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Chemical Composition of *Anthocleista vogelii* and Anti-oxidant Effect of Its Methanolic and Acetone Extracts

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Authors' contributions

This work was carried out in collaboration among all Authors. Author EEI designed the study and perform the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors UDA, EGU and CUM managed the analyses of the study and literature searches. All Authors read and approved the final manuscript.

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ABSTRACT

This study entailed determination of chemical composition of *Anthocleista vogelii* and anti-oxidant effect of its methanolic and acetone extract. The phytochemical screening of *Anthocleista vogelii* showed quantitatively the presence of alkaloids, Tannins, Flavonoids, Saponins, Glycoside and phenols. The results from the proximate analysis indicated that the plant contained some important nutrient such as carbohydrate with percentage content of 16.07 – 34.67%, lipid 5.40 – 7.35%, and crude protein 2.23 – 2.41%. The mineral element analysis showed that potassium (K) was 32.6 – 41.1 mg/kg, sodium (Na) 5.41- 6.21 mg/kg, calcium 9.74 – 17.81 mg/kg, magnesium (mg) 5.71 – 32.0 mg/kg and Zn 0.32 – 0.68 mg/kg. The acetone and methanol leaves, stem bark and roots extracts of *Anthocleista Vogelii* exhibited reasonable scavenging activity in the DPPH (1, 1- diphenyl – 2- picrlhydrazyl) assay. The methnaolic extracts had a better inhibiting ability than acetone extract

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which could be attributed to higher content of tannin and Saponin in methanolic than acetone extract. The results were discussed with referenced to World Health Organization (WHO) standard of nutritional and medicinal values.

Keywords: Analyses; Anthocleista vogelii; chemical composition; methanolic extracts.

1. INTRODUCTION

A versatile application of substances derived from plant has been of great interest to scientist in recent times. These could be attributed to the fact that medicinal plants are the richest bio-resources of drugs for traditional system of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs as well as phytochemical constituents [1].

Phytochemical are naturally occurring chemicals found in plants, which protect plants against bacteria, viruses and fungi and are responsible for plant colours and other organoleptic properties, such as deep purple of blue berries and the smell of garlic [2]. Phytochemical are non-nutritive plant chemicals that have protective or disease preventive or diseases preventive properties. They are non-essential nutrients meaning that they are not required by human body for sustaining life. It is well-known that plant produces these chemicals to protect them, but researches demonstrate that they can also protect human against diseases [3].

Scientist estimated that there may be as many as ten thousand different phytochemical having the potentials to affect diseases such as cancer, strokes autoimmune disorders, rheumatoid arthritis, cataract, aging, and diabetes, cardiovascular and neuron-degenerative diseases [4]. Other important plant constituent is the proximate analysis according to Ikpe and Akpabio [5] is defined as the partitioning of compound in a feed into six categories based on the chemical properties of the compound such as moisture, Ash, Crude protein, crude lipid, crude fibre and Nitrogen free extract (carbohydrate). The essence of this of work is to investigate the phytochemical proximate content and antioxidant activity of the leaves, stem bark and root of the methanolic and acetone extracts of *Anthocleista vogelii.*

2. MATERIALS AND METHODS

2.1 Sampling Area

The samples used in this study are leave, stem bark, and root of *Anthocleista vogelii.* These were obtained from the Forestry Department, Faculty of Agriculture Science, University of Benin, Benin City, Edo State, Nigeria.

2.2 Materials

The samples used in this study are: The leaves, stem bark, and root of *Anthocleista vogelii.*

Reagent: All reagents obtained for the study is of Analytical grades, obtained from sigma Aldrich and Lobal Chemie.

2.3 Apparatus

I. Oven
II. Muffle furnace
III. 2ml pipette
IV. Test tubes
V. water bath
VI. Analytical balance
VII. UV/ visible spectrophotometer. Jenway 6715

2.4 Methods

2.4.1 Sample preparation for proximate and phytochemical analysis

The plant leaves, stem bark and root were carefully separated, washed with distilled water to remove dust and air-dried at room temperature for two weeks. The separated plant samples were then pulverized into powder using blender and stored in an air-tight containers, protected from sunlight until required for proximate analysis.

Fresh plant sample(leaves, stem bark, root) of 10 g each were obtained for phytochemical analysis, crush using porcelain mortar and pestle, soak in a 500 ml glass beaker with 200 cm^3 of methanol and acetone separately at room temperature for

twenty four hours(24hrs) . Each extract was filtered using filter paper. The filtrates were concentrated between 45-50°C over water bath until a paste was obtained. The paste extract were labeled accordingly, cooled and stored in desiccators for further analysis in the laboratory [5].

2.5 Proximate Analysis

The determinations of proximate composition of the samples were carried out using standard analytical method described by Ikpe *et al.* [5]; and [6].

2.5.1 Moisture content determination

Water or moisture in the plant was determined by drying a known weight in an oven to a constant weight at a suitable temperature (90-105°c). The loss in weight was regarded as being due to moisture loss and was calculated in terms of percentage weight of the plant sample taken [5]. i.e.

The weighing crucible was washed and dried in an oven at 105°c for two hours, this was cooled in a desiccator and weighed as (a) three (3g) of the fine-ground plant samples (leaves, stem, bark, root) were weighed into the weighing crucible, weight of the crucible and the sample was taken as (b). The weighing crucible and the sample was then dried in an oven at the temperature of 105°c for twenty-four hours (24hrs), after which was quickly transferred to the desiccator to cool, weight was taken with a minimum exposure to atmosphere to avoid Adsorption of moisture. The procedure was repeated till a constant weight was obtained as (C).

Calculation:

$$\%\ \text{Moisture content} = \frac{\text{loss in weight due to drying}}{\text{nitial weight of sample}} \text{x} \frac{100}{1}$$

OR

$$\frac{\text{b} - \text{c}}{\text{b} - \text{a}} \text{x} \frac{100}{1}$$

Where

a=Initial weight of empty crucible
b=weight of crucible + sample before drying
c=weight of sample + crucible after drying

2.5.2 Ash content determination

The ash content of the plant sample was determined by burning the sample in an enclosed muffle furnace at temperature not below 500^0c until the plant sample turned at temperature value expressed in terms of dry weight of plant sample. The organic matter was obtained by subtracting the amount of ash from the dry plant sample i.e.

One gram (1.0 g) of the oven-dried sample in triplicate were weighed into porcelain crucibles of known weight and covered. The porcelain crucibles and their contents were ignited for four (4) hours at 500°c in a muffle furnace till the material was completely white. They were cooled in a desiccator and weighed soon after it reached room temperature. The ash content was then, calculated as follows

Calculation:

$$\%\ \text{Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of oven dried sample (g)}} \text{X100}$$

2.5.3 Crude fibre content estimation

Crude fibre determination involved dafating the sample and boiling in turn with tetraoxosulphate (iv) acid (H_2SO_4) and sodium hydroxide (NaOH) with suitable washing of the insoluble residue obtained at the different stages with distilled water.

The following basic procedures were involved during estimation of crude fibre content.

(a) Defating
(b) Acid digestion
(c) Filtration
(d) Base digestion
(e) Ignition process

(a) Acid Digestion

Two grams (2 g) of the ground plant sample was accurately weighed into 400ml beaker, which has been previously marked at 200 ml level. Fifty ml (50 ml) of 1.25% H_2SO_4 was added, and the mixture made up to 200 ml level with distilled water. The content of the beaker was boiled for thirty (30) minutes. During boiling the mixture was stirred with a rubber-tipped glass rod, removing all particles from the side and keeping the volume constant by adding hot water from time to time (occasionally).

(b) Filtration

The content of the beaker was fettered through a Buchner funnel with the aid of suction pump. The residue was washed with hot water until it was free from acid (tested with blue litmus paper).

(c) Base Digestion

The residue left after digestion was quantitatively transferred into the 400m beaker. Fifty ml (50 ml) of 1.25% NaOH solution was added and made up to 200ml with distilled water. The mixture was again heated for 30 minutes with constant stirring. The contents of the beaker was filtered through the Buchner funnel and washed several times with hot water until it was free from NaOH. The residue was washed twice with 95% methanol and transferred into a porcelain crucible and dried in an oven at 100°c to constant weight. The oven-dried residue was later ignited in a furnace at 550°c for 3hrs later transferred to desiccator to cool. The weight of the ash left after ignition was noted.

Calculation:

Let

l_0= Weight of empty crucible
l_a= Weight of empty crucible after ignition
2_g= Weight of the plant sample taken

$$\therefore \%\ \text{of crude fibre content} = \frac{1a - 10}{\text{Weight of sample taken}} \text{x} \frac{100}{1}$$

2.5.4 Lipid content determination

Here, petroleum ether (b.p 60-80°c) was used to extract lipid exhaustively, using soxhlet apparatus and weighing the lipid after evaporating the solvent i.e.

This method was carried out by continuously extracting the sample with organic solvent for six (6) hours. Three grams (3.0 g) of the oven-dried sample was accurately weighed into a soxhlet thimble. Petroleum either of 200 ml with boiling point (60-80°c) was poured into a 500 ml round bottom flask containing anti-bumping granules (for uniform boiling and prevention of bumping). The soxhlet apparatus was then assembled for distillation. The temperature of the heating mantle was slowly raised until the ether boiled. The flask was heated for about six (6) hours to ensure complete extraction. The solvent was then distilled till about 50 ml of the extract left. The flask content was then poured into a weighed beaker and the flask washed with some either into the beaker and was later evaporated to dryness on a hot water bath and the lipid left in the beaker was placed in a desiccator for further drying and then weighed. Amount of lipid extracted was obtained from the difference between the weight of the empty beaker and the weight of beaker containing the extract.

Calculation:

$$\%\ \text{crude fat (lipid)} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

OR

$$\left(\frac{w_2 - w_1}{weight\ of\ sample}\right) \text{g x } 100$$

Where

w_1=weight of empty beaker
w_2=weight of empty beaker + sample

2.5.5 Crude protein content determination

Crude protein content determination was done by analyzing the plant sample for nitrogen and multiplying the value for nitrogen by a protein factor (6.25). This involved three processes (digestion, distillation, titration), such as digestion of a known weight of the sample with H_2SO_4. The treatment converted nitrogen components in the sample into ammonium sulphate. Ammonia liberated from the ammonium salt by heating (distillation) with sodium hydroxide. The ammonia generated was collected in an excess of boric acid and the nitrogen was estimated by titration of the ammonium borate produced with standardized 0.1 m HCL.

Equation of Reaction

$$NH_4HBO_3 + HCl \rightarrow NH_4Cl + H_2BO_3$$

However, Half gram (0.5 g) of the sample was accurately weighed into a standard 250 ml Kjedahl flask containing 1.5 g $CuSO_4$ and 1.5 g Na_2SO_4 as catalyst and 5ml concentrated H_2SO_4 as catalyst and 5ml concentrated H_2SO_4. The digestion flask and all its content was then place into the digestion rack and heated gently for one hour to prevent vigorous charring and frothing. The flask and its content were then subjected to vigorous heating in a fume cupboard until a clear bluish solution was obtained. After digestion, the

digest was cooled and quantitatively transferred into a standard 100 ml volumetric flask and made up to the mark with distilled water.

Twenty ml (20 ml) of this digest was pipetted into a micro-kjedahl distillation apparatus and treated with 30 ml of 40% (w/v) NaOH solution and heated. The ammonia evolved steam distilled into 100 ml conical flask containing 10 ml 5% boric acid solution and four (4) drops of double indicator added (Tashirus indicator).

The tip of the condenser was immersed into the boric and double indicator solution and the distillation continued until two third ($^2/_3$) of the original volume of this solution turned green. The tip of the condenser was rinsed with few ml of distilled water. The distillate was then titrated with 0.1 MHCL solutions until a purple, pink colour was obtained.

A blank determination was performed in a similar way as described above without the sample in the flask.

Calculation:

$$\%\ \text{crude fat (lipid)} = \frac{\text{VA} - \text{VB x Macid x 14}}{\text{W x 1000}} \text{x} \frac{100}{1}$$

Where

V_A = Volume (ml) of acid required to titrate the sample
V_B =Volume of acid required to titrate the blank
M acid = Molarity of acid
W =Weight of the sample taken
% = Crude protein = % Nitrogen x F
W here F= Conversion factor, $\frac{100}{16}$ (16 is % nitrogen in food protein)
The common factor used for most food is 6.25

2.5.6 Estimation of carbohydrate content

Carbohydrate content was obtained by subtracting the values obtained for fat and protein from organic matter.

The percentage of organic matter was calculated by subtracting the percentage of ash from one hundred (100) [6].

The carbohydrate content of the sample was determined by the difference method after obtaining the values of organic matter, protein, lipid and ash.

Amount of protein= a%
Amount of lipid = b%
Protein + lipid = (a + b) %
Organic matter (%) = (100 - % ash)

Carbohydrate = organic matter – (protein + lipid + fibre)

2.6 Phytochemical Screening of the Sample Extracts

The freshly prepared methanolic and acetone extracts of *Anthocleista vogelii* leave, steam bark and root were tested for the presence of phytochemical constituents using standard procedures [5, 7].

2.6.1 Test for alkaloids

Half gram (0.5g) of the methanolic and acetone extracts were each measured into three separate test tubes for the test and colour change for each test was noted. A few drops of freshly prepared Dragendorff reagent were added to the first test tube. A pink or red precipitate was an indication of a positive test. A freshly prepared Mayer's reagent was added to each of the extracts in the second test tubes. A milky or green colour was an indication of a positive test. A few drops of picric reagent were added to the extract in the third test tube. A white or yellow precipitate indicated a positive test.

2.6.2 Test for tannins

The plant extracts of weight 0.5 g were taken into 100 ml beaker was stirred. 10.0 ml of distilled water and 2 mls of bromine water was added and stirred. For a positive test, tannin decolourised bromine water. The plant extract of weight 0.5 g was dissolved in distilled water and ferric chloride reagent was added to the filtrate. A blue black, green or blue green precipitate was taken as evidence for the presence of tannins.

2.6.3 Test for phenols

This was done by treating 1.0 ml plant extract with 4 drops of ferric chloride solution. Formation of a bluish black colour indicates the presence of phenols.

2.6.4 Test for saponins

(a) **Frothing Test**: 0.5 g of each extract was shaken vigorously with distilled water in a test tube. Frothing which persisted on

warming was taken as preliminary evidence for the presence of saponins.

Fehlings solution was added to 0.5 g of each extract and warmed. The presence of brick red precipitate confirmed the presence of saponins. 10 ml Na_2CO_3 was added to 0.5 g of each extract and fehlings solution was also added and warmed, brown precipitate confirmed the presence of saponins.

2.6.5 Test for flavonoids

This was done using Shinoda's test. The plant extract was dissolved in concentrated hydrochloric acid. Few pieces of magnesium metal were added to 5 mls of the extract. The formation of orange, red, crimson or magenta was taken as a positive test for the presence of flavonoid. Ammonia test was carried out by weighing 0.2 g of plant extract into a test tube. 5 ml of ethyl acetate was added and heated. It was cooled and filtered; 4.0 ml of the filtrate was shaken with 1.0 ml of dilute ammonia solution. The colour changed was observed.

2.6.6 Test for steroids terpenes

To 0.05 g of each extracts, 3.0 ml chloroform was added and flittered, 10 drops of ethanolic anhydride and 2 drops of concentrated H_2SO_4 were added to the filtrate and the colour change was observed. Pink colour at interphase was taken as positive test for terpenes, bluish green interphase was positive test for steroids.

2.6.7 Test for cardiac glycosides

Liberman's Test was carried out by dissolving 0.5 g of plant extract in 2 mls of ethanolic anhydride and cooled in ice. Sulphuric acid was then carefully added. A colour change from violent to blue then to green indicated the presence of a steroidal nucleus i.e. aglycone portion of the cardiac glycoside.

Salkowski Test was carried out by dissolving 0.5 g of the plant extract in 2 mls of chloroform; sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicated a positive test.

2.7 Measurement of Free Radical Scavenging Activities of Sample Extracts

The free radical scavenging activities of each of the plants extracts were assayed using a stable DPPH standard method [8, and 9] with slight modification.

The reaction mixtures, control and blank were allowed to incubate in the dark for 30 minutes.

The absorbance of the reaction mixtures was measured using UV/visible spectrophotometer at 518nm wavelength and the ability of the extract to scavenge DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical were calculated by the following equation:

$$\%\ \text{Free Radical Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample/standard}}{\text{Absorbance of control}} \times \frac{100}{1}$$

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

3.1.1 Moisture content

The moisture content of *Anthocleista vogelii* ranged from 53.13 – 66.18% (w/w). This result indicated low shelf life of the fresh plant hence long storage would lead to spoilage due to its susceptibility to microbial attack. This supports the practice of storage in dry form by users. Moisture content is among the most vital and mostly used measurement in the processing, preservation and storage of food [5].

Table 1. Result of proximate analysis of *Anthocleista vogelii*

S/N	Parameter	Leaves	Composition	
			Stem	Root
1.	Moisture content (w/w)	63.34±0.03	53.13±0.03	66.18±0.02
2.	Ash (% dry matter)	8.33±0.03	2.55±0.04	9.82±0.06
3.	Crude fibre (% dry matter)	4.45±0.01	0.80±0.03	1.50±0.01
4.	Crude lipid (% dry matter)	5.40±0.02	7.35±0.00	6.00±0.07
5.	Crude protein (% dry matter)	2.41±0.01	2.23±0.02	2.25±0.01
6.	Carbohydrate (%dry matter)	16.07±0.07	34.67±0.04	14.24±0.04

Results are mean of triplicate determinations ± SD

3.1.2 Ash content

Ash content represents the index of mineral element present in a sample; its amount is useful in assessing a sample and gives an idea of the minerals available in a sample [10]. Ash in food or any sample constitutes the residue remaining when these material are incinerated at about 550^0c in a muffle furnace. This result in the oxidation of organic constituents to volatile material considered as carbon (iv) oxide, nitrogen oxide and sulphur (iv) oxide etc. Ash residue is considered to be a measure of mineral content of the original sample [6].

Generally, the ash content of the plant root had the highest value of $9.82 \pm 0.06\%$, while the stem recorded the least value of $2.55 \pm 0.004\%$. This implies that the root of the plant has higher mineral content, which is corroborated by a similar study carried out by [11].

3.1.3 Crude fiber

The crude fibre in food or plant is an indication of the leave of non-digestible carbohydrate and linger. The result of crude fibre in the samples (table 1) follows a decreasing order of leaves > Root > stem. This implies that the leave sample has the highest crude fibre content and it play significant roles in human nutrition.

According to Gwarzo US et al. [12] fibre helps to maintain the health of gastrointestinal tracts, in excess may bind trace elements leading to deficiencies of iron and Zinc. Moreso, fibre lowers the body cholesterol leave and consequently decreases the cardiovascular disease.

On the whole, fibres appears to inhibit many cancers especially colon cancer by binding the carcinogens and prevent them from entering the body while they pass through the system [13].

3.1.4 Crude lipid

The crude lipid in the samples investigated, ranged from 5.40 - 7.35% as showed in Table 1. Crude lipid can provide a very good source of energy, it aid in transport of fat soluble vitamins, insulates and protects the internal tissues, it contributes to important cell processes [14] fat and oil are insoluble hydrophobic substances of vegetable and animal origin, they consist of glycerol esters of fatty acid called triglycerides. They are divided into two groups, the saturated fat with only single bond and unsaturated fat with at least on double bond. Hence it is good to add lipid (fat) to most of our diets because numerous body function relied on lipid [11]

3.1.5 Crude protein

The *Anthocleista vogelii* showed (Table 1) protein content of 2.23 - 2.41%, which is considered to be of important to human health. Proteins are made up of polypeptide chains. Some protein, like myoglobin, consists of only one polypeptide chain. Others may consist of more one like or unlike polypeptide chains. Proteins are found in all living systems as structural components and as biologically important substances such as hormones, enzymes and pigments. Protein in our food can be divided into first class protein and second-class protein. First class protein contains essential amino-acids. These are mainly of animal origin. Examples of food, which contain first class proteins, are meat, fish, egg and cheese. Second class proteins are mainly vegetable proteins which are found in vegetables such as bean as reported by Trease et al. [15].

3.1.6 Carbohydrate

The high percentage of carbohydrate in sample is a signal that it can be used to regulate various metabolic processes in the body as vital molecules in the central metabolic path ways of the body. Carbohydrate serves as stored form of energy, as glycogen in liver muscles. It also provides most important source of energy and responsible for breaking down fatty acids and preventing ketosis as reported by Gwarzo US et al.[12]. From the analysis, stem of *Anthocleista vogelii* had the highest composition of 34.67% compared to leave and root which stood at 16.07 and 14.25% respectively. The presence of carbohydrate in the investigated samples supports assertion by several researchers, that there are several benefits which mangrove forest provides.

3.2 Results of the Phytochemical Screening for Plant Extracts

3.2.1 Discussion of phytochemical screening

Phytochemical are bioactive chemical compounds that occur naturally in plants and which have beneficial effect on human health. They act as deterrents to insect and microbial

attack many of them have been found to have medicinal properties in clinical trials for a variety of diseases [1]. Deficiency of phytochemical in processed food may contribute to increased risk of preventable diseases [16]. The major phytochemical compounds found in plants include, alkaloid, Tannins, flavonoids, saponins, glycoside and phenols.

The stem and leave of both methanolic and Acetone extracts were found to contain a higher composition of saponins with double positive sign. saponins are known to make bronchial secretion more liquid, reduce the congestion of the bronchi and ease coughing [17]. The presence of saponin in the leaves and stem indicate that the extract from the plant can be used to stop bleeding and in treating wounds.

The extract of both samples contain Alkaloid with a single positive sign which indicate its availability or presence. Plants that contain alkaloid are used as muscle relaxant, pain relief and in the treatment of malaria [1]. Isolated plant alkaloids are used as basic medicinal agent for their analgesic, antispasmodic and anti-bacterial effect [1; 13]. This may be the reason the plant is used in herbal medicine to treat chest pain and hepatitis.

The presence of flavonoid is also observed in the plant extract. Flavonoid are known to posses antioxidant property, protect against allergies, inflammation, microbes, ulcer, viruses and tumor [1]. Flavonoids are also known to prevent oxidative cell damage, have strong anti-cancer activity and inhibit all stages of carcinogenesis [18].

Tannin is another important phytochemical found in the plant extracts. Tannins are organic substances of diverse composition with pronounced astringent properties that promote the healing of wounds and inflamed mucus membrane [1]. Tannin gives a slight bitter to food, and so can counterbalance too much acidity [19]. It can also be effective in protecting the kidney. The use of *Anthocleista vogelii* to treat diarrhea could also be emphasize because of the presence of tannin and flavonoids.

The plants extracts also contain phenols; this could be used as anti-inflammatory, immune enhancers and hormone modulators [20]. Phenols are also known to have the ability to block specific enzymes that cause inflammation and to prevent diseases.

Investigation showed that Eugenoid, steroid and terpenoids were not found in the plant extracts.

3.3 Inhibition Effect of Phytochemical

Tables 4 and 5 shows that the methanolic extracts have better inhibiting ability than the acetone extracts. This could be attributed to the higher content of tannin and saponins in methanolic than in acetone extract.

The half maximal inhibitory concentration ($1C_{50}$) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process of component of a process i.e. an enzyme, cell, cell receptor or microorganism by half. The values are typically expressed as molar concentration.

Table 2. Results of the photochemical screening for plant extracts

		Composition					
S/N	**Parameters**	**SA**	**RA**	**LA**	**SM**	**RM**	**LM**
1.	Alkaloid	+	+	+	+	+	+
2.	Tannins	+	+	+	+	+	+
3.	Flavonoids	+	+	+	+	+	+
4.	Steroids	-	-	-	-	-	-
5.	Saponins	++	+	+	+	+	++
6.	Glycoside	+	+	+	+	+	+
7.	Eugenols	-	-	-	-	-	-
8.	Phenols	+	+	+	+	+	+
9.	Terpenoids	-	-	-	-	-	-

Keys
SA = Stem Acetone extract; SM = Stem Methanolic extract
RA = Root Acetone extract; RM = Root Methanolic extract
LA = Leave Acetone extract; LM = Leave Methanolic extract

Table 3. Concentration of ascorbic acid versus % inhibition

Concentration in Ng/ml	% Inhibition
250	88.911±0.04
200	81.760±0.02
150	69.780±0.02
100	47.578±0.01
50	15.988±0.03
0	0.000±0.00

Results are mean of triplicate determination ± SD

Table 4. Percentage (%) inhibition of the methanolic extracts

	Percentage Inhibition (%)		
Concentration in µg/ml	**RM**	**LM**	**SM**
250	16.54±0.06	16.99±0.01	16.64±0.03
200	16.04±0.08	16.49±0.01	16.69±0.07
150	15.83±0.01	16.24±0.03	16.34±0.01
100	15.43±0.01	15.68±0.02	16.09±0.04
50	14.68±0.02	15.18±0.04	15.88±0.01

Results are mean of triplicate determination ± SD

Table 5. % Inhibition of acetone extracts

	Percentage Inhibition (%)		
Concentration in µg/ml	**RA**	**LA**	**SA**
250	12. 46±0.08	12.20±0.02	11.62±0.03
200	12.04±0.01	11.73±0.01	11.46±0.05
150	11.94±0.05	11.52±0.02	11.10±0.01
100	11.31±0.01	11.20±0.03	10.10±0.03
50	10.73±0.02	10.89±0.01	10.78±0.04

Table 6. The half maximal inhibitory concentration ($1.C_{50}$) values of methanolic and acetone extracts of the leaf, stem bark and root

	RM	LM	SM	RA	LA	SA	AA
1.C50 (µg/ml)	123.1± 0.04	135.7± 0.01	131.5±0.04	122.7±0.05	150.2± 0.04	140.8± 0.05	92.07± 0.09

Table 6 Show the $1C_{50}$ of the percentage inhibition of the extracts and Ascorbic Acid (AA). It can be seen that Ascorbic Acid (AA) which is a known standard inhibitor, has a better inhibitory property than any of the extracts. The Table (6) also show that the methanolic extracts have better inhibitory properties than the acetone extracts with exception of the root acetone extract which seems to be slightly higher potent than the root methanol extract (Table 6), because the lower the $1C_{50}$ the more potent the extract is [21].

It has been reported that flavonoids is the main polyphenolic compound associated with the inhibitory properties of most medicinal plant [11].

3.4 Mineral Element Composition (mg/kg, Dry Matter) of *Anthocleista vogelii*

3.4.1 Potassium (k)

Potassium is very vital in regulation of water and electrolyte balance and acid-base balance in the body, as well as responsible for nerve action and functioning of muscles.

Table 7 Mineral element composition of *Anthocleista vogelii*

Mineral	Concentration mg/kg		
	L	S	R
K	33.1±0.03	41.1±0.08	32.6±0.05
Na	5.41±0.01	6.21±0.04	5.54±0.09
Ca	13.50±0.03	17.81±0.01	9.74±0.00
Mg	32.0±0.01	7.0±0.03	5.75±0.04
Zn	0.68±0.01	0.60±0.00	0.35±0.02

Results are mean of triplicate determination± SD
L=Leaves
S=Stem bark
R=Root

Deficiency of potassium leads to muscle paralysis [22]. Potassium deficiency and excess can result in numerous signs and symptoms, including an abnormal heart rhythm and various electrocardiographic abnormalities. Fresh fruits and vegetable are good dietary sources of potassium. The body responds to the influx of dietary potassium, which raises serum potassium levels, with a shift of potassium from outside to inside cells and an increase in potassium excretion by the kidneys. Diets low in potassium can lead to hypertension and hypokalemia [23]. The WHO [24] recommended standard for potassium in food/vegetables is 35 /mg/g while the recommended dietary allowance of potassium for adults by national research Council (1974) is 1875-5625mg.

The value of potassium in the present study of *Anthocleista vogelii* in Table 7 ranged from 32.6 – 41.1 mg/kg, which simply shows a satisfactory level of potassium present in the plant with the highest level recorded to be found in the stem.

3.4.2 Sodium (Na)

In human, sodium is an essential mineral that regulates blood volume, blood pressure, osmotic equilibrium and pH. Unusually low or high sodium levels in humans are recognized in medicine as hyponatremia and hypernatremia respectively. These may caused by genetic factors, ageing, or prolonged vomiting or diarrhea [25]. According to National Research Council (1974), the recommended daily allowance value for sodium is 1100-3300 mg/100 g for adults. Deficiency of sodium may lead to dehydration or muscle cramp [22]. The experimental analysis carried out shows that *Anthocleista vogelii* contains 5.41 mg/kg sodium in leaves extract 6.21 mg/kg in the stem and 5.54 mg/kg in the roots (Table.7). These levels are considerably low in comparison to the WHO's standard of 20 mg/kg. Hence *Anthocleista vogelii* is not a good source of sodium.

3.4.3 Calcium (Ca)

Calcium is essential for bone and teeth formation and development, blood clotting and for normal functioning of heart, nervous system and muscles. Calcium deficiency can lead to rickets, Osteomalacia and tooth decay [22]. However, calcium is the most abundant metal and fifth-most abundant element in the human body.

As electrolytes, calcium ions play a vital role in the physiological and biochemical processes of organisms and cells. In signal transduction pathways where they act as a second messenger, in neurotransmitter release from neurons, in contraction of all muscle cell types, as cofactors in many enzymes and in fertilization [26]. Calcium ions outside cells are important for maintaining the potential differences across excitable cell membranes as well as proper bone formation. Calcium is an essential element needed in large quantities.

Osteoporosis, which is a reduction in mineral content of bone per unit volume, can be treated by supplementation of calcium, vitamin D, and biphosphate. Inadequate amounts of calcium, vitamin D, or phosphate can lead to the softening of bones, known as Osteomalacia. The WHO's standard for calcium in food/ vegetable is 75 mg/kg which is higher than the content in the plant under study (Table 7). The stem bark of the plant recorded the highest level of calcium (17.81 mg/kg) among the parts analysed.

3.4.4 Magnesium (Mg)

The important interaction between phosphate and magnesium ions makes magnesium essential to the basic nucleic acid chemistry of all cells of known living organisms. More than 300 enzymes require magnesium ions for their catalytic action, including all enzymes using or synthesizing ATP and those that use other nucleotides to synthesize DNA and RNA. The ATP molecule is normally found in a chelate with a magnesium ion [27]

It was observed that the leaves of *Anthocleista vogelii* are rich in magnesium (32.0 mg/kg). Minimal levels are present in stem and root (7.01 and 5.75mg/g) respectively as stated in Table 7. The WHO recommended standard for magnesium is 50 mg/g. Hence, higher level of magnesium can be obtained from the leaves of *Anthocleista vogelii.*

3.4.5 Zinc (Zn)

Zinc is an essential trace element for humans and other animals for plants and organisms [28].

Zinc is required for the function of over 300 enzymes and 1000 transcription factors [29] which is stored and transferred in metallothioneins. It is the second most abundant trace metal in humans after iron it is the only metal which appears in all enzyme classes [30]. Roughly 2 - 4 grams of zinc are distributed throughout the human body. Most Zinc is in the brain, muscle, bones, kidney and liver, with the highest concentration in the prostate part of the eye [31].

Semen is particularly rich in Zinc, a key factor in prostate gland function and reproductive organ growth [32].

The zinc content of *Anthocleista vogelii* (Table 7) showed that leaves had 0.68 mg/kg while stem and root had 0.60 and 0.35 mg/kg respectively. From these analysis it shows that Zinc in the plant is below the WHO recommended standard for Zinc (30 mg/kg), hence the daily nutritional requirement for Zinc cannot be gotten from the consumption of *Anthocleista vogelii.*

4. CONCLUSION

This study has revealed that traditional usefulness of *Anthocleista vogelii* is still untapped, because most of the potent bioactive component (proximate and phytochemicals) has been discovered. As such the study has provided useful information for the maximum utilization of the plants parts, to better human and animal health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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