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Research Article

Phytochemical Profiling in Different Solvent Systems and DPPH Radical Scavenging Activity of *Cinchona pubescens* Leaf Extract obtained from Ughelli, Delta State

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ABSTRACT

The present research investigated the phytochemical composition and antioxidant potential of *Cinchona pubescens* leaf extracts obtained using methanol and n-hexane as extraction solvents. Qualitative phytochemical screening revealed the presence of flavonoids, phenolics, alkaloids, terpenoids, tannins, and saponins—compounds widely recognized for their antioxidant and therapeutic properties. The differences observed between the two solvent extracts indicated that solvent polarity significantly influenced extraction efficiency and the diversity of bioactive constituents. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which demonstrated strong antioxidant capacity in both extracts in a concentration-dependent manner. The methanol extract showed slightly higher scavenging activity (88.2–93.2%) compared to the n-hexane extract (82.6–86.8%), while ascorbic acid, used as the standard antioxidant, exhibited the highest inhibition (90.8–96.7%). These results suggest that both polar and non-polar phytoconstituents, including terpenoids and fatty acid derivatives, contribute to the plant's antioxidant activity. The findings validate the traditional medicinal use of *Cinchona pubescens* and highlight its significance as a potential natural source of antioxidant compounds. The coexistence of diverse metabolites in both polar and non-polar extracts underscores the plant's biochemical richness and its promise as a precursor for developing natural antioxidant agents suitable for pharmaceutical and nutraceutical applications. Overall, the study provides scientific evidence supporting the therapeutic relevance of *Cinchona pubescens* and emphasizes its potential contribution to the formulation of health-promoting products derived from plant-based antioxidants.

Keywords: Antioxidant; *Cinchona pubescens;* DPPH Assay; Phytochemical; Solvent Polarity

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INTRODUCTION

The increase in oxidative stress-related diseases around the world is a significant challenge to the current healthcare systems, already overstretched by environmental and socioeconomic factors. Oxidative damage to lipids, proteins, and DNA can be caused by free radicals and reactive oxygen species (ROS) formed during normal cellular metabolism and can cause degenerative conditions, including diabetes, cancer, cardiovascular, and neurodegenerative diseases (Bastos *et al*., 2022) Despite the human body having a complex antioxidant defense system, which includes superoxide dismutase, catalase, and glutathione peroxidase enzymes, this system may be overwhelmed during stress, and the search for exogenous antioxidants in the natural environment is required (Jongrungraungchok *et al*., 2023).

The recent years have seen a renewed interest in natural products as a potential alternative to the synthetic drugs, which are often quite expensive, not

biocompatible, and are accompanied by side effects. Secondary metabolites in medicinal plants include alkaloids, flavonoids, terpenoids, phenolic acids, and tannins, which are well-documented antioxidant, antimicrobial, and anti-inflammatory agents (Gadisa and Tadesse, 2021; Ugboko *et al*., 2020). These free radical scavengers, transition metallothiobacillus, and lipid peroxidation elimination prevent oxidative stress and inflammation (Hassan *et al*., 2024).

Cinchona pubescens is a plant that belongs to the Rubiaceae family and is endemic to the forests of the Andean mountain ranges in South America. It has been traditionally used as a medicinal plant. Extracts of the plant have been used to treat malaria, fever, and inflammatory conditions. The bark and leaves of the plant have been part of the drug preparations used for these ailments. The plant is the source of several biologically active alkaloids, such as quinine, quinidine, cinchonine, and cinchonidine that are noted for their antimalarial and antiarrhythmic activity (Ramić *et al*., 2021). In addition to the previously mentioned drug-like effects, there are also reports of antioxidant and hepatoprotective applications of *C. pubescens* in recent times. This is attributed to the high phenolic and flavonoid content of the plant (Singh et al., 2024). Cinchona species have a close relationship between the structural diversity of their bioactive compounds and the antioxidant potential of these plants. The phenolic compounds give away the hydrogen atoms to neutralize the production of free radicals, and alkaloids like quinine have the capacity to regulate the oxidative processes and prevent the formation of ROS. Moreover, the application of *Cinchona* extracts as nanomaterials, e.g., carbon dots (CDs), has recently been demonstrated to increase their antioxidant and antimicrobial properties, providing new opportunities in the food, biotechnological, and biomedical domains (Gonzalez-Reyna et al., 2022). Although there is a lot of research about the bark of Cinchona, not many studies have been conducted on the antioxidants of its leaves. Leaf extracts are renewable, non-pollutant, and environmentally friendly, and can offer another source of intensive antioxidants without affecting the ecological balance of the plant. Furthermore, the antioxidant mechanisms of *C. pubescens* may be used to develop natural therapeutic agents that have the potential to reduce oxidative stress and its related pathologies. In this context, the present work addresses the antioxidant potential of *Cinchona pubescens* leaves extracts that are extracted by different solvents. This study is aimed at providing a scientific basis for the traditional uses and promoting the use of this plant in the preparation of natural-derived antioxidants, by determination of its phytochemical content and evaluation its potential antioxidant activity with radical scavenging assays. The results of these studies will contribute to the growing field of natural product chemistry, identifying bioactive compounds for prevention against oxidative damage.

MATERIALS AND METHODS

Collection And Identification of Plant

Cinchona pubescens leaves were collected in Oteri-Ughelli, Ughelli- north local government area of Delta state. The plant was identified by a botanists named, Mr. MICHAEL, O.E. in the Department of Biological Science, Faculty of Sciences, Delta State University, Abraka, Nigeria with the voucher number DELSUH-379 (Figure 1).

Preparation and extraction of plant material

The plant leaves were air dried under shade at room temperature. The dried material was pulverized using motorized miller. The dried powder (650g) of the leaves was used for the total extraction of crude extracts (Soxhlet extraction). N-Hexane and methanol were employed as the extraction solvents, based on the increasing polarity. 60g of the powdered plant material in each occasion was placed in the thimble compartment of the Soxhlet extractor, and a condensed vapor of the extraction solvent is allowed to drain through the thimble at a maintained temperature of about 60 ^{o}C, for a period of 5 to 6 hours. The distillates, which contains the crude extract and the extraction solvent drained into the extraction flask. Based on differences in the boiling points between the extraction solvent and the crude extract, the crude extract remains in the extraction flask while the extraction solvent vaporizes again. The process is repeatedly carried out until a total extraction is obtained. The crude extracts were individually concentrated by partial evaporation and dried in a desiccator over anhydrous copper sulphate to obtain dry solid of extracts required for subsequent analysis. The percentage recovery was calculated using Equation 1 below:

$$\text{Percentge yield} = \frac{mass\ of\ extract}{mass\ of\ sample} \times 100 \quad (1)$$

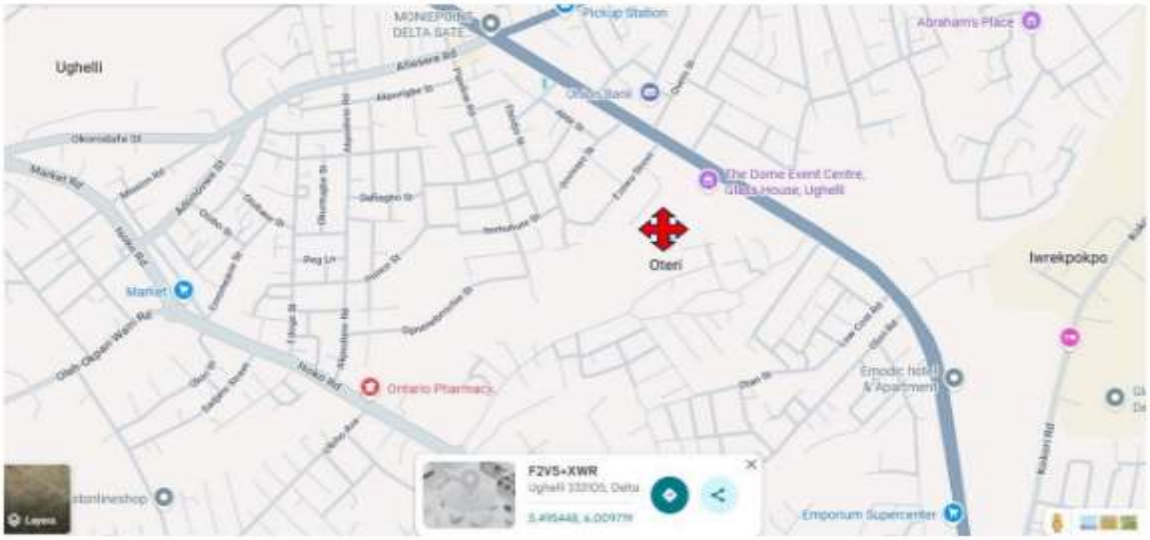

Fig 1:

Figure 1. Google map of the location showing where plant was obtained

Phytochemical Screening

Standard phytochemical methods were used to detect the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and phenolics. The procedure for phytochemical screening follows established methodologies as reported by Ali, *et al*. (2020).

Test for Alkaloids

5 mL of each of the extracts was treated with 2ml of Mayer's reagent (1.36 g of $HgCl_2$ + 5 g of KI in 100 ml of water). The formation of a cream-colored precipitate confirms the presence of alkaloids.

Test for Flavonoids

2 mL each of the extracts was treated with a few drops of sodium hydroxide (NaOH) solution. The formation of an intense yellow colour that becomes colourless upon the addition of a few drops of dilute HCl indicates the presence of flavonoids.

Test for Tannins

2 mL of each of the extracts was added 5 drops of dilute HCl and boiled for 5 minutes. Red precipitate is observed for condensed tannin.

Test for Saponins

Foam Test: 2 mL of each of the extracts was diluted with distilled water to 20 ml and shaken thoroughly in a graduated cylinder for 15 minutes. Persistent frothing indicates the presence of saponins.

Test for Steroids

Ten drops of concentrated H_2SO_4 were added to 2 mL of each of the extracts. The development of a red coloration indicates the presence of steroids.

Test for Glycosides

2 mL of each of the extracts was treated with Keller-Kiliani reagent (glacial acetic acid + few drops H_2SO_4 + few drops 5% $FeCl_3$), and a reddish-brown ring formation indicate the presence of glycosides.

Test for Terpenoids

0.5 mL of chloroform was added to 2 mL of each of the extracts, followed by 1 mL of concentrated H_2SO_4. A reddish-brown coloration at the interface indicates the presence of terpenoids.

Antioxidant analysis

DPPH Radical scavenging method

The procedure of Khatua and Acharya (2017) was followed to conduct the DPPH assay using 0.004 g of DPPH reagent dissolved in 100 ml of methanol. Scientists prepared different solutions of sample which extended from 10-150 µg/ml. An experiment was conducted in 96-well plates by adding 50 µl sample solutions at different concentrations and 150 µl DPPH solutions. The incubation occurred for 30 min at room temperature while under dark conditions and led to the measurement of 595 nm wavelength absorbance. Ascorbic acid served as the Standard using different concentrations between 10 to 150 µg/ml. The experiment used methanol as its Negative

Control solution. Three identical runs were performed for each Set of the Sample, Standard and Control. The degree of scavenging was calculated from Equation 2:

$$\% \, Scavenging \, Activity = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Results of Extraction

The Percentage recovery of the extracts ranged from 0.83 % to 2.07 % with n-hexane extract being the least while methanol extract gave the highest yield as shown in Table 1.

Table 1: Percentage yield of the plant extract

Solvent	Yield (g)	Color	Texture	Percentage recovery (%)
n-Hexane	5.43	Yellowish	Semi oily	0.83
Methanol	13.49	Dark brown	Solid	2.07

Results of preliminary phytochemicals screening

Qualitative phytochemical screening of the rhizome of *C. pubescens* was conducted using two organic solvent extracts (n-hexane and Methanol) and revealed the status (presence and absence) of different phytochemicals presented in Table 2. The test revealed the presence of steroids, terpenoids, cardiac glycosides, anthraquinones, saponins, tannins, flavonoids, and alkaloids whereas tannins, and were absent. It was obvious that *Cinchona pubescens* was rich in phytochemicals with higher polarity. Alkaloids are a significant group of phytochemicals in *C. pubescens*. According to a study by Verma *et al*. (2021), alkaloids such as crinine and lycorine are present in the plant. These compounds have been noted for their anti-inflammatory, analgesic, and antitumor activities (Verma *et al*., 2021). Lycorine, in particular, has been studied for its potential in cancer therapy due to its ability to inhibit protein synthesis in cancer cells (Chen *et al*., 2020). Alkaloid was absent in the n-hexane extract but present in the extract of methanol. Saponins were found in both extracts. Saponins are another major group of phytochemicals identified in *C. pubescens*. These compounds have been linked to various pharmacological activities, including antidiabetic and antioxidant effects (Ndiaye *et al*., 2022). Saponins' ability to modulate glucose absorption and enhance insulin sensitivity makes them relevant for diabetes management (Gao *et al*., 2023). Flavonoid was present only in the methanol extract. Flavonoids are known for their antioxidant properties and have been identified in *Cinchona pubescens*. The antioxidant activity of flavonoids can help protect cells from oxidative stress and reduce the risk of chronic diseases (Kumar *et al*., 2023). In *C. pubescens*, flavonoids such as quercetin and kaempferol have been reported, contributing to its overall antioxidant potential (Jiang *et al*., 2022). Tannins were also found only in the methanol extracts. Tannins are polyphenolic compounds with significant astringent and antimicrobial properties. In *Cinchona pubescens*, tannins contribute to the plant's ability to treat gastrointestinal issues and exhibit antimicrobial activity (Olaniyi *et al*., 2024). Their astringent properties help in the treatment of diarrhea and other digestive disorders. Terpenoids and steroids were present in all the extracts. Terpenoids and steroids in *C. pubescens* have shown promise in various biological activities, including antimicrobial and anti-inflammatory effects. These compounds, such as those found in the essential oils of the plant, have been shown to exhibit activity against a range of pathogens (Singh *et al*., 2023). Cardiac glycosides were present in the extracts. Cardiac glycosides, which helps preserve the heart's strength and rate of contraction while the heart is malfunctioning (Rohini *et al*., 2019; Lasisi and Adesomoju, 2015) were found in ethyl acetate and methanol extracts. As constituents of nutrients, they assumed to be protectants against cardiovascular diseases and cancers (Fayaz et al., 2018; Castano et al., 2019). Anthraquinones were also found to be present in the extracts. These phytochemicals found gave a preliminary idea about the relationship between the biological activity and phytochemicals present in the plant’s extracts (Table 2).

Table 2: Phytochemical constituents of *Cinchona pubescens* extract

Test Compounds Extract	N-Hexane	Methanol E
Saponins	+	+
Cardiac glycosides	-	-
Alkaloids	-	+
Anthraquinones	+	+
Steroids	+	+
Terpenoids	+	+
Flavonoids	-	+
Tannins	-	+

Key: (+) = Present and (-) = Absent

Antioxidant Assay

The antioxidant properties of the *C. pubescens* leaf extracts were measured using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay test and the obtained results are shown in Table 3 and Figures 1-3. The assay involved the measurement of the percentage inhibition of the DPPH radicals in different concentrations (0.125-1.00 mol/dm3) of the n-hexane and methanol extracts in comparison with ascorbic acid which was the standard antioxidant. Table 3 indicates that the two extracts had a concentration-dependent antioxidant response whereby the higher the concentration of the extracts, the higher the inhibition. The greatest percentage of inhibition was observed in the methanol extract, that is, 88.2-93.2 %, but the n-hexane extract was slightly less active (82.6 % - 86.8 %). Conversely, ascorbic acid had the highest radical-scavenging activity as its values of inhibition ranged between 90.8 and 96.7%. These results showed that the two extracts had a high antioxidant potential, though their effectiveness differed in terms of polarity of the solvent. This tendency was proved by the graphical presentations in Figures 1-3, as the trend was to a stable increase in radical scavenging activity as the concentration of all samples tested increased. The n-hexane extract activity indicated the possibility of the presence of non-polar phytoconstituents like terpenoids, sesquiterpenes, and fatty acid derivatives in the n-hexane extract, which contributed to its radical-scavenging activity (Jolly *et al*., 2024). The methanol extract had phenolics and flavonoids, which are well known to have hydrogen or electron-giving potential and thus stabilize DPPH radical (Yakubu et al., 2024). The antioxidant activity of the two extracts was high in comparison to the standard ascorbic acid and it indicates that *C. pubescens* extracts have bioactive compounds that can counter the effects of free radicals via redox mechanism. Phenolics and terpenoids have been attributed to significant antioxidant activities by previous studies (Câmara *et al*., 2024). The described activities also indicate that the plant might be a promising source of natural anti-oxidative substances to be used in pharmaceutical and nutraceutical studies to address the issue of oxidative stress (Kukharenko *et al*., 2019).

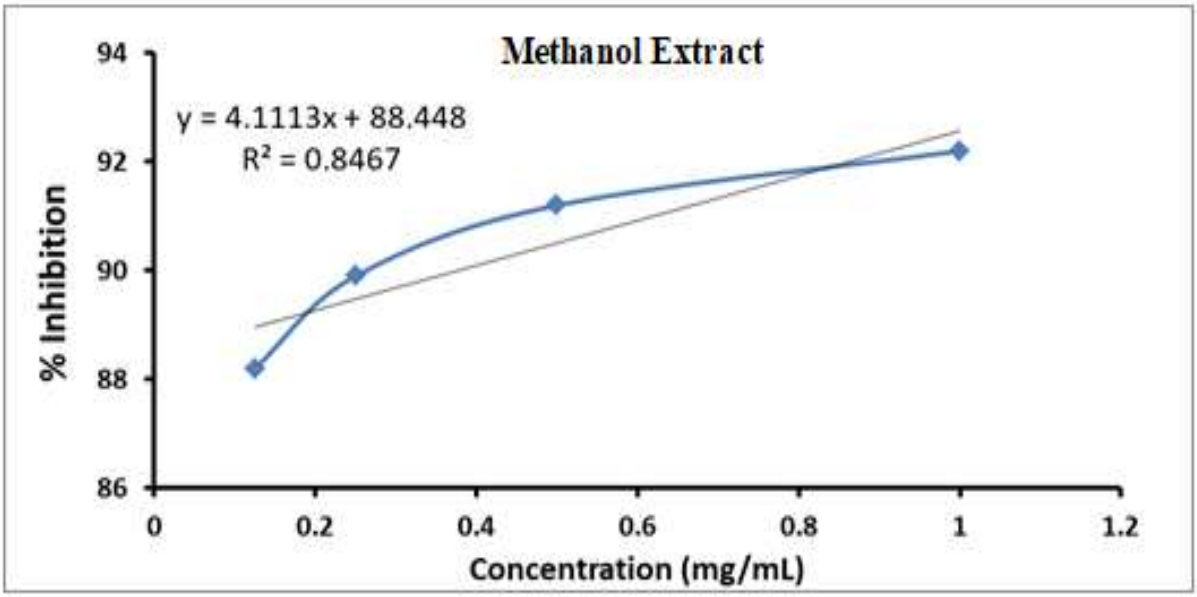

FIGURE 2: DPPH ACTIVITY GRAPH FOR METHANOL EXTRACT

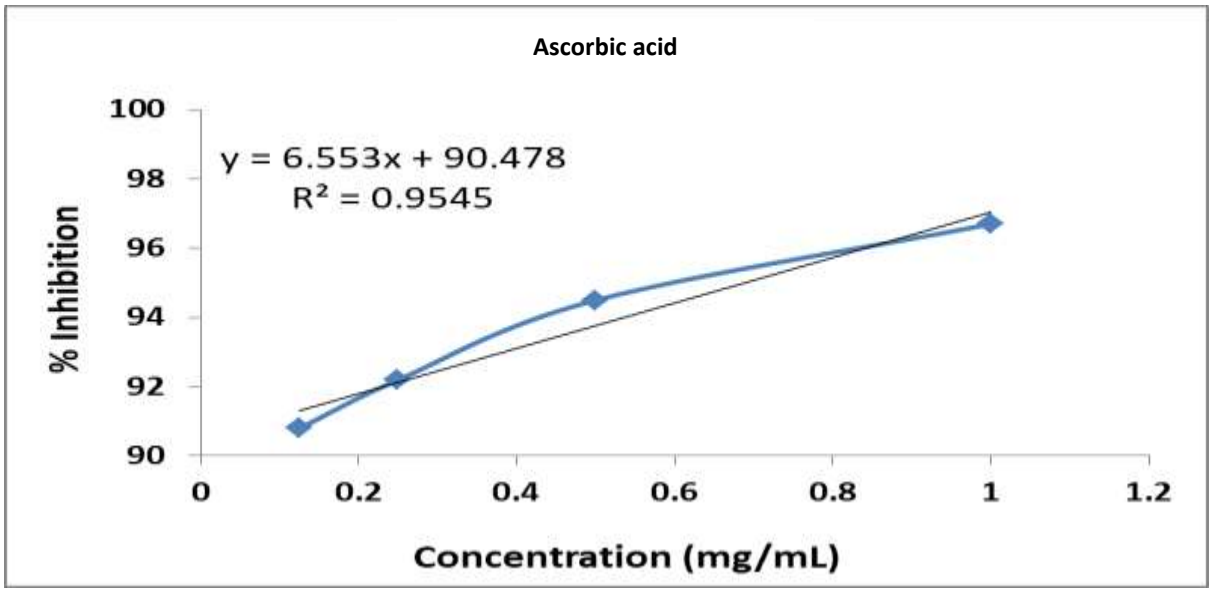

FIGURE 3: DPPH ACTIVITY GRAPH FOR CONTROL

Table 3: % Antioxidant Inhibition

Conc.(mol/dm^3)	Methanol	Hexane	Ascobic acid
1.00	93.2	86.8	96.7
0.50	91.4	85.6	94.5
0.25	89.9	84.2	92.2
0.125	88.2	82.6	90.8

CONCLUSION

The present study investigated the antioxidant activity and composition of phytochemicals of *C. pubescens* leaf extracts using methanol and n-hexane as extracting solvents. The phytochemical screening was done qualitatively, where various secondary metabolites were found, including flavonoids, phenolics, alkaloids, terpenoids, tannins, and saponins. They are recommended to be good antioxidants, anti-inflammatories, and therapeutic compounds. The disparity in the phytochemical content of the two extracts also implied that polarity of the solvent would have a significant influence on the efficacy of the extraction procedure and quality of bioactive compounds to be extracted. The DPPH radical scavenging test also showed that the two extracts had a high antioxidant activity dependent on the concentration. Methanol extract exhibited a little higher inhibition value (88.2-93.2 %) than the n-Hexane extract (82.6-86.8 %). In spite of higher level of inhibition (90.8-96.7 %) observed with the standard antioxidant, ascorbic acid, the findings of both extracts showed similar antioxidant potential, which confirmed the use of *C. pubescens* as a potent source of natural antioxidant. The evidence supported the classic medicinal applicability of *C. pubescens* and gave a scientific basis of using it as a solution to the problem of oxidative stress-induced diseases. The simultaneous existence of polar and non-polar antioxidant compounds in the extracts indicated the versatility of the plant and its use in natural product-based drug discovery and nutraceuticals. In order to expand the existing results, the next generation of studies must be concerned with the isolation and characterization of the active antioxidant compounds with the help of chromatographic and spectroscopic techniques that could be applied, including HPLC, NMR, and MS. Also, the computational Evaluation that entails Density Functional Theory (DFT) and molecular docking ought to be utilized to elucidate the electronic behavior, reactive sites and molecular interactions of isolated compounds with oxidative stress-relevant targets.

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