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Phytochemical, Proximate, and Mineral Analysis of Anchomanes Difformis: Unveiling its Nutritional and Medicinal Potentials

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Abstract

This comprehensive study investigates the phytochemical, proximate and mineral compositions of *Anchomanes difformis*, a plant species with potential nutritional and medicinal applications. Fresh tubers of *Anchomanes difformis* plant were obtained from a natural habitat, washed, chopped and air dried for two weeks. The size was reduced with mortar and pestle into fine powder. The proximate compositions were determined according to AOAC method. Our results revealed a rich presence of bioactive compounds, including flavonoids, phenolic acids, tannin, and saponin, which may contribute to its antioxidant and therapeutic properties.

The proximate analysis highlights significant amounts of carbohydrates, proteins, and dietary fibres, indicating its potential as nutritious food source. Furthermore, the mineral composition analysis showed the presence of essential minerals like calcium, potassium, magnesium, iron, zinc, phosphorous, and manganese. These findings suggest that *Anchomanes difformis* may be a valuable resource for the development of natural remedies and function foods.

Keywords

Phytochemical; Proximate; Mineral; *Anchomanes difformis*; Nutritional; Medicinal.

Introduction

Since the dawn of time, people have used medicinal plants as a source of healing from a variety of maladies. Many modern medications have their roots in medicinal plants, which have been used for primary healthcare in numerous civilizations around the world [1]. Secondary metabolites found in plant extract are thought to be responsible for the therapeutic efficacy of plants. Leaf, bark, root, and even whole plant extracts are utilized in treatment, depending on the ailment. The 21,000 plants that are utilized as medicines worldwide are recorded by the WHO. 800 of these plants have reportedly been shown to have anti-diabetic properties [2]. Both rural and urban residents of Nigeria frequently use herbal medicine. The main justification is given as their alleged price, accessibility, and efficacy in comparison to traditional medications. Numerous medicinal plants have been increasingly well-liked over time for treating both human and animal illnesses [3,4].

One of those native medicinal plants, *Anchomanes difformis*, has been used by traditional herbalists to cure a variety of ailments and disorders. It is a member of the ARACEAE family. In English, it is referred to as woodland anchomanes. It is a prickly-stemmed herbaceous plant [5,6]. The genus *Anchomanes difformis* a large herbaceous plant is a member of the Araceae family. It is a significant medicinal plant that grows in tropical regions throughout Africa, but it is particularly prevalent in West African forests [7]. It is a substantial herbaceous plant. The plant root is also referred to as Chakara (Hausa) in Northern Nigeria, Oje in Eastern Nigeria, and Ishu agan (Yoruba) in South Western Nigeria. Locals frequently refer to it as bush cocoyam [8]. A root decoction is used to cure diabetes mellitus, according to ethno-medicinal information from herbal practitioners in Zaria, Nigeria. The root or tuber is used as a diuretic and to treat diabetes mellitus in the Republic of Benin.

There is currently no scientific evidence to back up this assertion, though. Additionally, it has been stated that the powdered root of *Anchomanes difformis* combined with palm oil is used as a treatment for respiratory illnesses in children in Zaire (DRC) and South Western Nigeria, respectively. The herb is also said to possess anti-microbial qualities [9,10].

Materials and Methods

Plant collection

Four kilogrammes of fresh tubers of *Anchomanes difformis* plant were collected from a natural habitat at Okpella, Etsako East LGA of Edo State and authenticated in the Department of Botany, Ambrose Alli University, Ekpoma, Edo State.

Preparation of extraction

Extraction was done at the University of Lagos (UNILAG), Department of Pharmacology, College of Medicine, Lagos state. The fresh tubers of *Anchomanes difformis* plant were obtained, washed, chopped and air dried for two weeks. The size was reduced with mortar and pestle into fine powder. About 2.0 kg of the powder was extracted with distilled ethanol (7000ml) by soaking for three days with periodic stirring. The samples were filtered with sintered glass funnel to eliminate particles. The filtrates collected were then concentrated using a rotary evaporator to give brownish viscous pastes which were then weighed directly. The aqueous extract was treated the same way, although distilled water (7000ml) was used in place of ethanol. The brownish pastes (both extracts) were kept in the freezer at -21°C prior to use. The yields were 23.6gm and 21.3gm for ethanol and aqueous extracts respectively.

Proximate analysis of the plant powder (samples)

- **Moisture content determination:** An evaporating crucible was washed and dried in the oven for 30 minutes at a temperature of 105°C. It was then cooled in the desiccator for 30 minutes. The empty crucible was weighed on a balance. Exactly 2 grams of the sample was added to the crucible and its weight was recorded. The crucible and sample were then placed in the oven for one hour maintaining the temperature of 105°C. The crucible and content were weighed at 30 minutes interval, until a constant weight was attained. The result was expressed as a percentage of the weight of moisture loss over the original weight of the sample [11].
- **Ash content determination:** The ash content of the samples was determined according to [11] method. A porcelain crucible was washed and dried in a hot air oven for 30 minutes at a temperature of 105°C. After heating, it was then cooled in a desiccator for 30 minutes. The crucible was weighed using analytical balance. Exactly 2 grams of sample was weighed into the porcelain crucible and its weight was recorded. It was then charred using a Bunsen flame to de-carbonize it after which it was transferred to a muffle furnace maintaining a temperature of 500°C for one hour, for complete de-carbonization to obtain white ash. The crucible and ash were cooled and the weight was recorded. The result was expressed as percentage of the weight of ash over the original weight of sample.
- **Fat content determination:** The fat content of the tuber of *Anchomanes difformis* was determined according to [11] method. A 250 ml round bottom flask was washed and dried in oven for one hour at 105°C. It was then cooled in the desiccator and weighed. The thimbles were washed with the solvent used for extraction, dried in the oven and cooled in the desiccator. Exactly 2 grams of moisture free samples was weighed, wrapped, with filter paper and placed in the thimble which was then plugged with defatted (soaked in ether) cotton wool with 200 ml of petroleum spirit in a round bottom flask, and the extraction was carried out for six hours. After extraction, the solvent was distilled off and the weight of residual fat was noted, and this was calculated as a percentage of the sample used.
- **Protein determination:** Protein content of the samples was determined using micro-Kjeldahl method [12]. Exactly 2 grams of sample and 8 grams of catalyst mixture were weighed in a filter paper and placed in a digestion flask. Exactly 20 ml of conc. H₂SO₄ solution was poured into the flask and the flask was heated in an inclined position. Heating continued with occasional swirling until solution turned colorless, the resulting solution was then diluted to 100 ml in a volumetric

flask with distilled water. The digested sample was distilled, using 10 ml solution of 50 % NaOH and the distillate in the beaker containing 10 ml of 1% boric acid with screened methyl red as indicator. This was titrated with 0.05M H₂SO₄ solution. This result was expressed as;

$$\% \text{ Nitrogen} = \frac{\text{Sample titre} - \text{Blank titre} \times 0.0014 \times 100}{\text{Sample weight} \times 1}$$

Where 0.0014 is the gram of nitrogen in 0.05M H₂SO₄ and 10 ml is the dilution factor.

Crude protein (%) = % Nitrogen x 6.15.

- **Crude fibre determination:** This was carried out according to [11] method. Exactly 3 grams of the defatted sample was placed in the round bottom flask containing 200 ml of 0.13M H₂SO₄ and was refluxed for 30 minutes. The solution was filtered through a linen cloth on a funnel and washed with boiling water. The residue was transferred to a beaker and boiled for 30 minutes with 200 ml 0.313M NaOH. The residue was washed with 50 ml of acetone to remove any further trace of oil, and placed in a crucible. This was then dried in the oven at 105°C for one hour and then incinerated in the Muffle Furnace. The result was expressed as;

$$\% \text{ Crude fibre} = \frac{\text{Weight of crude fibre} \times 100}{\text{Weight of Sample} \times 1}$$

- **Carbohydrate content determination:** The carbohydrate content is usually calculated as total carbohydrate by difference. That is the percentage of water, protein, crude fibre, fat and ash subtracted from 100%.

Carbohydrate content = 100% - (% moisture + %protein + %ash + %fat + %fibre)

Qualitative phytochemical screening of the root extracts of *A. difformis*

After the extraction with the solvents, the extracts derived from *A. difformis* were analysed for the presence of phytochemicals such as flavonoids, alkaloids, saponins, tannins, phlobatannins, anthraquinones, and cardiac glycosides. Harborne methods [13,14,15]. Were used to assess for alkaloids, flavonoids, cardiac glycosides and phlobatannins. For the presence of saponins, the method used by [16,17], was employed while tannins and anthraquinones were also screened as done by [18,19].

Test for Alkaloids: About 0.2 gram of the extract was warmed with 2% of H₂SO₄ for two minutes, it was then filtered and few drops of Dragendoff's reagent were added. The appearance of orange red precipitate indicated the presence of alkaloids [13,14].

- **Test for Anthroquinones:** One millilitre of the extract was shaken with 10 ml of benzene; the mixture was filtered and 5 ml of 10% (v/v) ammonia were added, then shaken and observed. The formation of a pinkish solution indicated a positive test [18,19].
- **Test for Cardiac Glycosides:** Added 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully. The formation of blue colour in acetic acid layer confirmed the test [13,14].

- **Test for Flavonoids:** Exactly 1ml of the plant powder was mixed with 2 ml of 10% lead acetate, a brownish precipitate indicated a positive test for the phenolic flavonoids. For the other flavonoids, 1 ml of the plant extract were mixed with 2 ml of dilute NaOH, a golden yellow colour indicated the presence of flavonoids [13,14].
- **Test for Saponins:** Exactly 1 ml of the plant filtrate was diluted with 2 ml of distilled water; the mixture was vigorously shaken, and left to stand for 10 minutes. The development of foam on the surface of the mixture lasting for more than 10 minutes, indicated the presence of saponins [16,17].
- **Test for Tannins:** Exactly 1 gram of the plant extract was mixed with 2 ml of FeCl₃, a dark green colour indicated positive test for tannins [18,19].

Quantitative analysis of the phytochemicals of the root extracts of *A. difformis*

- **Determination of Total Phenols:** Total phenolic content of the extracts was determined according to the method reported by [20].
- **Procedure:** The crude extract was heated with 50 mL of ether to extract the phenolic components for 15 min. A volume of 5 ml extract was pipetted into a 50 ml flask with additional 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol. The sample was left to react for 30 min for colour development. Result was read on the spectrophotometer at 505 nm.
- **Calculation**

$$\text{Phenol (mg/100)} = \frac{\text{Absorbance of sample} \times 100}{\text{Weight of extract / sample}}$$

- **Determination of Tannins:** Total tannin content of the extracts was determined according to the method reported by [21].
- **Procedure:** Five hundred milligrams of extract was poured into 50 ml of distilled water and shaken for 1 h in a mechanical shaker and strained into a 50 ml volumetric flask. Exactly 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 mol/l FeCl₃ in 0.1 mol/l HCl and 0.008 mol/l potassium ferrocyanide. Absorbance was read on the spectrophotometer at 120 nm within 10 min.
- **Calculation**

$$\text{Tannin as Tannic acid \%} = \frac{\text{Mg of tannic acid} \times \text{Dilution} \times 100}{\text{Vol. of sample taken} \times \text{wt. of sample taken} \times 1000}$$

- **Determination of Saponins:** Total saponins content of the extracts was determined according to the method reported by [22,23].
- **Procedure:** Dried tubers of *A. difformis* were ground and 20 g of each was put into a conical flask and 100 ml of 20% aqueous ethanol was added. Mixture was heated over a hot water bath for 4 h with continuous stirring at about 55 °C and then filtered. The residue was re-extracted with 200 ml 20% ethanol. Both filtrates were concentrated over a water bath at 90 °C. The concentrate was poured into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken. The aqueous layer was recuperated while the ether layer discarded. A total of 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium

chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage.

$$\% \text{Saponin} = \frac{\text{Weight of saponin} \times 100}{\text{Weight of sample}}$$

- **Determination of Flavonoids:** Total flavonoid content of the extracts was determined according to the method reported by [25].
- **Procedure:** The plant sample (10 g) was soaked in 100 mL of water at room temperature. The mixture was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was evaporated to dryness over a water bath and weighed to a constant weight in a crucible.

The percentage of flavonoid was calculated as:

$$\% \text{Flavonoid} = \frac{\text{Weight of flavonoid} \times 100}{\text{Weight of sample}}$$

- **Determination of Oxalate:** Oxalate level was determined according to the method described by [25,26].
- **Procedure:** Exactly 1 gram of the sample was weighed and placed into a 250ml conical flask. About 75ml of 3N H₂SO₄ was added. The mixture was filtered using Whatman No 1 filter paper. About 25ml of the filtrate was pipetted into a beaker and 2 drops of methyl red indicator was added. It was then heated to boil. While still hot the solution was titrated against a 0.05M KMnO₄ solution until a faint pink colour that persisted for at least 30 seconds was obtained. The oxalate content was calculated by taking 1ml of 0.05M KMnO₄ as an equivalent to 2.2mg oxalate.
- **Calculation:**

$$\text{Oxalate (mg/100g)} = \frac{\text{Titre value} \times 2.2 \times \text{DF}}{\text{Weight of sample}}$$

Where 2.2mg = Mass equivalent oxalate value of 1ml of 0.05M KMnO₄ solution.

DF= Dilution Factor. (That is, total volume of sample divided by volume of portion used for titration).

- **Determination of Phytate:** Phytate content of the sample was quantified according to the method reported by [27].
- **Procedure:** Exactly 2 grams of the sample was weighed and placed into a 250ml conical flask. 100ml of 2% concentrated HCl was added into the flask. The mixture was allowed to soak for 3hours. It was then filtered. About 50ml filtrate was pipetted into a 250ml beaker. 107ml of distilled water was added to the solution in order to improve its acidity. About 10ml of 0.3% ammonium thiocyanate solution was also added as an indicator. This solution was titrated against a standard iron iii chloride (FeCl₃) solution which contained 0.00195g iron/ml until a brownish yellow colour appeared, persisting for 5minutes. Calculation of the phytate content was made as shown below;

$$\text{Phytic acid g/kg} = \frac{0.00195 \times \text{volume of FeCl}_3 \text{ consumed} \times \text{DF}}{\text{Sample weight}}$$

DF: Total volume of extraction solvent added/volume of aliquot taken for the titration.

- **Determination of Trypsin Inhibitor Content:** Trypsin inhibitor content of the sample was quantified following the method of [28,29].
- **Procedure:** Exactly 1 gram of sample was extracted with 50 ml of 0.01N NaOH (pH of 8.4 - 10.0) for 3 hours, while stirring intermittently. About 2 ml of diluted extract was then dispensed into test tubes to which 2 ml of cold trypsin solution (4 mg in 200 ml of 0.001M HCl) was added, and the tubes were placed in water bath at 37°C. Afterwards 5 ml of Benzoyl-DL-arginine-P-nitroanilide hydrochloride, (BAPNA), (40 mg dissolved in 1 ml of dimethyl sulfoxide and diluted to 100 ml with tris-buffer - 0.05M, pH 8.2; pre-warmed to 37°C) was added as substrate to each tube. After 10 minutes the reaction was terminated by adding 1 ml of 30% acetic acid, and the content of each tube was thoroughly mixed. Thereafter, the content of each tube was filtered using Whatman filter paper No. 3, and the absorbance of the filtrate was measured at 410 nm against a reagent blank and standard. The reference was prepared in the same way as the sample assay except that 2 ml of distilled water was added in the place of the extract.

Results

Table 1 showed the result of the proximate compositions of *A. difformis* tuber. Ash content was 11.19%, Moisture content 9.6%, Crude protein 12.87%, Fat 6.46%. Crude fibre 11.4% and Carbohydrate 47.12%. The result of the phytochemical screening of the aqueous and ethanol extracts of *A. difformis* in **table 2** showed that alkaloids and terpenoid are absent in both aqueous and ethanol extracts. Both aqueous and ethanol extracts contain Phenol, Tannins, Flavonoid, Saponin, Glycoside, Oxalate, Phytate and Trypsin inhibitors. In **table 3**, the quantitative analysis of the secondary metabolites (mg/kg) in this study showed that the aqueous extract, has higher number of tannins and flavonoid (4529.41 ± 56.12 and 1914.19 ± 22.70), while phenol and trypsin inhibitor (1043.14 ± 25.00 and 2215.10 ± 31.00) respectively are more in the ethanol extracts. Table 4.4 revealed that the mineral compositions (mg/100g) of tuber of *A. difformis* are Sodium 20.56 ± 0.21 , Potassium 22.34 ± 0.15 , Calcium 21.89 ± 0.50 , Magnesium 22.10 ± 0.20 , Zinc 20.89 ± 0.35 , Iron 5.92 ± 0.05 , Copper 0.04 ± 0.00 , Manganese 6.34 ± 0.23 and Phosphorous 23.91 ± 2.01 .

Parameters (%)	Value
Ash content	11.9 ± 0.02
Moisture content	9.60 ± 0.11
Crude proteins	12.82 ± 0.10
Fat	6.46 ± 0.02
Crude fibre	11.4 ± 0.11
Carbohydrate	47.12 ± 0.00

Table 1: Quantitative analysis of the proximate compositions of the tubers of *Anchomanes difformis*. Result represented as Mean $\pm$ S.D of triplicate analysis.

Parameters	Aqueous extract	Ethanol extract
Phenol	+	+
Tannin	+	+
Flavonoids	+	+
Saponin	+	+
Glycosides	+	+
Oxalate	+	+
Phylate	+	+
Alkaloids	-	-
Terpenoid	-	-
Trypsin inhibitor	+	+

Table 2: Qualitative analysis of the phytochemicals of the aqueous and ethanol extracts of the tubers of *Anchomanes difformis*.

Key: Positive = (+), Negative = (-)

Parameters (mg/kg)	Aqueous extract	Ethanol extract
Tannin	4529.41±56.12	3609.50 ±34.05
Phenol	890.20 ± 21.30	1043.14±25.00
Oxalate	787.30 ±12.07	751.50 ±15.01
Phytate	7.80 ± 0.12	9.27 ±0.50
Trypsin inhibitor	2117.82 ±22.20	2215.10 ±31.00
Glycosides	28.94 ±0.35	26.25 ±0.62
Flavonoids	1914.19± 22.70	1589.11±16.00
Saponin	0.842 ± 0.00	0.677 ± 0.01
Terpenoids	Absent	Absent
Alkaloid	absent	Absent

Table 3: Quantitative analysis of the phytochemicals of aqueous and ethanol extract of the tubers of *Anchomanes difformis*.

Result represented as Mean ±S.D of duplicate analysis.

Parameters	Value
Sodium (Na ⁺)	20.56 ± 0.21
Potassium (K ⁺)	22.34 ±0.15
Calcium (Ca ⁺⁺)	21.89 ± 0.50
Magnesium (Mg ⁺)	22.10 ± 0.20
Zinc (Zn)	20.89 ±0.35
Iron (Fe ⁺)	5.92 ± 0.05
Copper (Cu ⁺⁺)	0.04 ± 0.00
Manganese (Mn)	6.34 ± 0.23
Phosphorous (P)	25.91 ± 2.01

Table 4: Quantitative analysis of the mineral present in the tubers of *Anchomanes difformis* (mg/100g). Results represented as mean ±S.D of triplicate analysis.

Discussion

The present study showed that *A. difformis* tuber is composed of Ash, Moisture, Crude Protein, Fat, crude fibre and carbohydrates. The phytochemistry of the extracts revealed the presence of phenol, tannins, flavonoid, saponin, glycoside, oxalate, phytate, and trypsin inhibitors in both aqueous and ethanol extracts while alkaloids and terpenoid are absent. Quantitatively, aqueous extract has higher amounts of tannins and flavonoids, while phenol and trypsin inhibitors are more in the ethanol extract. The greater yield of tannins and flavonoids in aqueous extract could potentially be attributed to its high polarity, enabling it to aggregate a wide range of plant constituents compared to other extracts [30]. This finding agrees with the work of [31], who noted in their study that the extraction of the secondary metabolites may have been greatly influenced by the polarity level.

This study further revealed that *A. difformis* tuber is a very rich source of potassium, sodium, calcium, magnesium, iron and manganese. These mineral elements are very important in human nutrition. Calcium, Potassium, and Magnesium are required for repair of worn cells, strong bone and teeth in humans, building of red blood cells and for body mechanisms. The biological roles for K⁺ and Ca²⁺ are important for disease prevention and control, and may therefore, contribute to some of the traditional medicinal influences of the plant. Magnesium is an important modulator for cell functions and plays vital role in the control of diabetes mellitus. These minerals play a significant role in human nutrition. Humans need calcium, potassium, and magnesium for healthy bones, teeth, and to repair damaged cells. Red blood cell production and for bodily functions [32,33]. K⁺ and Ca²⁺ have crucial biological functions in the

prevention and management of disease, which may explain some of the plant's traditional medicinal effects. Iron, zinc, and magnesium are crucial for the metabolism of enzymes. According to [34], magnesium is a key regulator of cellular processes and is essential for the management of diabetes mellitus. The presence of these minerals indicates that this plant has a great deal of potential for producing the changeable secondary metabolites and mineral supplies that could accelerate the healing process for illnesses.

Magnesium is known to be present in a number of enzymes involved in the processes for oxidizing glucose and also contributes to the mechanism of transporting glucose. According to [35], zinc (Zn) is necessary for preserving the structural integrity of insulin; a shortage results in decreased pancreatic insulin content, poor glucose tolerance, and insulin breakdown. According to [36], copper (Cu) is recognized to have an insulin-like effect and induce lipogenesis. Manganese (Mn) can reduce glucose intolerance, while iron (Fe) can affect insulin action and glucose metabolism [37]. According to [39,40], phosphorus (P) has the ability to maintain normal blood glucose levels in an intact organism and potassium is a well-established insulin secretagogue. It is possible therefore, that the aforementioned nutrients present in the root extract of *A. difformis* might have contributed either directly or indirectly in maintaining glucose levels in the diabetic rats.

Conclusion

This study shows that *Anchomanes difformis* tuber extracts contain phytochemicals like flavonoids, saponins, and phenols, and as well as minerals like magnesium, iron, zinc, potassium, and manganese which have been shown in previous studies to have blood glucose-lowering effect in the experimental animals. The low level of ash content observed in this study shows that the plant is relatively non-toxic.

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Disclosure of conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Availability of data and materials

The authors declare consent for all available data present in this study.

Authors' contribution

The entire study procedure was conducted with the involvement of all writers.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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