serve as safer, more effective alternatives to synthetic compounds. The process of extracting these bioactive compounds is critical to harnessing their full therapeutic potential. Extraction methods, particularly those utilizing polar solvents like methanol, are designed to efficiently isolate a wide range of phytochemicals from plant matrices.[11] Subsequent fractionation techniques further refine these extracts, allowing for the separation of compounds based on their polarity and chemical characteristics.[11] This systematic approach not only enhances the purity of the isolated compounds but also facilitates a more accurate evaluation of their bioactivities.[11-16] Overall, the integration of traditional knowledge with modern scientific inquiry has paved the way for a deeper understanding of medicinal plants and their role in healthcare. The literature underscores the need for comprehensive phytochemical screening and the development of robust extraction and fractionation methods to fully exploit the therapeutic potential of these natural products. By bridging the gap between age-old traditional practices and contemporary pharmacological research, the study of plants like *Pyrenacantha staudtii* offers promising prospects for the discovery of new, naturally derived therapeutic agents that can address modern health challenges. This synthesis of traditional wisdom and scientific exploration do not only validate the medicinal uses of *P. staudtii* but also highlights the broader significance of medicinal plants in global healthcare. As researchers continue to unravel the complex phytochemical profiles of these plants, their contributions to the development of novel drugs and therapeutic strategies remain an area of immense potential and ongoing interest. This study therefore aims to determine the phytoconstituents of *P. staudtii* leaves and evaluate the antioxidant activity of its solvent fractions using different *in vitro* assays.

Materials and Methods

Chemicals and Equipment

The chemicals used included methanol (99.8%, Loba Chemie®, India), n-hexane, ethyl acetate, distilled water, hydrochloric acid (Loba Chemie®, India), ferric chloride, chloroform, Dragendorff's reagent, alpha-naphthol, sulphuric acid (Molychem®, India), Benedict's reagent, gelatin (Kermel®, China), sodium chloride, lead acetate, dichloromethane, TPTZ (Molychem®, India), potassium persulfate, DPPH (Molychem®, India), ABTS (Molychem®, India), ascorbic acid (Sigma Aldrich®, Germany), glacial acetic acid (GHTECH®, China), sodium acetate (Merck®, Germany), and ferrous sulphate heptahydrate (Xi'an tian mao chemicals®, China). Equipment employed comprised a rotary evaporator (Stuart®, UK), condenser, T80 UV/Visible spectrophotometer (PG Instrument Ltd®, England), temperature-controlled water bath (Bibby Scientific Limited®, UK), weighing balance (Ohaus®, USA), and a vacuum pump (Stuart®, UK).

Plant collection and identification

Fresh leaves of *Pyrenacantha staudtii* Hutch. and Dalz (Icacinaceae) were collected from Igbekhue village along the Benin–Akure road in Benin City, Edo State, Nigeria (Latitude 6°09′04″N, Longitude 5°53′56″E) in October 2023. The plant material was identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. Herbarium specimen was prepared and deposited at the herbarium unit of the department with voucher No. UBH–P634.

Extraction and fractionation of the plant sample

Powdered *Pyrenacantha staudtii* leaves were extracted by maceration in 1 L of 99.8% methanol at room temperature for 7 days with occasional stirring. The extract was filtered, and the filtrate was concentrated *in vacuo* using a rotary evaporator at 45°C, air-dried, weighed, and stored in an airtight container until ready for use.

The crude methanol extract (20 g) was dissolved in methanol:water mixture (4:1) and partitioned in a separating funnel using sequential additions of n-hexane, dichloromethane, and ethyl acetate, with the remaining aqueous layer retained. Each fraction was then concentrated at 45°C, air dried, weighed, and stored. Percentage yields were calculated based on the weight of the original extract.

Phytochemical screening

An aqueous extract was prepared by boiling 5 g of powdered plant material in 75 mL of distilled water for 30 minutes. The extract was filtered, and the filtrate was used for qualitative phytochemical tests for the presence or absence of secondary metabolites, including alkaloids, saponins, tannins, phenols, terpenoids, and flavonoids.[17,18]

Determination of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging activity of the fractions of *P. staudtii* was evaluated using the method described by Jain *et al*. (2008).[19] DPPH solution (0.1 mM) was prepared in methanol. Stock solution of the plant fractions (1000 µg/mL) were serially diluted to achieve concentrations ranging from 1 to 200 µg/mL. One milliliter of the DPPH solution was added to 3 mL of each dilution, and the mixtures were incubated at room temperature in the dark for 30 minutes. The decrease in absorbance at 517 nm was recorded using a T80 UV/Visible spectrophotometer (PG Instruments Ltd®, UK). The percentage of radical scavenging activity was calculated using the following equation 1:

$$\text{Percentage DPPH scavenging activity (\%)} = \left(\frac{A_b - A_s}{A_b}\right) \times 100 \quad (1)$$

Where; Ab is the absorbance of blank (DPPH in methanol), and As is the absorbance of the DPPH with extract.

From these data, the IC_{50} value, indicating the concentration required to inhibit 50% of the DPPH radicals, was determined.

ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay

The ABTS radical cation was generated by mixing equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate and allowing the reaction to proceed in the dark for 12–16 hours. The solution was then diluted with methanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Stock solutions of the fractions (0.01 g in 10 mL methanol) were diluted to obtain final concentrations of 50–250 µg/mL. A 0.1 mL aliquot of each dilution was added to 1 mL of the $ABTS^{\bullet+}$ solution and incubated for 5 minutes at room temperature. The percentage ABTS radical scavenging activity was calculated for the different fractions using the formula (equation 2);

$$ABTS\ radical\ scavenging\ activity\ (\%) = \left[\frac{(A0 - A1)}{A0}\right] \times 100 \quad (2)$$

Where; A0 is the absorbance of the control at 5 min and A1 is the absorbance of the sample at 5 min. IC_{50} values were determined from plots of percentage inhibition versus concentration.[20]

Ferric reducing antioxidant power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted following a modified method of Benzie and Strain (1996).[21] The FRAP reagent was prepared by mixing 300 mM acetate buffer (adjusted to pH 3.6 with glacial acetic acid), 10 mM TPTZ (dissolved in 40 mM HCl), and 20 mM ferric chloride hexahydrate in a 10:1:1 ratio A standard calibration curve was constructed using $FeSO_4 \cdot 7H_2O$ at concentrations of 0, 5, 10, 20, 40, 60, 80, and 100 µM.

Each plant fraction (hexane, dichloromethane, ethyl acetate, and aqueous) at a concentration of 0.1 mg/mL was combined with the reagent, incubated in the dark for 30 minutes at room temperature, and then the absorbance was measured at 593 nm. Antioxidant activity was expressed as millimolar $FeSO_4$ equivalents per gram of extract, which was extrapolated from a calibration curve of ferrous sulphate.

Statistical analysis

Values were presented as mean ± standard deviation (Mean ± SD) of triplicate measurement. Microsoft Excel 2019 (Microsoft Corp., USA, 2019) was used for plotting all graphs.

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Results and Discussion

Phytoconstituents of Pyrenacantha staudtii leaf extract

The phytochemical analysis of *Pyrenacantha staudtii* leaf extracts revealed the presence of several secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids (Table 1). These compounds are known to contribute to various pharmacological activities, particularly antioxidant properties.[4,5] Flavonoids and phenolic compounds are widely recognized for their ability to scavenge free radicals, and reduce oxidative stress.[8,28]

The detection of alkaloids in *P. staudtii* is significant, as these compounds are known for their diverse biological activities, including anti-inflammatory and neuroprotective properties.[9] Similarly, the presence of saponins and tannins suggests potential antimicrobial and cytoprotective effects.[6,22] The observed high flavonoid content aligns with previous studies on medicinal plants with strong antioxidant activity.[8,29]

Table 1: Phytochemical constituents of *Pyrenacantha staudtii* leaf

Phytoconstituent	Observation	Result
Alkaloids	Orange precipitate	+
Saponins	Frothing	+
Tannins	White precipitate	+
Phenols	Dark green colour	+
Terpenoids	Reddish brown colour	+
Carbohydrates	Purple ring formed	+
Reducing sugars	Reddish-brown colour	+
Flavonoids	Yellow precipitate	+

Key: '+' indicate presence of phytoconstituent

Antioxidant activity of the fractions

The antioxidant activity of different solvent fractions of *P. staudtii* leaf was evaluated using three assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Ferric Reducing Antioxidant Power (FRAP), assays.

DPPH radical scavenging activity

The DPPH assay evaluates the ability of an extract to donate hydrogen atoms to stabilize free radicals, with lower IC_{50} values indicating stronger antioxidant activity.[27] The ethyl acetate fraction showed the highest radical scavenging ability, with an IC_{50} value of 1.77 µg/mL, followed by the dichloromethane fraction (15.60 µg/mL) and aqueous fraction (17.75 µg/mL), while the n-hexane fraction had the weakest activity (348.04 µg/mL) as shown in Figure 1.

The strong radical scavenging activity observed in the ethyl acetate fraction further supports its high phenolic content.[8,9] Phenolic compounds have been extensively studied for their ability to donate hydrogen atoms, effectively neutralizing reactive oxygen species.[5,28] The aqueous fraction, which also demonstrated considerable activity, likely contains polar antioxidants such as flavonoids and tannins.[6,22]

While the ethyl acetate fraction exhibited notable antioxidant activity, its IC_{50} value was slightly higher than that of ascorbic acid (1.73 µg/mL), suggesting that while effective, its radical scavenging potential is slightly lower than that of standard synthetic antioxidants.[27] This trend has been observed in previous studies, where plant extracts often exhibit lower activity than purified antioxidant compounds due to the presence of non-antioxidant components.[26,29]

ABTS radical scavenging activity

The ABTS assay is another widely used method to assess the antioxidant activity of plant extracts.[20] The ethyl acetate fraction again demonstrated the highest radical scavenging activity, with an IC_{50} value of 575.04 µg/mL, followed by the dichloromethane fraction (922.13 µg/mL), aqueous (methanol + water) fraction (1108.88 µg/mL), and n-hexane fraction (5459.09 µg/mL) (Figure 2).

These findings reinforce the results obtained from the DPPH assays, highlighting the strong antioxidant potential of the ethyl acetate and aqueous fractions. [8,9] The lower IC_{50} values of these fractions suggest that they contain potent antioxidant compounds capable of neutralizing free radicals.[28,29]

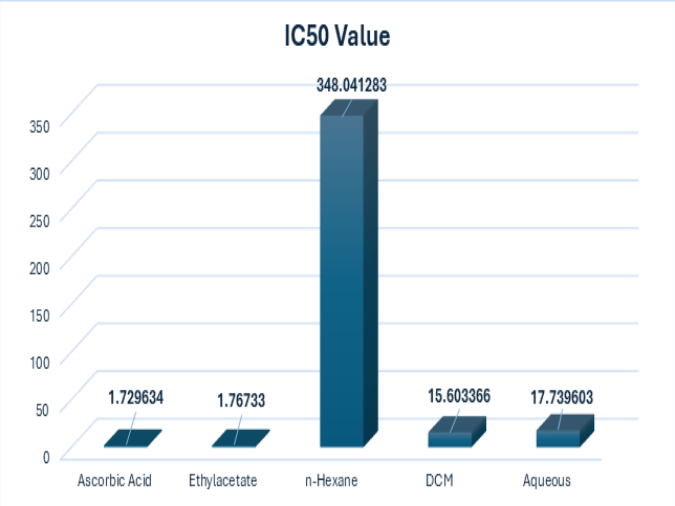

Figure 1: DPPH radical scavenging activity of different solvent fractions of *Pyrenacantha staudtii* leaf.

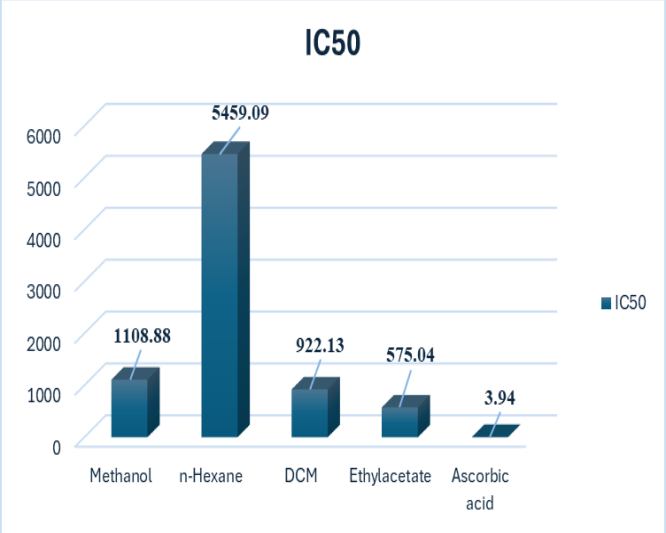

Figure 2: ABTS radical scavenging activity of different solvent fractions of *Pyrenacantha staudtii* leaf

Ferric reducing antioxidant power

The FRAP assay measures the ability of an extract to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), indicating its antioxidant potential.[21] The ethyl acetate fraction exhibited the highest reducing power, with a FRAP value of 189.58 mM Fe^{2+} equivalents per gram. This was followed by the dichloromethane fraction (187.63 mM Fe^{2+}/g) and aqueous fraction (140.85 mM Fe^{2+}/g), while the n-hexane fraction had the lowest reducing power (114.37 mM Fe^{2+}/g) (Figure 3). These results suggest that the highest concentration of redox-active compounds, particularly phenolics and flavonoids, is found in the ethyl acetate fraction.[8]

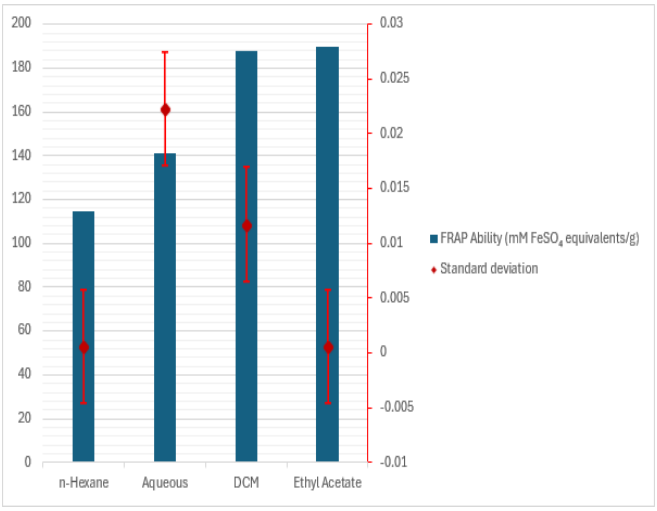

Figure 3: Ferric reducing antioxidant power of different solvent fractions *of Pyrenacantha staudtii* leaf

The trend observed in the FRAP results aligns with previous studies on medicinal plants, where polyphenol-rich fractions exhibit stronger reducing capacity.[28,29] The higher reducing potential of the ethyl acetate and aqueous fractions suggests that they contain compounds capable of donating electrons, thereby neutralizing free radicals and preventing oxidative damage.[8,26]

The results of this study suggest that *P. staudtii* possesses significant antioxidant activity, with the ethyl acetate fraction being the most potent. The presence of flavonoids and phenolic compounds likely contributes to its strong radical scavenging and reducing power.[5,8,9] Given the growing interest in natural antioxidants as safer alternatives to synthetic compounds, *P. staudtii* could serve as a potential source of natural antioxidant agents.[26,29]

The observed variation in antioxidant activity across solvent fractions highlights the importance of solvent selection in phytochemical extraction.[12,24] Ethyl acetate, a moderately polar solvent, was the most effective in extracting bioactive compounds, followed by water and dichloromethane. This suggests that optimizing extraction techniques could further enhance the yield of potent antioxidant compounds.[23]

Conclusion

The phytochemical screening of *Pyrenacantha staudtii* leaf extracts revealed the presence of essential bioactive compounds, including flavonoids, phenols, alkaloids, tannins, and saponins, which are known contributors to antioxidant activity. The antioxidant activity evaluation of the different fractions showed that the ethyl acetate fraction exhibited the highest potency across all assays (DPPH, ABTS, and FRAP), followed by DCM, aqueous, and n-hexane fractions in decreasing order. These findings indicate that the distribution of bioactive compounds in *P. staudtii* leaves varies across solvent fractions, with the ethyl acetate extract containing the highest concentration of antioxidants. On the basis of the present findings, *P. staudtii* could potentially serve as a source of natural antioxidant compounds.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

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