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**PYHYTOCHEMICAL ANALYSIS AND PROXIMATE SCREENING OF
(*ACANTUS MONTANUS*, *MORINGA OLEIFERA* AND *VERNONIA
AMYGDALINA*)**

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DOI: <https://doi.org/10.51193/IJAER.2024.10106>

Received: 27 Jan. 2024 / Accepted: 06 Feb. 2024 / Published: 22 Feb. 2024

ABSTRACT

Plants generally contain phytochemical compounds which are both nutritional and non-nutritional and may be used and applied in nutritional and pharmaceutical industries for production of drugs and beverages. This study was aimed to evaluate phytochemical constituents and proximate screening of *Acantus montanus*, *Moringa oleifera* and *Vernonia amygdalina* using standard laboratory approaches. The result of the Phytochemical screening showed that of *Moringa oleifera* contains Alkaloids, Flavonoids, Saponins, Phytate, Cyanogenic glycoside is moderately present. *Vernonia amygdalina* contains alkaloids, flavonoids, cyanogenic glycosides, phytate and saponins in different proportions. While *Acantus montanus* contains Alkaloids, Flavonoids, Saponins, Phytate and cyanogenic glycoside.

The proximate composition of the leaf extracts shows that *Acantus montanus*, *Moringa oleifera* and *Vernonia amygdalina* has carbohydrate content of 30.30%, 25.99% and 25.10%. Protein content of 44.80%, 38.10%, and 42.30%, Fat content 5.40%, 6.70% and 3.45%, Fibre content of 10.00%, 15.2% and 16.00%, Ash content of 7.40%, 5.10% and 9.95%, moisture content of 2.1%, 8.90% and 3.2%. The presence of the phytochemicals in the leaf extracts has authenticated its usefulness in ethno medicine and its potentials use in drug formulation as well as use for food beverage production.

Keywords: *Vernonia amygdalina*, *Acantus montanus*, *Moringa oleifera* phytochemicals, Proximate analysis.

INTRODUCTION

Over the years due to the increase in world population and the acceleration in the requirement of life and health of individuals, there is reason to meet up with human challenges hence the depletion of natural resources. Herbal medicinal practice makes use of phytochemicals found in plants; therefore, understanding and characterizing phytochemicals found in medicinal plants is critical for effective consumption and conservation (Alabi and Adeyemi, 2021).

Scientist has explored the use of Medicinal plants to fulfill the necessity of both man and livestock in an economical way without degrading natural resources. The use of plant derived natural compounds as part of herbal preparations for alternate source of medicament continues to play major roles in chemotherapy especially in third world countries (Joy *et al.*, 1998).

Polyherbal therapy which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects (Ebong *et al.*, 2008).

Vernonia amygdalina is an angiosperm belonging to the order, Asterales (Toyang and Verpoorte, 2013). In Africa, *V. amygdalina* is the common name for this bitter-tasting plant (Abosi and Raseroka, 2003). The plant is predominantly cultivated in the tropical regions of Africa, especially in the West African (Tekou *et al.*, 2018). In Igbo, Yoruba, and Hausa tribes of Nigeria, it is called as “Olugbu”, “Ewuro” and “Fetefete” respectively. It is a soft woody shrub that grows perpetually to a height of 1 m to 6 m (Ifedibalu Chukwu *et al.*, 2020). This shrub can withstand a broad range of weather conditions (Tekou *et al.*, 2018). It is commonly called “bitter leaf” due to its characteristic bitter taste and this may be attributed to its anti-nutritional contents (Ifedibalu Chukwu *et al.*, 2020).

Acanthus montanus, commonly known in English as “mountain thistle” or “devil’s fig”, is a perennial plant that belongs to the *Acanthaceae* family. It is known as *Ogwuduburu-Okukọ*, or *Agamebu*, among the Igbos of South Eastern Nigeria. The plant extract is reported to be diuretic, purgative and anthelmintic. The leaves are taken by post-natal mothers in southern Nigeria to ensure health and vitality. The roots are used for the treatment of furuncles, leaves for boils on the fingers, and also for cough. (Igoli *et al.*, 2005). The central portion of the twigs and leaves is applied as a hot poultice to mature abscesses and decocted leaves are used as purgative and also used for the treatment of gonorrhea, syphilis, wounds. Also used for treating cardiac dysfunctions and hepatitis. (Okenwa & Jude, 2014).

Moringa oleifera, Lam (*M. oleifera*), also known as *Moringa pterygosperma* Gaertn, is a member of the *Moringaceae* family of perennial angiosperm plants, which includes 12 other species (Olson, 2002). Native of the sub-Himalayan northern parts of India, it is cultivated

throughout tropical and sub-tropical areas of the world, where it is known by various vernacular names (Ramachandran *et al.*, 1980), with drumstick tree, horseradish tree, and malunggay being the most commonly found in the literature. The medicinal value of *Moringa oleifera* is due to the presence of bioactive components called phytochemicals. (Bamishaiye *et al.*, 2011)

Moringa oleifera is an edible plant. A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Ramachandran *et al.*, 1980; Anwar *et al.*, 2007; Kumar *et al.*, 2010). Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β -carotene, vitamin C, and flavonoids (Bennett *et al.*, 2003; Aslam *et al.*, 2005; Manguro and Lemmen, 2007; Amaglo *et al.*, 2010; Gowrishankar *et al.*, 2010).

MATERIALS AND METHOD

COLLECTION AND PREPARATION OF PLANT MATERIALS

The plant leaves were collected and prepared using the botanical field collection methodology (Ojiako and Nwanjo, 2009; Humphry *et al.*, 1993).

Fresh leaves of *Acantus montanus*, *Moringa oleifera*, and *Vernonia amygdalina* was bought from New Benin Market, Benin City, Nigeria. The leaves were taken to the University of Benin (Botany Department) for proper identification and authentication before analysis.

The fresh leaves of the plants were washed under running tap water; the fresh leaves were left over the night to get dried at a constant weight in an oven at 60⁰c.

Hundred grams (100g) of each set of dried leaves were grinded into a fine powder using laboratory blender and stored at 4⁰c in a labelled, air-tight glass container.

A 10g portion of each sample powder was extracted overnight at room temperature with 150ml of distilled water.

Each extract was then filtered through whatmann No. 1 Filter paper.

Preliminary Phytochemical Screening

Preliminary Phytochemical screening was carried out using the method described by Sofowora, (1994)

Test for Saponins

The grounded plant extract (20g) were put into a conical flask after which 100ml of (20%) aqueous ethanol was added. The mixture was put in hot water bath at about 55⁰C after which it was filtered and the residue re-extracted further in 200ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at about 90⁰C. and transferred into a 250ml separation funnel. Diethyl ether was added and shaken vigorously.

The aqueous layer was then recovered while the ether layer was discarded. The purification process was repeated three times and 60ml of n-butanol was added. The combined n-butanol extracts were then washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was also heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material.

Calculation

$$\% \text{ Saponin} = \frac{W_3 - W_2}{W_1} \times \frac{100}{1}$$

Were

W_3 = weight of beaker and residue after evaporation to dryness

W_2 = weight of beaker alone

W_1 = weight of sample

100 = scaling factor to convert to percentage

Test for Flavonoids

10g of the plant samples were extracted repeatedly with 100ml of 80% aqueous methanol, at a room temperature after which the solution was filtered through with whatman filter paper No 42. The filtrate was transferred into a crucible and evaporated into dryness over a water bath, the dry content was weighed to a constant weight (Edeoga *et al*; 2005).

Calculation

$$\% \text{ Flavonoid} = \frac{W_3 - W_2}{W_1} \times \frac{100}{1}$$

Where:

W_3 = weight of beaker and residue after evaporation to dryness

W_2 = weight of beaker alone

W_1 = weight of sample

100 = scaling factor to convert to percentage

Test for Alkaloids

5g of the plant samples were weighed into a 250ml beaker after which 200ml of 10% acetic acid in ethanol was added, the reaction mixture was covered and allowed to stand for 4 hours. Then it was filtered and the extract was then concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was also added drop-wise to the extract until the precipitation was complete. The solution was then allowed to settle and the precipitate collected, washed, with dilute ammonium hydroxide and then filtered, the residue was the alkaloid which was dried and weighed to a constant mass.

(Trease and Evans, 1989).

Calculation

$$\% \text{ Alkaloid} = \frac{W_3 - W_2}{W_1} \times 100$$

Where:

W_3 = weight of beaker and residue after evaporation to dryness

W_2 = weight of beaker alone

W_1 = weight of sample

100 = scaling factor to convert to percentage.

Test for Tannins

500mg of the plant samples were weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the marked level. Then, 5ml of the filtrate was then transferred into a test tube and mixed with 2ml of 0.1m FeCl in 0.1m HCl and 0.008m potassium Ferro cyanide. The absorbance was measured at 120nm within 10mins. The tannins content was calculated using a standard curve of extract.

Calculation:

$$\text{Mg/cm}^3 \text{ Tannin} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \frac{\text{Concentration of Std}}{1}$$

Test for Cyanogenic Glycoside

10.g of the plant sample is placed in a quick fit round bottom flask. 200cm³ of deionized distilled water was added into the flask and the contents allowed standing for 2hours. The flask was then connected to complete the distillation set up and about 150cm³ of the reaction mixture is distilled into a 250cm³Erlenmeyer flask (receiver flask) containing 20cm³ of 2.5% w/v sodium hydroxide solution. Little quantity (0.5-1.0g or cm³) of anti-foaming agent (tannic acid) was added. 100cm³ of the receiver flask content was measured into a 250cm³ Erlenmeyer flask and added to it is 8.0cm³ of 6.0m ammonium hydroxide solution followed by 2.0cm³ of 5.0% potassium iodide solution. The contents of the flask were mixed and titrated with 0.02N silver nitrate solution from a burette till there is was no further turbidity upon the addition of the silver nitrate solution. The flask was placed on a black background during the titration.

Calculation:

The Cyanogenic glycoside concentration can be obtained using the formula;

$$X = T \times \frac{108}{1} \times \frac{N}{1000} \times \frac{V_e}{V_a} \times \frac{100}{W}$$

Where:

X = % cyanogenic glycoside

T = Titre value

108 = Equivalent weight of silver nitrate

N = Normality of silver nitrate solution

1000 = scaling factor to obtain silver nitrate content of 1.0cm³ of AgNO₃

V_e = Extract volume

V_a = aliquot volume

100 = scaling factor to convert value to percentage

W = weight of sample in grams

The test Phytate

10.g of the powdered plant sample was placed in a thimble and extracted was the fat content using hexane; by the soxhlet extraction method. The defatted sample was then dried in an oven set at 70⁰c and allowed to cool to room temperature in a desiccator.

2g of the defatted sample was thus placed in a 100cm³ flask and 50cm³ of 0.18M trichloroacetic acid solution was added to it. The mixture was then stirred for 1 hour with a magnetic stirrer. The reaction mixture was centrifuged for 10mins at 3000 r.p.m. I added 10cm³ to the centrifugate to 4cm³ of 0.036M Iron (iii) chloride solution in a boiling tube. Placed the boiling tube in boiling water bath for 45mins and the resulting mixture was centrifuged and the resulting Ferric phytate collected. The phytate residue was then washed twice with 20cm³ of 0.18m trichloroacetic acid solution and also twice with 30cm³ of water each time. 3-5cm³ of 1.5M hydroxide solution was added to the residue. 25cm³ of water was also added to the reaction mixture and placed in a boiling water bath till coagulation of ferric hydroxide was completed. I collected the ferric hydroxide by centrifugation and washed twice with water. I dissolve the ferric hydroxide thus obtained in 40cm³ of 3.2M trioxonitrate (v) acid added little at a time making up the final volume to 100cm³ using distilled water.

Proximate Analyses of the plant extracts

Determination of Moisture Content by Oven Method

A glass of petri dish/water glass was placed on a tarred analytical balance and recorded the weight (W₁). 5-10g of the plant sample was placed in the container and weighed (W₃), after which i placed the container and its contents in an aircirculating oven set at 150⁰c. The sample was allowed to dry for 3-5hrs and at the end of heating, it was placed in a desiccator to cool down to room temperature. With minimum exposure to air, the container and its contents was weighted again (W₂). The container and its contents was weighed again in the oven and heated for 1 hour. Cooled and weighed again as before (W₂). The process was repeated till two consecutive weighing gave a constant value (AOAC, 2000).

Calculation:

Use the equation

$$X\% = \frac{W_3 - W_2}{W_3 - W_1} \times \frac{100}{1}$$

Where,

X% = percentage moisture content

W₃ = weight of container and sample before drying

W₂ = weight of container and sample after drying

W₁ = weight of container alone

W₃ – W₂ = weight of moisture

W₃ – W₁ = weight of sample

Determination of Ash Content by the Muffle Furnace Method

Porcelain/platinum was placed in a dish on a tarred analytical balance, the weight was recorded (W₂) after which the dish and its contents was placed in a muffle furnace set at 500-600⁰c / hours till it ashed. it was placed in a desiccator to cool to room temperature. I quickly weighed and recorded the weight (W₃).

Calculation:

Using the equation

$$X\% = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where,

X% = percentage ash content

W₃ = weight of dish and ash

W₂ = weight of dish and sample

W₁ = weight of dish alone

100 = scaling factor for conversion to percent.

Determination of protein content by Kjeldahl Method

0.3g of each plant sample was weighed and put in a conical flask, 4ml conc sulphuric acid and kjeldahl was added to it. Heat was applied to the sample till it became colorless. After which reading was taken with a spectrophotometer at 360nm.

Nitrogen content = $\frac{\text{Abs} \times \text{dilution volume (digest)}}{\text{Weight of sample} \times \text{color developer}}$

Determination of Total Lipid Content by Soxhlet Extraction Method

The measured sample was placed in a thimble plugged with cotton wool on a tarred analytical balance. Recorded the weight (W_1) and placed about 10-20g of sample particles or powder into the thimble, plugged with cotton wool and weighed again (W_2). The thimble and its contents were placed in a Soxhlet extractor and connected the extractor to a round bottom flask containing the extraction solvent. At the end of the extraction, the thimble and its contents were transferred to an oven set the evaporation temperature of the extraction solvent for about 1-2 hours (or as deemed proper). Cooled the thimble and its contents in a desiccator to room temperature and quickly weighed again (W_3). Also, I transferred the contents of the extraction flask into another fore-weight round bottom flask (W_4). Rinsed the extraction flask with 10cm³ of the extraction solvent and added the rinsing into the fore-weighted round bottom flask. I connected the fore-weighted flask and contents to a rotary evaporator and evaporated off the solvent. Dried the flask, cooled and weighed again (W_5).

Calculation

Using the formula,

$$X\% = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where,

X% = % Lipid content

W_3 = weight of thimble, cotton wool and sample after extraction

W_2 = weight of thimble, cotton wool & sample before extraction

W_1 = weight of thimble and cotton wool alone

Determination of Fibre content

Weighed 3.0g of the defatted sample into a 500ml beaker. Added 200ml of 1.25 sulphuric acid solution. Boiled for 30mins, making up the volume of reaction mixture to 200ml with hot water during the boiling. Filtered the mixture using a weighed number 42 filter paper dried at 60⁰c. Transferred all the residue to the filter paper and washed with distilled water till filtrate was neutral to litmus paper. Transferred carefully, the residue in the filter paper to a 500ml beaker, added 200ml of 1.25% NaOH solution and boiled for 30mins as before. Filtered, using the previous filter paper and washed residue till it was neutral. Dried the filter paper with residue at 60⁰c and weighed.

Placed the filter paper with residue into a weighed crucible and weighed again. The contents of the crucible were ashed at 50⁰c for 5hrs. Cooled the crucible and contents to room temperature in a dessicator and weighed again.

Calculation:

$$\% \text{ Fibre} = \frac{W_3 - W_2}{W_1} \times 100$$

Where,

W₃ = weight of residue after acid/base digestion

W₂ = weight of ash

W₁ = weight of sample

Determination of Carbohydrate content

The carbohydrate content of was determined by calculating the percent remaining after all the other components have been measured.

$$\% \text{ carbohydrate} = 100 - \% \text{ moisture} - \% \text{ protein} - \% \text{ lipid} - \% \text{ ash} - \% \text{ fibre}$$

RESULTS

The result of the phytochemical screening of the plant extracts of *Moringa oleifera*, *Vernonia amygdalina* and *Acantus montanus* is presented in Table 1. The result indicates the presence of Alkaloids, Saponins, Phytate, Flavonoids and Cyanogenic glycosides.

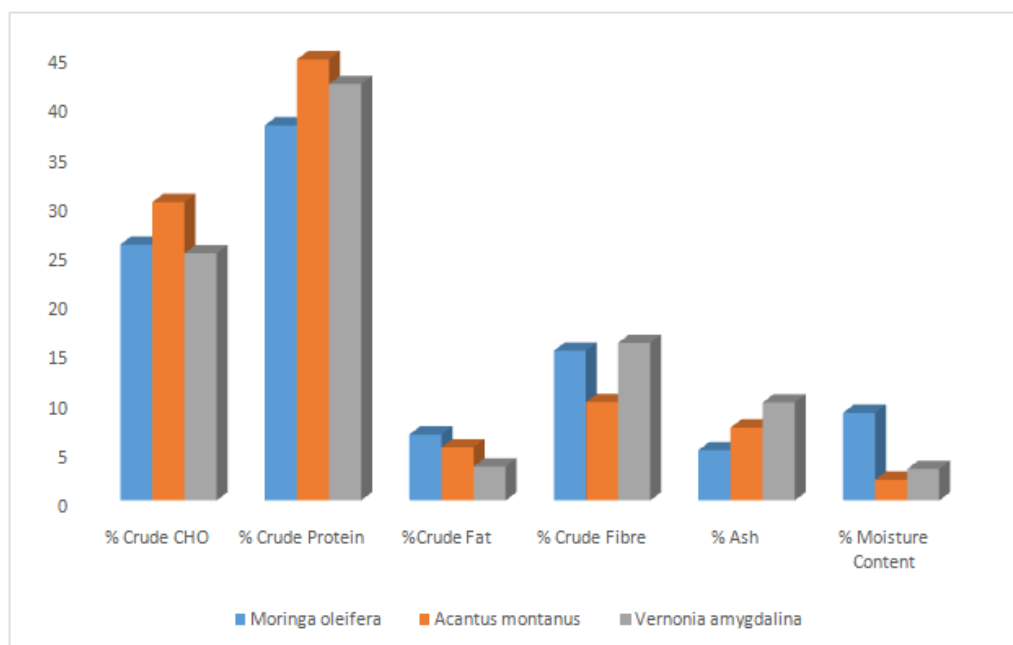
Table 1: phytochemical composition *Moringa oleifera*, *Vernonia amygdalina* and *Acantus montanus*.

Plant Species	Alkaloids	Flavonoids	Saponins	Phytate	Cyanogenic glycoside
<i>Moringa. Oleifera</i>	+++	++	++	+	+++
<i>Acantus montanus</i>	+	++	++	+	++
<i>Vernonia amygdalina</i>	++	++++	+++	+	++++

KEY: + + + + = **Highly Present**; + + + = **Moderately Present**; + + = **Present**; + = **Scarcely Present**; - = **Absent**.

Proximate Composition of *Moringa oleifera*, *Vernonia amygdalina* and *Acantus montanus*.

The qualitative and quantitative proximate composition of the plant extracts of *Moringa oleifera*, *Vernonia amygdalina* and *Acantus montanus* is presented in FIG 2. The qualitative proximate composition of *Moringa oleifera*, *Vernonia amygdalina* and *Acantus montanus* in g/100g showed that they contain carbohydrate, protein, fats, fibre, moisture and ash. While the quantitative screening shows the present of **Carbohydrate** 25.99% (*Moringa oleifera*), 30.30% (*Vernonia amygdalina*) and 25.10% (*Acantus montanus*). **Crude Protein** 38.10% (*Moringa oleifera*), 44.80% (*Vernonia amygdalina*), 42.30% (*Acantus montanus*). **Crude Fat** 6.70% (*Moringa oleifera*), 5.40% (*Vernonia amygdalina*), 3.45% (*Acantus montanus*). **Crude Fibre** 15.21% (*Moringa oleifera*), 10.00% (*Vernonia amygdalina*), 16.00% (*Acantus montanus*). **Ash content** 5.10% (*Moringa oleifera*), 7.40% (*Vernonia amygdalina*), 9.95% (*Acantus montanus*). **Moisture content** 8.90% (*Moringa oleifera*), 2.1% (*Vernonia amygdalina*), 3.2% (*Acantus montanus*).



DISCUSSION

Phytochemicals are secondary metabolites and non-nutritive chemicals produced by plants to fight against diseases and also for protection.

The study of the *Moringa oleifera*, *Vernonia amygdalina* and *Acanthus montanus* leaf extracts revealed that they contain Alkaloids, Saponins, flavonoids, phytate and cyanogenic glycoside. Several phytochemical studies carried on this individual plant extracts shows a conformity with the studies conducted of phytochemical composition of the leaf. Phytochemicals have several medicinal properties used in pharmaceutical companies for manufacturing of drugs. The bioactive components contained in the leaf extracts accounts for it been used in treatment of various diseases like Diabetes, Asthma, Nausea, Diarrhea, etc.

Alkaloids which contain nitrogenous compounds can be used as chemotherapeutic agents, and anaesthetics. (Williams *et al.*, 2004). while flavonoids have the ability to scavenge hydroxyl radicals and lipid peroxide radicals. Medically saponins has the ability to reduce cholesterol level and stimulation of immune system (Prince *et al.*, 1987). Saponins can also be used for manufacturing of soap because of its ability to form froth. However, since the late 1980s, several studies have indicated that Phytate has beneficial effects such as antioxidant, anticarcinogenic, and antidiabetic properties (Omoruyi *et al.*, 2013). It is mainly present as a salt of mono-valent and divalent cations such as K^+ , Mg^{2+} , and Ca^{2+} . It is accumulated into the seed during the ripening period. In dormant seeds, phytate represents 60-90% of total phosphate.

The proximate analyses of the three herbal plants are as reported in table 2 where protein contents were considerably high. Other nutrients in the leaves were total carbohydrates, fats, fibre, minerals or ash content and moisture.

Generally, the fiber contents are high as compared to other plant leaves and seeds as reported by Elegbede, 1998. Although high fiber contents increase digestibility, but on the other hand high level of fibers in the diet can produce intestinal irritation, which ultimately decrease nutrients utilization (Oyenuga and Fetuga, 1975). It can be deduced that the fiber contents of these formulation are mild in concentration and have more beneficial effects rather than hazardous. The ash and moisture content are comparatively low in *Vernonia amygdalina*.

CONCLUSION

The presence of the phytochemicals has authenticated its usefulness in ethno medicine and its potentials in drug formulation and development. In addition to that, the presence of nutrients proves why leaf extracts of *Moringa oleifera*, *Vernonia amygdalina* and *Acanthus montanus* can be used as food supplement.

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