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Phytochemical composition and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of *Anthocleista vogelii* (Gentianaceae) root extract

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ABSTRACT

Background: *Anthocleista vogelii* Planch. (Gentianaceae) is an evergreen tree with a stilt root system, native to West Africa. It is a rich source of polyphenols and has been traditionally used in medicine to treat ailments associated with oxidative stress caused by free radicals generated during metabolic processes. This study aimed to screen for phytochemicals in the crude extract of *A. vogelii* root, quantify its polyphenolic contents, and evaluate its radical scavenging potential.

Methods: Standard protocols involving colorimetric and precipitation reactions were used to screen for phytochemicals in the crude root extract of *A. vogelii*. The Folin–Ciocalteu reagent was used to quantify phenolic compounds, while aluminum chloride was employed to determine flavonoid content. The antioxidant potential was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Results: Preliminary screening revealed the presence of alkaloids, tannins, phenolics, flavonoids, glycosides, terpenoids, and saponins. The crude extract yielded a total phenolic content of 24.87 ± 2.12 mg GAE/g and a total flavonoid content of 16.18 ± 0.54 mg QE/g. The radical scavenging potential of the extract compared favorably with ascorbic acid, with an IC_{50} value of 14.08 µg/mL.

Conclusion: The methanol root extract of *A. vogelii* contains significant phytochemicals, particularly flavonoids and phenolics, which may be responsible for its radical scavenging activity.

Keywords: 2,2-Diphenyl-1-picrylhydrazyl radical scavenger, *Anthocleista vogelii,* Total flavonoid, Total phenolic content

1.0 INTRODUCTION

Anthocleista vogelii Planch. belongs to the family Gentianaceae, formerly classified under Loganiaceae. It is an evergreen rainforest tree that grows up to 20 m in height, with a stilt root system [1]. The species is native to West Africa and widely distributed across tropical Africa, the Comoros, and Madagascar [2]. In Nigeria, it is found predominantly in the southern region [3], where it is called *Mpoto* in Igbo and *Sapo* in Yoruba [4], and *Okhurho* in Edo. Its common name is "Murderer's Mat." Traditionally, it is used in West African medicine for treating oxidative stress-related illnesses, as a resilient bowel cleanser, for managing metabolic disorders, and as a diuretic [5]. A decoction of the root is used as an abortifacient, a menstrual regulator, and a treatment for constipation. In Sierra Leone, hepatitis is treated by combining the root decoction with lemon juice [6, 7]. harmacologically, different parts of the plant have been shown to possess activities that validate its traditional uses. The root bark is used as a diuretic and laxative [2], while the stem bark has demonstrated anti-trypanosomal and anti-inflammatory properties [8, 9]. Phytochemicals are groups of bioactive compounds produced by plants to protect against environmental stress or predators in their surroundings [10]. Many of these compounds have proven useful as medicinal agents for both humans and animals. Their presence in a cell can

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significantly alter physiological states by influencing cell membrane permeability, signaling pathways, and metabolic routes [11]. Phytochemicals are broadly categorized based on their biological activities, such as anti-inflammatory, anticancer, and antioxidant effects. This suggests that they are largely responsible for the pharmacological properties of plant extracts or decoctions. Screening and quantifying phytochemicals allows for proper classification into relevant groups and helps determine their concentration, which is essential in assessing the medicinal potential of the plant. Oxidative stress results from an imbalance between free radical production and antioxidant defense mechanisms. This imbalance can lead to various ailments, including cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders [12]. Antioxidants act as free radical scavengers, preventing cellular damage and halting the onset of degenerative diseases. Their effects are exerted by terminating oxidative chain reactions and supporting the biosynthesis of endogenous antioxidant enzymes [13]. Physiologically, antioxidants slow down the ageing process, promote healthy ageing, and protect normal cells from oxidative damage. Previous literature indicates that aqueous extracts from dried pulverized root powder [14], fresh root aliquots [15], and 70% hydro-ethanol extracts [16] of *A. vogelii* roots have been evaluated for radical scavenging potential. Methanol's versatile nature—its ability to dissolve in both water and organic solvents—suggests that it can extract compounds within a broad polarity range. This study aims to address the gap in research regarding the use of methanol root extracts of *A. vogelii* for free radical scavenging. Specifically, the research seeks to identify the phytochemical constituents, quantify selected compounds, and assess the antioxidant capacity of the methanol extract obtained from the roots of *A. vogelii*.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

The whole plant of *A. vogelii*

2.1.2 Equipment and apparatus

Rotary evaporator (Büchi, Switzerland), UV/Visible spectrophotometer (Shimadzu, Japan), Refrigerator (Thermocool).

2.1.3 Chemicals and reagents

Folin-Ciocalteu's reagent, DPPH (Molychem, India), Ascorbic acid, Gallic acid (Molychem, India), Quercetin, Methanol (Sigma-Aldrich (USA), Chloroform (BDH Chemicals (UK), Acetic anhydride (BDH Chemicals (UK), Potassium acetate, Aluminum chloride, Ferric chloride, Sulphuric acid (Conc.), Sodium hydroxide solution, Hydrochloride (Dilute), Lead acetate solution, Salkowski reagent, Dragendorff's reagent, Sodium carbonate

All solvents and reagents used during this study were of analytical-grade methanol. The rotary evaporator (Büchi, Switzerland) was used for extractions and UV–UV-Visible spectrophotometer (Shimadzu, Japan) was used for absorbance measurements.

2.2 Methods

2.2.1 Collection and Identification of Plant Materials

The whole plant of *A. vogelii* was collected in June 2023 from uncultivated land within the vicinity of Precious Palm Royal Hotel, Isihor, Benin City. Identification and authentication were conducted by Prof. H. A. Akinnibosun, a taxonomist from the Department of Plant Biology and Biotechnology, University of Benin. A herbarium specimen, labeled UBH-A258, was prepared and deposited in the Departmental Herbarium for future reference.

2.2.2 Preparation of Plant Extract

The roots were carefully cut, rinsed under running tap water, chopped into small pieces, and air-dried for 21 days. Once dried, they were ground into fine powder using a mechanical grinder and stored in an airtight container. A total of 180 g of the pulverized powder was macerated with 1.5 L of 99.5% methanol for three days, with initial agitation every 30 minutes for 2 hours. After three days, the extract was decanted and filtered through Whatman filter paper (size 1). The filtrate was concentrated under reduced pressure using a rotary evaporator at 50°C, and the resulting extract was stored at 4°C.

2.2.3 Phytochemical Screening

Preliminary phytochemical screening of the crude extract was carried out according to the methods described in [17–19]. The phytochemicals tested for included saponins, glycosides, flavonoids, tannins, phenolic compounds, steroids, terpenoids, and alkaloids.

2.2.4 Total Phenolic Content

The total phenolic content of the extract was determined using the method described in [20]. A 0.5 mL portion of the extract solution (1000 µg/mL) was mixed with 4.5 mL of distilled water, followed by the addition of 0.5 mL Folin–Ciocalteu's reagent. The mixture was vortexed thoroughly and allowed to stand at room temperature for 5 minutes. Subsequently, 5 mL of 7% sodium carbonate solution and 2 mL of distilled water were added. The mixture was allowed to stand at room temperature for 90 minutes, after which the absorbance was measured at 750 nm using a spectrophotometer. The total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract. A standard calibration curve was generated using gallic acid at concentrations of 12.5, 25, 50, 75, 100, and 150 mg/L.

2.2.5 Total Flavonoid Content

The total flavonoid content was determined using the method outlined in [21]. A 0.5 mL portion of the extract (1 mg/mL) was mixed with 1.5 mL of methanol, followed by the addition of 2.8 mL of distilled water, 0.1 mL of 1 M potassium acetate, and 0.1 mL of 10% aluminum chloride. The mixture was incubated at room temperature for 30 minutes, after which the absorbance was measured at 415 nm using a spectrophotometer. The total flavonoid content was expressed as milligrams of quercetin equivalents (mg QE) per gram of extract. A standard calibration curve was generated using quercetin at concentrations of 12.5, 25, 50, 75, 100, and 150 mg/L.

2.2.6 DPPH Radical Scavenging Assay

The antioxidant activity of the crude methanol extract was determined using the DPPH radical scavenging method described in [22]. A 0.1 mM DPPH solution was prepared in methanol. One milliliter of this solution was mixed with 3 mL of the extract at concentrations ranging from 0.01 to 0.2 mg/mL. The mixture was vortexed and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a spectrophotometer, with ascorbic acid serving as the reference standard. The percentage DPPH radical scavenging activity was calculated using the equation:

$(\%) = [(A_0 - A_1) / A_0] \times 100$

where:

A_0 = absorbance of DPPH solution + methanol (control)

A_1 = absorbance of DPPH solution + sample extract or standard.

3.0 RESULTS

3.1 Phytochemical screening

The phytochemical screening of the methanol extract from *A. vogelii* roots revealed saponins, glycosides, flavonoids, phenolic compounds, terpenoids and alkaloids. Notably, tannins and steroids were not found in the extract (Table 1).

Table 1 Phytochemical screening

Constituents	Test	Results
Glycoside	General test	+
Saponins	Frothing	+
Phenols	Ferric Chloride	+
Terpenoids	Salkowski reagent	+
Steroids	Acetic acid/H_2SO_4	-
Alkaloids	Dragendoff's reagent	+
Flavonoids	Lead Acetate	+
Tannins	Ferric Chloride	-

Keys: + = Present - = Absent

3.2 Total phenolic and flavonoid content

From the calibration plot (figure 1), total phenolic content of 24.87±2.12 mgGAE/g was obtained from the equation of the curve, y = 0.0065x + 0.1137 and $R^2 = 0.9224$. y = absorbance of the sample and x is the concentration (phenolic content).

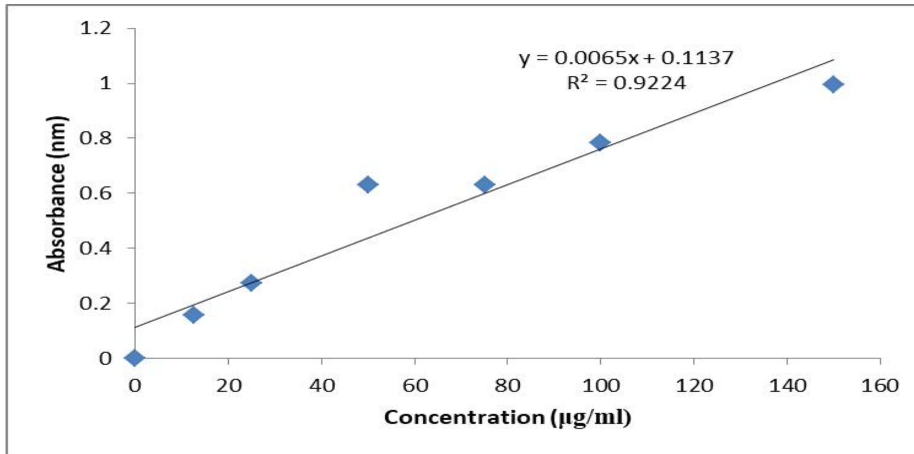

Figure 1: Calibration plot of Gallic acid, showing the absorbance against concentration

Similarly the total flavonoid content (16.18±0.54 mgQE/g) was obtained from the calibration plot (figure 2). From the equation of the curve, $y = 0.0196x + 0.0393$ and $R^2 = 0.9702$. y = absorbance of the sample and x is the concentration (flavonoid content).

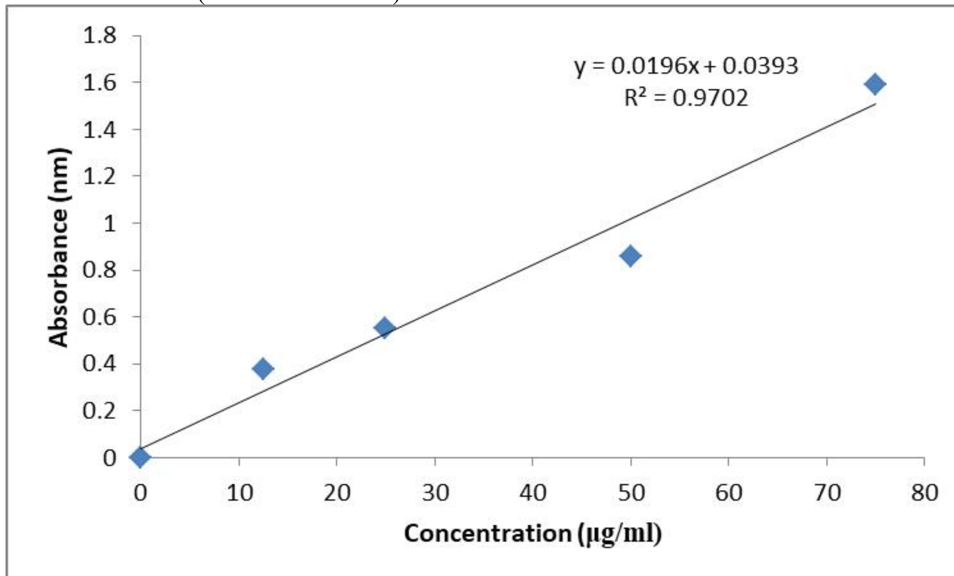

Figure 2 Calibration plot of Quercetin, showing absorbance against concentration

3.3 DPPH free radical scavenging activity

From the plot, the mean percentage radical scavenging activity for *A. vogelii* compare with ascorbic acid at the concentration range of 1 – 50 µg/mL is embedded in figure 3. The graph shows a progressive inhibition of DPPH from 0-50 µg/mL by ascorbic acid and *A. vogelii* extract, the curves show optimal inhibition of DPPH radical at 50 µg/mL.

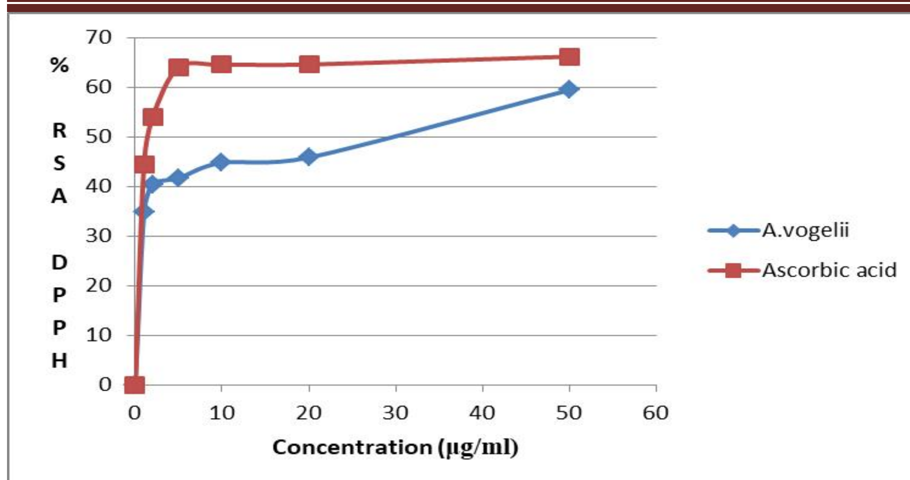

Figure 3. Curve of DPPH radical scavenging activity for ascorbic acid and *A. vogelii* extract

3.4 IC_{50} of Ascorbic Acid and Extract

From Figure 4, the equation of the curve was determined to be $\mathbf{y = 47.677e^{0.0359x}}$. Here, y is set at 50, and x represents the concentration (IC_{50} value). Substituting these values into the equation yields an IC_{50} value for ascorbic acid of **1.33 µg/mL**.

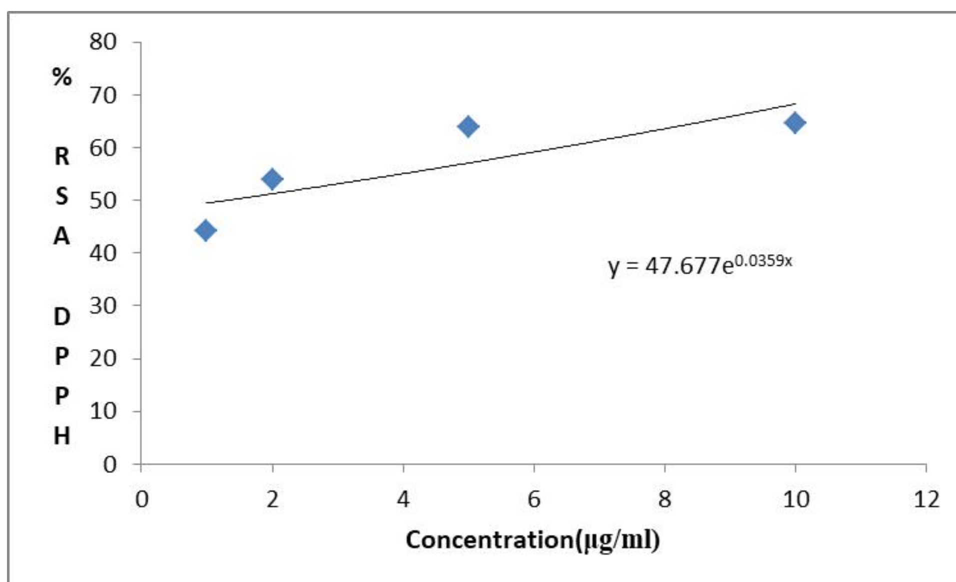

Figure 4. IC_{50} plot for ascorbic acid

For the extract, as shown in Figure 5, the equation of the curve was $\mathbf{y} = 36.369e^{0.0226x.}$ Similarly, y was 50, and x represented the concentration (IC_{50} value). The IC_{50} value for the extract was calculated as 14.08 µg/mL. Although ascorbic acid exhibited a stronger inhibitory effect than the *A. vogelii* extract, the extract also demonstrated notable antioxidant potency.

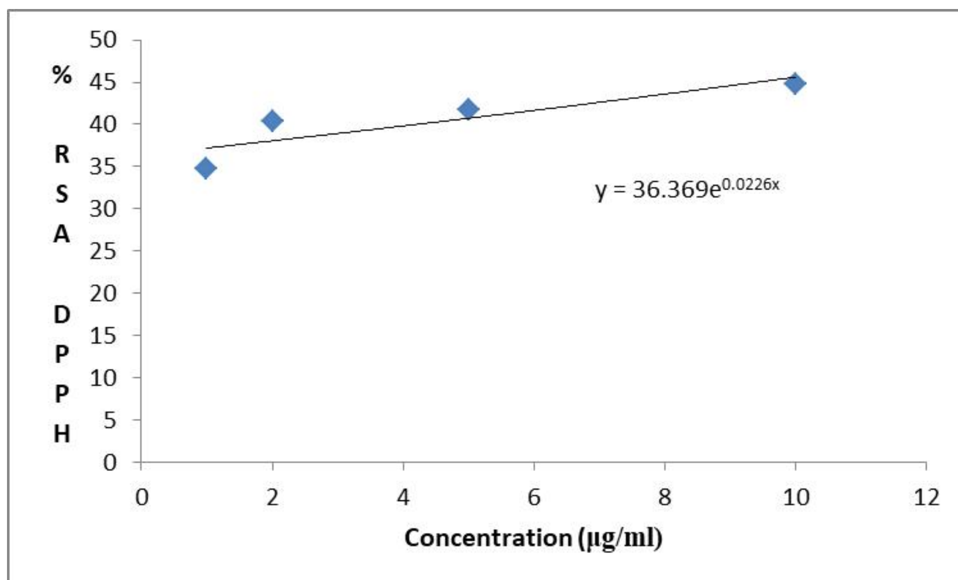

Figure 3: IC_{50} plot for *A. vogelii* extract

4.0 DISCUSSION

The ability of bioactive compounds to alter or modulate various biological pathways suggests that plant extracts may play a significant role in addressing a range of health-related conditions, particularly those associated with oxidative stress. Antioxidant activity in plants is often attributed to the presence of polyphenols such as flavonoids, stilbenes, phenolic acids, coumarins, and tannins. These compounds typically possess one or more hydroxyl groups attached to a benzene ring [23]. Previous phytochemical screening of *A. vogelii* root extract reported the presence of flavonoids, alkaloids, tannins, glycosides, saponins, and phenols [24]. This contrasts with the present study, which identified triterpenoids and did not detect tannins. The variation may be attributed to differences in environmental stress factors within the plant's growing environment [25]. Quantifying flavonoids and phenols helps to evaluate antioxidant potential, identify possible health benefits, support pharmacological discovery, and ensure quality control of *A. vogelii* preparations [26, 27]. Sunday et al. previously reported total phenolic and flavonoid contents of 20.51 ± 2.88 mg GAE/g and 52.90 ± 4.18 mg QE/g, respectively, for ethanolic extracts of *A. vogelii* [16]. Similarly, Apiamu et al. reported 2.81 ± 0.50 mg GAE/g phenolic content and 503 ± 15.28 mg QE/g flavonoid content for leaf extracts [28]. In contrast, the present study found higher phenolic content (24.87 ± 2.12 mg GAE/g) and lower flavonoid content (16.18 ± 0.54 mg QE/g), indicating that the root extract is rich in phenols but relatively low in flavonoids. Although total phenolic and flavonoid assays may lack specificity—sometimes overestimating due to the wide range of compounds measured—they still provide a valuable basis for further research. Studies have consistently shown that the presence of phenols and flavonoids in plant extracts is an indicator of potential antioxidant activity [29, 30]. Antioxidants prevent lipid peroxidation primarily by neutralizing free radicals [31]. In this study, the antioxidant activity of *A. vogelii* root extract was assessed using the DPPH assay—a standard method involving a stable nitrogen-centered free radical. When DPPH interacts with reducing agents, it undergoes a color change due to the acceptance of electrons or hydrogen atoms. The degree of color change depends on the number of electrons transferred and their efficiency in neutralizing free radicals [32]. The antioxidant activity of *A. vogelii* compared favorably with that of ascorbic acid, although it was less potent. This suggests that the methanol extract of *A. vogelii* may act via a similar mechanism, as both produced comparable dose–response patterns. This is promising, as the extract demonstrated the ability to scavenge free radicals and could potentially be used in the management of oxidative stress-related conditions such as diabetes, cancer, and inflammatory disorders. Once antioxidant activity was confirmed, the IC_{50} value—representing the half-maximal inhibitory concentration—was determined. IC_{50} is a quantitative measure of the potency of a compound in inhibiting a specific biological or biochemical function. In the context of the DPPH assay, it indicates the concentration of extract required to achieve 50% inhibition of DPPH radicals through proton donation. A lower IC_{50} value signifies higher antioxidant potency [33, 34]. In this study, *A. vogelii* extract had a higher IC_{50} value than ascorbic acid, indicating it was less potent, yet still demonstrated significant radical scavenging capacity.

5.0 CONCLUSION

This study found that *A. vogelii* root extract contains most of the phytochemicals previously reported, with the addition of triterpenoids and the absence of tannins. Phenolic and flavonoid levels, determined using Folin–Ciocalteu and aluminum chloride methods, revealed a higher phenolic content than flavonoid content—an unusual pattern that may indicate the presence of other polyphenols such as phenolic acids, stilbenes, and coumarins. The antioxidant activity of the extract, confirmed via the DPPH radical scavenging assay, demonstrated its ability to neutralize free radicals, although it was less potent than ascorbic acid. These findings validate the traditional use of *A. vogelii* in managing oxidative stress-related illnesses and support further investigation into its potential therapeutic applications.

DECLARATIONS

Acknowledgement

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Conflict of Interest

The authors wish to declare no conflict of interest

Contribution of Authors

AEA conceptualized, supervised and edited the manuscript. EEO wrote, edited and analyzed data from the study. OSO and OFA were involved in data collection and edited the manuscript.

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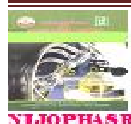

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