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Research Paper

Proximate and Phytochemical Analysis of leaves of *Anthocleista vogelii* (Cabbage palm)

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Abstract

The proximate and phytochemical analysis of leaves of *Anthocleista vogelii* was studied. The phytochemical screening indicated qualitatively the presence of alkaloids, saponins, flavonoids, polyphenols, tannins, reducing sugars, and the absence of phlobatanins, anthranoids. The results from the proximate composition indicate that the leaves contained some important nutrients such as Carbohydrate 52.50±0.60, Fatty acid 4.40±0.11, Fiber content 17.30±0.22 and Protein content 17.30±0.11. The presence of the phytoconstituents in *Anthocleista vogelii* shows that the plant contains essential nutrients, hence, it can be viewed as a potential component for the production of drugs.

Introduction

Over the years, there has been strong emphasis on the usage of medicinal plants for disease prevention, cure and health promotion (Anita et al., 2022). Varieties of natural herbs are used by different ethnic groups, and they serve as potential component for development of synthetic drugs (Priyanka et al., 2016). These plants consist of secondary metabolites which serves as a key protector from biotic and abiotic stress, they are classified based on the biosynthesis and the appearance of their functional group (Naqui et al., 2021).

Phytochemicals are chemical compounds found in plants, and are responsible for the antioxidant activity in plants. The consist of flavonoids, phenols, and polyphenols. Previous study on the phytochemicals of 'Rio Red' Grapefruit (Citrus *paradisi* Macf.) which includes vit C, carotenoids, flavonoids indicated a decrease in content of phytochemicals in the developing stage of the plant (Naqui et al., 2021 & Notup et al., 2024). According to Mohammed *et al*. (2023) the phytochemical composition as established in A. *polystachyus* has the antimicrobial property which may be used in reducing microbial population load and fight pathogens. Studies on the epidemiology of plants suggested the curative ability of consuming some plants, especially nuts which is noted to reduce severity of human disease due to the high phytochemicals and antioxidant potentials, with other functions like high mineral and vitamin concentrations (Apiamu et al., 2023). The research done on buckwheat (*Fagopyrum sp.)* and barley *(Hordeum sp*.) of the trans Himalayan region indicated significant values in the phytochemical in each species, highlighting their great potential as nutraceutical food (Notup et al., 2024).

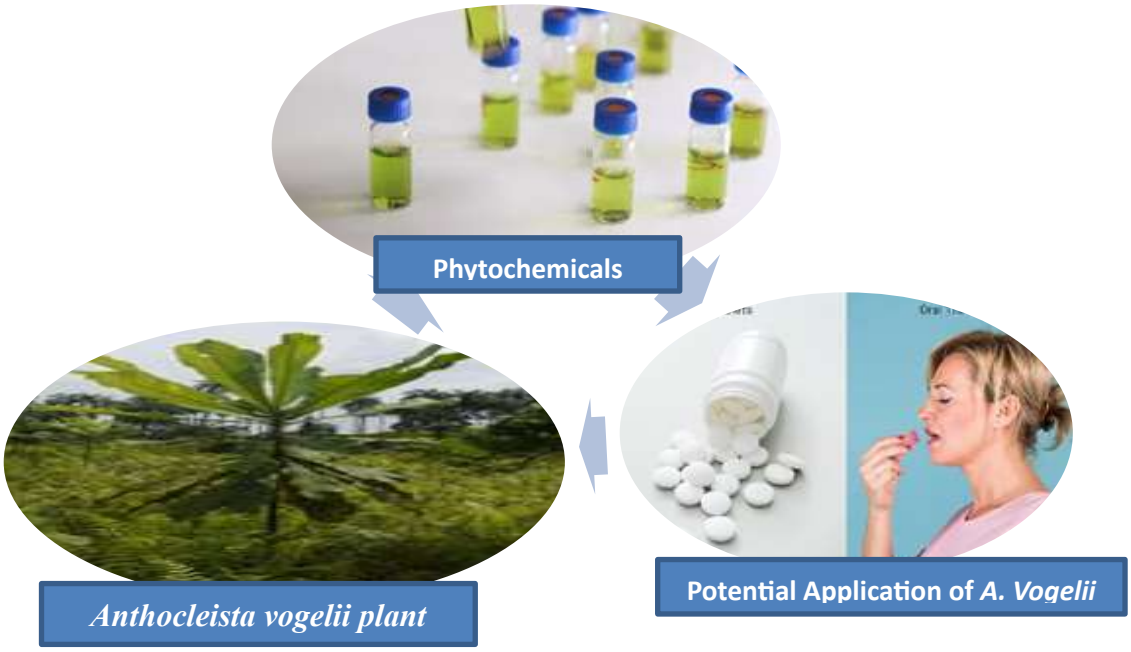

Fig 1. **Application of leaves extracts of *Anthocleista vogelii***

Anthocleista vogelii is of the family Gentianaceae, commonly called "*Cabbage Tree"* In several countries in Africa is a rainforest tree ultimately reaching eighty feet (24 meters) in height, with leaves in opposite pairs up to 14 inches (36 centimeters) long and five inches (12 centimeters) in width in adult trees (Menninger and Edwin,1976). These juvenile trees are mostly found in tropical Africa like Nigeria, South Sudan, South Kenya, South Angola, Kenya and Tanzania, they are noteworthy in bearing enormous leaves up to 7.5 feet (2.3 meters) in

length by about 18 inches (46 centimeters) wide with edges in opposite pairs with nodes spread about four inches apart.

Fig. 1 shows the application of *vogelii.* The leaves extract of A. vogelii has proven to have an antidiabetic and antioxidating potential. The leaves, played a potential antioxidating role in the reform of Cd-induced oxidative stress in the serum of male Wistar rats (Apiamu et al., 2019). Kwanabie *et al.* (2022) suggested that A. nobilis leaf extract has a potential inhibitory effect on activity of the enzyme α-amylase, which indicated a non-mixed inhibition, thereby concluding that the plant has the potential to be used a component for antidiabetic drug production. In relation to Meron et al. (2023), there has been previous records that the extract of *Anthocleista grandiflora* leaves contained an antimicrobial inhibitor (Ellof, 1998).

Phytochemicals screening is the scientific method of extracting, experimenting and analyzing plants, to spot out the different phytoconstituents that are present in various part of a plant. This method is vital for drug discovery, as the actives identified during this process can be further researched on for potential therapeutic purpose for human. It is recorded that they are numerous phytochemicals with a potential to impact on a wide range of life-threatening diseases, including cancer, diabetes, stroke, arthritis (Ebrahimzadeh et al., 2010).

Previous research highlights the various methods used in verifying and identifying the presence of phytonutrients and its potentiality in disease prevention and cure. These methods includes the estimation of phytoconstituent (starch and total protein, crude fiber, moisture content, volatile and ash content, mineral content), bioactive compounds (phenolic content, flavonoid content, polyphenol content) GC-MS spectrometry, HPLC, antioxidant analysis (DPPH,ABTS, Reducing sugar assays),antidiabetic analysis (α-glucosidase, α-amylase assays), molecular docking(CtBP, SOX2) (Hafix et al.,2022 ; Meron et al., 2023; & Notup et al., 2024).This study aims at verifying the phytonutrients present in the leave extract of *A*. *vogelii* through the proximate and phytochemical analysis, and comparing the results obtained with previous work, to ascertain its potency.

Materials and Methods

Fresh leaves of Anthocleista *vogelii* were collected from Akamkpa Local Government area of Cross River State and its identity confirmed by an herbarium in Botany department of the University of Calabar, Cross River State Nigeria. Collected plant leaves were washed with distilled water, the veins carefully detached and leaves chopped into smaller pieces, and placed

in an oven at the temperature of 150°C for six hours to dry. Dried plant parts were grounded into fine powder using an electric grinder.

Proximate Screening

The proximate composition of the ground plant was determined using the methods as described by (A.O.A.C 1990). The proximate analysis includes moisture, crude protein, fibre and ash content.

Moisture Content

Five grams of fresh sample was placed in an oven for six hours at the temperature of 100°C. The weight was taken after drying. The loss in weight was expressed as a percentage of the initial weight. The mixture was heated with continuously stirred for thirty minutes and allowed to cool and settle. Distilled water was added and allowed to settle and was decanted. The process of decantation was repeated for six consecutive times to separate liquids of different densities. All the laboratory activities were performed in the laboratory of the Chemistry Department, University of Calabar.

The Mixture was added to 50 mL of 1.25% NaOH, and made up to 200 mL with distilled water in a beaker and was heated and continuously stirred for thirty minutes. It was allowed to cool and settle. Distilled water was added and decanted for six consecutive times. After filtration, the mixture was kept for 45minutes for water to drain completely and the weight was taken.

Crude Fat Content

Five grams of ground sample was placed in a beaker, the crude fat was extracted in a Soxhlet extractor using petroleum ether (B.P 40-60°C) as solvent. When the extraction was completed, the solvent was evaporated off by placing in an oven at the temperature of 150°C. The weight of the extract left was taken as the weight of crude fat in the sample.

$$\frac{\%\ fat\ weight\ of\ extract}{Weight\ of\ sample\ used} \times 100 - - - - - - - - - - - - - (1)$$

Determination of Crudes Protein

Five grams of the sample was diluted with 30 mL of concentrated sulphuric acid using 2g of sulphate and 16g of sodium sulphate salt until clear green solution was observed. This was further dissolved in a 100 mL of distilled water placed in a volumetric flask. 12.5 mL of the digest was measured into a semi-micro kjedal markham distillation apparatus and treated with 12.5 mL of 1.25% of sodium hydroxide solution. This was digested with 10 mL of boric acid

and double indicator. The distillate was then titrated with 0.1% HCl solution until a light pink colour was observed. A blank titration was carried out in triplicate and the percentage of nitrogen was obtained by appropriate calculation.

$$\%\ crude\ protein = ATV - TOTB \text{ x } 0.1\text{N HCL x } 0.014 \text{ x CF weight of the sample x } 100 \quad ---(2)$$

Where; ATV = Actual titre value;

$TOTB$ = Titre of the blank

CF = Conversion factor

HCL = Hydrogen chloride (mL)

Phytochemical Analysis of the Sample

Phytochemical screening procedures carried out were adapted from previous work on plant analysis. This analysis determined the biologically active non-nutritive compound which contributes to the flavour, colour and characteristics of plant parts. The extracts were used for the following plant constituents: cardiac glycosides, alkaloids, saponins, tannins, flavonoids, polyphenols, using the methods described below;

Test for Alkaloids

A freshly prepared Mayer's reagent was added to 2mL of the extracts in a 10mL test tube. A milky or green colour was an indication of a positive test. To the second test tube, 2mL of the extract was treated with 10mL 1% HCL for 10 minutes in a water bath, the presence of turbidity or precipitate indicated the presence of alkaloid.

Test for Saponins

Two mL of the aqueous extract was shaken vigorously with distilled water in a test tube. Frothing which persisted on warming was taken as evidence for the presence of saponin.

Test for Flavonoids

This was done using Shinoda's test. 2mL of the aqueous extract was dissolved in concentrated hydrochloric acid. Few pieces of magnesium metal were added to 2mL of the extract. The formation of orange, red, crimson or magenta was taken as a positive test for the presence of flavonoid.

Test for Polyphenols

To 2mL of the extract, 10mls of distilled water was added and heated for 30 minutes, 1mL of 1% of $FeCl_2$ was added to the mixture by the addition of 1mL of 1% potassium ferrocyanide to the solution. This was filtered and the formation of a blue-green colour indicated the presence of polyphenol.

Test for Anthranoids

2mL of the aqueous extract was heated with 5mL of potassium hydroxide (KOH). The solution was filtered with a glass wool. The filtrate was treated with 7% acetic acid, the result solution was mixed with toluene. The upper layer was transferred to another test tube and potassium added. The presence of a red colour indicated the presence of anthranoid.

Test for Anthraquinone

10mL of benzene was added to 2mL of t plant extract, the mixture was shaken and filtered. 5mL of 7% ammonia solution was added to the filtrate and shaken. The presence of a pink, red or violet colour in ammonical (lower) phase indicated the presence of anthraquinone.

Test for Cardiac Glycoside

Two mL of the extract was dissolved in 2mL chloroform, concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicated the presence of aglycone of the cardiac glycoside.

Test for Tannin

Two mL of the aqueous extract was stirred with 10mL of distilled water and filtered. 1% ferric chloride was added to the filtrate, blue-black precipitate indicates the presence of condensed tannins.

Test for Reducing Sugar

To 2mL of the aqueous extract in a test tube, 5mL of Fehling's solution was added to it and heated in a water bath. The formation of a brick red precipitate was taken as an evidence for the presence of a reducing sugar.

Results and Discussion

Table 1. The results of the proximate composition of *Anrhocleista vogelii*

Parameters	Results (%)
Ash content	12.00±0.05
Moisture content	42.40±0.14
Fibre content	14.00±0.22
Fatty acid content	4.40±0.11
Protein content	17.30±0.11
Carbohydrate content	52.50±0.60

Table 2. Proximate composition of grains of fagopyrum and Hordeum species

Data is represented as mean ± SD (n=5). Values showing different letters indicate differences among species according to the Tukeys's multiple comparison test.

Note: *Adapted from Nutritional potential and phytochemical screening of traditional buckwheat and barley crops of the Trans-Himalaya region.by Notup, T (2024), pp1071–1083, Vegetos.*

Plant species	Crude fibre (%)	Moisture (%)	Volatile matter (%)	Ash content (mg g-1)
F.*tataricum*	0.977±0.0004b	8.91±0.141a	80.31±0.11a	1.75±0.01b
F. *esculentum*	1.112±0.0018c	10.75±0.919a	79.01±0.51a	1.50±0.03a
F. *sp wild variety*	0.901±0.0021a	9.35±0.212a	80.52±0.12a	3.13±0.02c
H. *himalayens*	0.925±0.0004a	7.45±0.071b	82.23±0.04a	2.35±0.10a
H. *vulgare*	0.930±0.0003b	7.15±0.112a	83.47±0.10b	3.36±0.35

Table 3. Pharmacopoeia standards of the leaves and stem bark of Anthocleista *vogelii*

Note: *Adapted from Phytochemical and pharmacognostic studies of the leaf and stem-bark of Anthocleista vogelii (Planch) by Jegede I. (2011), pp. 6136-6139, Journal of Medicinal Plants Research.*

Parameters	Leaf value (%)	Stem bark value (%)
Moisture content	9.3	8.9
Total ash	5.5	2.5
Acid insoluble ash	0.5	1.0
Water soluble ash	4.0	1.5
Alcohol extractive	18.8	11.1
Water extractive	19.3	11.2

Table 4. *Results of Phytochemical Composition of Anthocleitsa vogelii*

Parameter	Water Composition	Petroleum ether composition
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	++
Polyphenols	++	+
Phlobatanins	+	+
Anthranoids	-	-
Anthraquinones	-	-
Cardiac glycosides	+	+
Tannins	+	+
Reducing Sugar	++	+

++ = Positive in high quantity
\+ = Positive with moderate quantity
\- = Negative (absence)

Discussion of Results of Proximate analysis of Anthocleista *vogelii*

The ash content of leaves of *Anthocleista vogelii* as analysed was 12.00±0.05 from table 1. On the basis of this study, it showed that the ash content of the leaves is low. Ash content represents the index of mineral element present in a sample, detecting the foreign organic matter of that sample, and its amount is useful in assessing a sample and gives an idea of the minerals available in a sample. The low value of ash content may imply that leaves of *vogelii* has lower mineral content. A similar analysis from Table 3. by Jegede *et al* on the ash content of leaves and stem back of *vogelii*, using water and ethanol extract, indicated that the leaves had a higher ash content in both water and ethanol compared to the stem back of the plant [Micheal and David, 2002; Jegede et al., 2011).

Another important parameter as analyzed in this study was moisture content, having the value 42.40±0.14 as seen in table 1. This indicated that the moisture content of the leaves extract is high. Similarly, Apiamu *et al.* (2023), in his study concluded that the moisture content in C. nitida was drastically higher than that of G. kola. species. The high content of moisture of the leaves of *A. vogelii* revealed that it has a low shelf life, as storage over a long period of time may show contamination. Moisture content of food is usually the measure of stability, shelf life and susceptibility of microbial contamination (Jegede et al., 2011).

Further analysis showed that the fibre content of *A. vogelii* leaves was 14.00±0.22 as seen in Table 1. This result implies that the fibre content is low. Reason for the low value may be attributed to environmental factors. Previous work done by Younas et al. (2024), also indicated a relatively low fibre content on both pea and chickpea flour. Study by Notup et al. (2024) as seen on table 2. recorded that F. esculentum had the highest fibre content compared to the other species of the plant. Moreso, fibre has the potential of lowering the body's cholesterol and also slows down cardiovascular disease (Gwarzo et al., 2014).

The fatty acid content from the analysis of A. *vogelii* was 4.40±0.11 as seen in table 1. This indicated that the fatty acid content is low. As highlighted in (Younas et al., 2024), the fat content for chickpea flour was higher as compared to pea flour. Plants generally use fatty acids to synthesize acyl lipids for many different cellular, physiological, and defensive roles (Rebecca et al., 2020).

The extract of A. *vogelii* as analysed showed that the protein content is 17.30±0.11, from table 1. This indicates that the protein content of this extract is low. As discussed in Notup et al.

(2024) from table 3, the total protein content as observed was significantly different among the three Fagopyrum sp., maximum in F. t*ataricum* (12.88%), followed by F. *esculentum* (10.77%), and F. sp. wild variety (8.16%) himalayens had significantly more protein (15.34%) than H. *vulgare* (10.22%). Fig. 2 shows the chart presentation of the results from the proximate composition of leaves extract of *vogelii.* 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 (Trease and Evans 2021).

In addition, the extract recorded that the carbohydrate content was 52.50±0.60 as seen in table 1 and Fig 2. It therefore indicated that the content of carbohydrate is high. The high value may be due to plants energy storage ability, which can serve as a great potential in drug formation. Highlight by Bakhtiar *et al*. (2024) shows that the leaf of Bushehr ecotype (6.15±0.25%) and the seed of Amol ecotype (50.5±1.90%) had the highest content of carbohydrate. The inhibition of carbohydrates hydrolyzing enzymes is seen as been beneficial in controlling hyperglycemia associated with type 2 diabetes mellitus. (Micheal and David 2022).

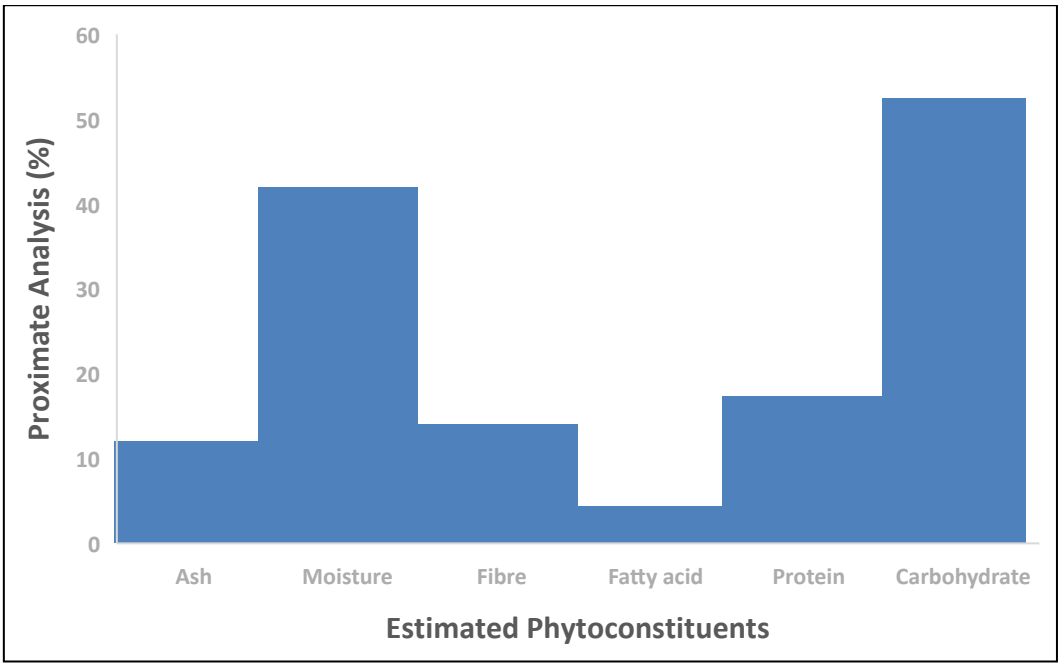

Figure 2. A Chart of Proximate Composition of leaves extract of *Anthocleista vogelii*

Discussion of the Results of Phytochemical Analysis of *Anthocleista vogelii* leaves extract

The identification of different phytoconstituent of plants is an indication of the various active metabolites present in it, making it a great potential for drug production. Phytochemicals are

bioactive chemicals present in plants, contributing significantly to human health through their anti-oxidant and disease preventive properties. The two solvents (water and petroleum ether) used in the extraction of leaves of *A.vogelii* were analyzed, and as seen in table 4, in them were the presence of saponins, alkaloids, flavonoids, polyphenols, phlobatanins, cardiac glycosides, tannins and reducing sugars. Anthraquinones and anthranoids were absent from the extract. The presence of saponins in *A*. *vogelii* may indicate the plant's potential of enhancing nutrients absorption, also highlighting its application as an additive or flavoring agent in food and pharmaceutical industries. In a study of other plant whose phytochemicals were checked, saponins present in acetone extract of *S. maritima* (SMAE) indicated an antidiabetic activity when compared to a standard acarbose. Saponins in leaves and stem indicates the plants potential for stopping bleeding and treatment of wounds.Saponins are known to liquefy bronchial secretion, decongestion of bronchi and ease coughing (Sampath et al., 2024).

The presence of Alkaloids in the leaves extract may show for its excellent analgesic ability, and as a potential respiratory and cardiac stimulant when applied in drug production. Alkaloids-containing plants are used for muscle relaxation, pain relief and malaria treatment. In comparison to this study, a previous analysis on other plant such as buckwheat and barley crops of Trans-himalaya region indicated the absence of alkaloids in *H. vulgare*, and its presence in *H. Himalaya*, which may be due to slight environmental factor (Kurek 2019).

Flavonoids was abundantly present in petroleum ether than water, the reverse was recorded for polyphenols. This may be due to the polarity properties of the solvents used. Because they are secondary metabolites, Polyphenols and Flavonoids are in abundance in plants, fruits and seeds. They also perform functions like regulating cell growth, improving pollination by attracting insect, and reducing plants biotic and abiotic stress. Similarly, assessment of the impact of flavonoids on the antioxidant activity of another plant such as peas and chickpeas on previous research, showed that chickpeas had the highest results. The presence of flavonoids in this study confirms the anticancer, antiviral and antiangiogenic potential of the plant as used in pharmaceutical industry. Secondary metabolites such as Anthranoids and Anthraquinone was found as non-essential part of the plant, with a non-significant contribution to the plant potential for the purpose of drug production (Luna et al., 2020 &Younas et al., 2024).

Different methods have been harnessed for the verification and validation of the antidiabetic, antidiuretic, anticancer and drug delivery potential of the phytochemicals present in plants. According to report from Apiamu *et al*. (2019) on the identification of the potential antioxidant

role of *A.vogelii* plant in ameliorating Cd-induced oxidative stress in the serum of male Wistar rats, the results was achieved through the application of response method methodology (RSM). In addition, a study done on a different plant on the chemical screening of halophyte *Suaeda maritima* (L.) Dumort by Sampath *et al*. (2024) discussed on the different methods employed to ascertain the phytochemicals and its binding capacity to a ligand. This includes the extraction methods, phytochemical profiling, invitro approach, stigmasterol derivatives, and the molecular docking approach, which was used to indicate delivery mode, should the plant be used as a component for drug production.

Conclusion

The phytochemical analysis of leaves extracts of *Anthocleista vogelii* was analyzed. From this study, it could be concluded that the claims from previous research by Jegede *et al.* (2011*)* which revealed the phytoconstituent and pharmacognostic properties of *vogelii* could be seen as valid. The phytoconstituents observed to be in abundance in this research were tannins, reducing sugar, cardiac glycosides, phlobatanins, polyphenols, flavonoids, saponin and alkaloids. The study was a step further to ascertain that *Anthocleista vogelii* can be used for nutritional and medicinal purposes, also, important basis for further screening into individual isolation and characterization of the phytoconstituents of the plant for drug development was provided. Furthermore, the screening was solely analyzed using qualitative method of analysis. It is recommended that subsequent works and more emphasis should be placed on the quantitative approach, different methods, which accounts for the binding mode and mechanism, assessment of free radical scavenging activity of the plant sample.

Recommendation

The analysis on this work has been narrowed only to the proximate and phytochemical constituent of the plant leaves. Further work should be done on the roots and stem extract to obtain the mineral and vitamin content.

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Authors Contribution

Emem Ikpeme Bassey: Proposed the idea, sample collection, methodology, data and sample analysis, writing of original draft, review and editing. Effiong Bassey Bassey: conceptualization, methodology, review and editing of the work. Ugbor Ofunna: resources,

methodology, review and editing. Pheobe Ikechwukwu: resources, methodology, review and editing. All authors read and approved the final manuscript.

Conflicting Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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