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PHYTOCHEMICAL SCREENING OF AQUEOUS AND METHANOL EXTRACTS OF LEAF AND ROOT BARK OF *Gardenia ternifolia* (SCHUMACK AND THONNS)

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ABSTRACT

Gardenia ternifolia is a shrub of the Rubiaceae family that is harvested in the wild for protection against evil spirit as it is believed that the plant has protective charm property. The study aims to determine the phytochemicals constituents and mineral compositions of aqueous and methanol extracts of the leaf and root bark of *G. ternifolia*. The leaves and root of *Gardenia ternifolia* were obtained from the wild in Akpegede in Otukpo Local Government Area of Benue State. The sample was rinsed and air dried in a shade, then pulverized. Extracts from the plant parts were gotten using water and methanol solvent. Thereafter, qualitative phytochemicals and minerals constituents was screened. The phytochemicals present in both aqueous and methanol extracts include carbohydrates, phenols, flavonoids, alkaloids, tannins, steroids, saponins, triterpenes and cardiac glycosides in different proportions. While, the minerals found include iron, copper, zinc, nickel, lead and cobalt.

Keywords: *Gardenia ternifolia*, Phytochemicals, Minerals, Aqueous and Methanol Solvent

INTRODUCTION

Medicinal plants remain a vital source of bioactive compounds and continue to play a significant role in primary healthcare systems, particularly in developing countries. These plants synthesize a wide range of secondary metabolites including alkaloids, flavonoids, tannins, phenolics, and terpenoids which exhibit antimicrobial, antioxidant, anti-inflammatory, and other pharmacological activities (Cowan, 1999; Ekor, 2014). The growing interest in plant-based therapeutics is driven by the need for safer, cost-effective alternatives to synthetic drugs, as well as increasing concerns about antimicrobial resistance (World Health Organization [WHO], 2019).

Gardenia ternifolia (Schumach. and Thonn.), a member of the Rubiaceae family, is widely distributed across tropical Africa and occurs abundantly in savannah regions of Nigeria, including Benue State. Ethnobotanical studies report its use in the treatment of infections, inflammation, and other ailments, as well as in cultural practices such as protection against perceived spiritual harm (Burkill, 2000; Sofowora *et al.*, 2013). Despite its widespread traditional use, there is limited scientific validation of its phytochemical composition, particularly with respect to different plant parts such as the leaf and root bark.

Previous studies have shown that extraction solvents significantly influence the yield and composition of phytochemicals. Polar solvents such as water and methanol are widely used due to their ability to extract a broad spectrum of bioactive compounds, especially phenolics and flavonoids (Do *et al.*, 2014; Azwanida, 2015). However, comparative evaluation of aqueous and methanol extracts of *G. ternifolia* remains limited, particularly in relation to both phytochemical composition and antioxidant activity. In addition, medicinal plants may accumulate heavy metals from their growing environment, which can pose serious health risks when consumed (Alloway, 2013). Therefore, assessing the mineral composition of medicinal plants is essential to ensure their safety and therapeutic applicability. Thus, this study provides scientific validation for the traditional use of *G. ternifolia* and contributes to the growing body of knowledge on its phytochemical constituents.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh leaves and root bark of Gardenia ternifolia were collected from the wild in Akpegede, Otukpo Local Government Area, Benue State, Nigeria. The plant materials were authenticated by a resident taxonomist in the Department of Biological Sciences, Nigerian Defence Academy, Kaduna, Nigeria. The samples were washed thoroughly under running tap water and rinsed with distilled water to remove adhering contaminants. The plant materials were air-dried at room temperature for six weeks and pulverized into coarse powder using a mechanical grinder. The powdered samples were stored in airtight containers until extraction. One hundred grams (100 g) of each powdered sample were separately cold-macerated in 1000 mL of methanol and distilled water for 72 h with intermittent shaking. The mixtures were filtered using Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator and further evaporated to dryness on a water bath maintained at 50°C. The dried extracts were properly labeled and stored in airtight containers at 4°C for subsequent analyses.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the extracts was carried out using standard procedures described by recent phytochemical analytical protocols (Maheshwaran et al., 2024; Shaikh & Patil, 2020).

Test for Alkaloids

Two milliliters of extract were treated with a few drops of Mayer's reagent. Formation of a creamy precipitate indicated the presence of alkaloids.

Test for Flavonoids

Two milliliters of extract were mixed with sodium hydroxide solution. The appearance of an intense yellow coloration that disappeared upon addition of dilute hydrochloric acid indicated flavonoids.

Test for Saponins
Five milliliters of extract were vigorously shaken with distilled water. Persistent frothing indicated the presence of saponins.

Test for Tannins
A few drops of 5% ferric chloride solution were added to the extract. Formation of a blue-black or green coloration indicated tannins.

Test for Anthraquinones
The extract was treated with benzene and ammonia solution. A pink or red coloration in the ammoniacal layer indicated anthraquinones.

Test for Cardiac Glycosides
The extract was treated with glacial acetic acid containing ferric chloride followed by concentrated sulfuric acid. Formation of a brown ring at the interface indicated cardiac glycosides.

Test for Terpenoids
The extract was mixed with acetic anhydride and concentrated sulfuric acid. Development of a deep green coloration indicated terpenoids.

Test for Steroids
The extract was mixed with chloroform and concentrated sulfuric acid. Formation of a reddish-brown coloration at the interface indicated steroids.

Test for Phenolic Compounds
Lead acetate solution was added to the extract. Formation of a white precipitate indicated phenolic compounds.

Test for Carbohydrates
Molisch's reagent and concentrated sulfuric acid were added to the extract. Formation of a violet ring indicated carbohydrates.

Quantitative Phytochemical Analysis
Determination of Total Phenolic Content
Total phenolic content was determined using the Folin–Ciocalteu spectrophotometric method. Briefly, 0.5 mL of extract was mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL sodium carbonate solution. The mixture was incubated for 30 min at room temperature, and absorbance was measured at 765 nm using a UV–Visible spectrophotometer. Gallic acid was used as standard, and results were expressed as mg gallic acid equivalent (GAE)/g extract.

Determination of Total Flavonoid Content
Total flavonoid content was determined using the aluminum chloride colorimetric method. One milliliter of extract was mixed with aluminum chloride reagent and incubated for 30 min. Absorbance was measured at 415 nm using a UV–Visible spectrophotometer. Quercetin was used as standard, and results were expressed as mg quercetin equivalent (QE)/g extract.

Determination of Total Alkaloid Content
Total alkaloid content was determined gravimetrically. Five grams of the sample were extracted with 10% acetic acid in ethanol and concentrated. Concentrated ammonium hydroxide was added dropwise to precipitate alkaloids. The precipitate was filtered, dried, and weighed.

Antioxidant Activity
DPPH Radical Scavenging Assay
Antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to validated analytical procedures (Munteanu & Apetrei, 2021). A 0.1 mM DPPH solution was prepared in methanol. One milliliter of extract at varying concentrations was mixed with 3 mL of DPPH solution and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm against a blank using a UV–Visible spectrophotometer. Ascorbic acid served as the standard antioxidant.

Mineral Determination
Sample Digestion
Mineral analysis was carried out according to the methods of the Association of Official Analytical Chemists (2003). One gram (1g) of powdered sample was digested using a mixture of concentrated nitric acid (HNO3_33), perchloric acid (HClO4 44), and sulfuric acid (H2 22SO4 44) in the ratio 5:1:1 on a hot plate until a clear solution was obtained. The digest was cooled, filtered, and diluted to 50 mL with distilled water.

Atomic Absorption Spectrophotometric Analysis
Mineral elements including Fe, Zn, Cu, Mn, Ca, Mg, Pb, and Cd were analyzed using Atomic Absorption Spectrophotometry (AAS) equipped with an automatic background corrector. Calibration curves were prepared using certified standard solutions for each element analyzed. Blank determinations were carried out for quality assurance purposes. Detection limits for the analyzed minerals ranged from 0.001 to 0.01 mg/L depending on the element analyzed. Results were expressed in mg/kg dry weight.

Statistical Analysis
The results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics version 25.0. Data were subjected to one-way analysis of variance (ANOVA), and significant differences between means were determined using Duncan's Multiple Range Test at $p<0.05p < 0.05p<0.05$.

RESULTS AND DISCUSSIONS
Percentage Yield of Extracts
The percentage yield from the root bark and leaves extracts using methanol and aqueous solvent as presented in Table 1. Aqueous leaf extract of *Gardenia ternifolia* had the highest yield with a value of 16.5 %. This is followed by methanol extract of the leaves which yielded 13.5%. The aqueous and methanol extracts of the root yielded 11.5% and 8.9% respectively. It was observed that the leaves yielded more than the roots. The leaves gave a mean yield of 15% while the root yielded 10.2%. Assessing the performance of the solvents used, aqueous solvent had a higher yield than methanol. Aqueous solvent returned a mean yield of 14% while methanol yielded 11.2%.

Table 1: Percentage Yield of Sample

Sample	Solvent	Weight of Sample	Weight of Extract	Percentage yield (%w/w)
Root	Methanol	250g	22.32g	8.9
Root	Aqueous	250g	28.79	11.5
Leaf	Methanol	250g	33.82	13.5
Leaf	Aqueous	250g	41.17	16.5

Differences were observed in the yield from the leaf and root bark. The aqueous leaf extract had the highest yield of 16.5% followed by the leaf methanol with 13.5% while root bark, aqueous and methanol yield 11.5and 8.9%respectively. A comparative analysis of the extraction yield showed that the extraction solvent influenced the yield. This is consistent with the finding of Klotoe *et al.,* (2020) who reported that aqueous solvent yield more from some medicinal plants. In this research the aqueous extract yield more than the methanol extract. It could be that the aqueous solvent has an inherent capacity to extract more yield. (Klotoe *et al.,* (2020); Mpiana *et al.* (2015) attributed the difference in yield to weight of powered sample, duration of the extraction process and solvent type used.

Qualitative Analysis

Ten phytochemicals were screened and nine were found to be present in the Leaf and Root bark of the plant. These are Alkaloids, Cardiac Glycosides, Saponins, Phenols, Tannins Triterpenes, Steroids, Carbohydrates, and Flavonoids. Anthraquinones was absent in the leaves and root bark extracts (Table 2).

Table 2: Qualitative Analysis

Phytoconstituents	Leaf Meth	Leaf Aque	Root Meth	Root Aque
Alkaloids	+	+	+	+
Cardiac Glycosides	+	+	+	+
Saponins	+	+	+	+
Phenolic compounds	+	+	+	+
Tannins	+	+	+	+
Steroids	+	+	+	+
Carbohydrates	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Anthroquinones	-	-	-	-

Key: + = Present and - = Absent

Phytochemical screening of plant material is a useful initial assay that facilitate the identification and quantification of bioactive components in the plant which may lead to drug discovery and development (Agrawal Pandey, 2019). The phytochemical screening of the root bark and leaf of *G. ternifolia* revealed the present of alkaloids, flavonoids, tannins, saponins, carbohydrate, steroids, triterpenes and cardiac glucoside however, anthraquinones were absent. The present of these phytochemicals is consistent with the findings of Dahiru (2015) who reported that the present of these phytochemicals in *G. ternifolia* collected at Jabillambi village in Adamawa state. Carbohydrate and phenol had the highest value of the phytochemicals analyzed. These results are inconsistent with the finding of Dahiru(2015) who reported that the leaves of *G. ternifolia* had the highest amount of saponins. The observed differences in the concentration of these phytochemical could be attributed to environmental factors. The finding of this research showed that the quantity of phenols extracted was higher when compared to methanol. This finding disagrees with the report of Klotoe (2020) who reported higher values of phenol from ethanolic extract. The quantification of flavonoids extracted with aqueous solution from the leaf was similarly, lower than the value gotten from ethanolic solvent. This disagrees with the findings of this research. We attribute this difference to the growing environment of the plant which could influence the internal physiological processes in the plant. Physiological process in plant is central to the type, nature and quantity of any phytochemical in the plant.

Quantitative Analysis

The result of the quantitative phytochemical analysis of aqueous and methanol extract of leaves and root bark of *gardenia ternifolia* as shown in table 3 revealed that in the methanol extract of the leaves, Carbohydrate yielded the highest percentage value in g/100g of 23.64, followed by phenol (13.19), and flavonoid with value of 11.41 percent. However, anthroquinones had the least percentage value of 0.05, steroid 2.07 and triterpenes 2.76percent whereas in the aqueous leaves extract (carbohydrate) reducing sugar yielded 24.05followed by phenols (14.36) and flavonoids (12.06) percent. Anthraquinones, steroids and triterpenes with least value of 0.06, 1.66 2.97percent respectively. Similarly, in the root bark, methanol extract Carbohydrate returned the highest value of 24.11, followed by phenols (11.42) and flavonoids (9.18) percent; The least value returned is anthraquinones (0.04), steroids(1.18) and triterpenes (3.54) while in the aqueous extract reducing sugar (carbohydrate) yielded 27.19percent followed by phenols(10.17), flavonoids (8.41)percent. Least value was recorded in steroids (1.31), triterpenes (2.54) and anthraquinones (0.05) percent. T-test analysis revealed a significant difference in the concentration of reducing sugar (carbohydrates), phenols and flavonoids, tannins, saponins, alkaloids in the leaves and root barks while no significant statistical differences was observed with triterpenes, cardiac glycoside and anthraquinones at $P<0.05$ (95%) confidence level. Analysis of variance revealed significant differences in the concentration of the most of the phytochemical's analysis in the four extract studied.

Table 3: Quantitative Phytochemical Analysis of Aqueous and Methanol Extract of *Gardenia ternifolia* % Concentration (g/100g)

	LEAF		ROOT BARK	
Phyto-constituents	**Aqueous**	**Methanol**	**Aqueous**	**Methanol**
Carbohydrates	24.05	23.64	27.20	24.11
Phenols	14.36	13.91	10.17	11.42
Flavonoids	12.07	11.42	8.62	9.18
C. Gycosides	6.35	6.22	6.68	6.05
Tannins	5.53	5.21	6.15	5.99
Saponins	4.07	3.11	6.30	4.24
Alkaloids	3.66	4.18	5.62	4.44
Triterpenes	2.93	2.77	3.54	2.54
Steroids	1.66	2.07	1.18	1.31
Arthraquinones	0.03	0.05	0.04	0.05

Table 4: Whole Plant Concentration of Phytochemicals

Phytochemical	**Concentration %**	**Standard deviation**
Alkaloid	4.48 ± 0.22	0.76
Saponin	4.43 ± 0.35	1.22
Flavonoid	10.27 ± 0.46	1.58
Phenol	12.46 ± 0.52	1.81
Tannin	5.72 ± 0.13	0.46
Carbohydrate	24.75 ± 0.45	1.56
Steroid	1.56 ± 0.11	0.39
Triterpene	2.91 ± 0.13	0.46
C/glycoside	6.32 ± 0.10	0.33
Antraquinone	0.05 ± 0.003	0.012

Mineral Elements in Leaf and Root Extracts of *Gardenia ternifolia*

The Mineral element content is presented in table 5 below. The element with the highest concentration in the leaves of *Gardenia ternifolia* was Fe with the value of 246.6 ± 0.03 mg/kg followed by Pb with value of 15.2 ± 1.9 mg/kg. Others are Zn 13.98, Cu 7.2 ± 0.01, Ni 3.3 ± 0.3, Co 1.4 ± 0.03 and Cd -7.4 ± 0.01mg/kg respectively. In the Root, the highest Mineral element content is Fe with the value of 162.4 ± 0.03 mg/kg, followed by Ni with 40.9 ± 0.6 mg/kg, Zn 36.14 ± 0.02, Cu 5.6 ± 0.01, Pb 0.2 ± 0.08, Cd -7.2 ± 0.01 and Co -8.6 ± 0.02mg/kg respectively.

Table 5: Mineral Elements of Leaf and Root of *Gardenia ternifolia* Extracts (mg/kg)

Mineral Elements	**Leaf**	**Root**
Iron (Fe)	246±0.03	162.3±0.03
Lead (Pb)	15.2±1.9	0.2±0.08
Zinc (Zn)	13.98±0.01	36.14±0.02
Cupper (Cu)	7.2±0.01	5.6±0.01
Nickel (Ni)	3.3±0.04	40.9±0.06
Cobalt (Co)	1.4±0.03	-8.6±0.02
Cadmium (Cd)	-7.4±0.01	-7.2±0.01

Comparing the leaf and root bark of the plant, it was observed that the leaves had a higher scavenging activity than the root. The radical scavenging activity of the leaves was 81.12% while that of the root bark was 70.05%. The difference in the activity of the leaves when compared to that of root bark was found to be significant at $p<0.05$, 95% confidence level. The activity of extracts based on the solvents used is presented in figure 4.8. Results revealed that methanol extracts had a higher activity than aqueous extracts. The results showed that aqueous extracts had a mean scavenging activity of 72.22% while Methanol extracts was 78.96%. The difference between the two was significant at $p<0.05$, 95% confidence level.

In the roots, aqueous extracts had a higher radical scavenging activity than methanol extracts, but this difference was not significant. In the leaves, however, methanol extracts had higher activity (88.01%) compared to aqueous extract which has a value of 74.24%. The difference in the results were significant. When compared to Ascorbic acid which was used as standard (94.13%), it was observed that the percentage radical scavenging activity values were lower and significantly different.

CONCLUSION

The preliminary phytochemical screening of *Gardenia ternifolia* revealed the presence of Alkaloids, Flavonoids, Saponins, Phenols, Tannins, Carbohydrates, Steroids, Triterpenes and Cardiac glycosides at varying concentrations in the aqueous and methanol extracts of the leaf and root bark with Carbohydrates as the most abundant, followed by Phenols and Flavonoids. Generally, the phyto-constituients were quantitatively higher in the leaf extracts than the root bark extracts. The leaf and root bark of *Gardenia ternifolia* have these mineral elements: Iron, Lead, Copper, Zinc, Cobalt, and Nickel. The presence of these elements with

exception of Lead, makes it a highly nutritional for metabolic processes. However, the Lead content makes it toxic to the body. It is recommended that further study should be carry out to isolate the actives phytochemicals, and pharmacological investigations to evaluate its safety, therapeutic potentials, and possible health risks associated with the detected lead content before its extensive medicinal or nutritional utilization.

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