Vitex doniana Fruit and *Milletia aboensis* Root; A Pharmacognostic Profiling

Ajaghaku Amara Anwuchaepe[1,2]*, Olanrewaju Mary Ololade[3], Ibe Chioma Ifeoma[3]

[1]Department of Pharmacognosy and Traditional Medicine, David Umahi Federal University of Health Science, Uburu, Ebonyi State, Nigeria.
[2]International Institute for Pharmaceutical Research and Innovation, David Umahi Federal University of Health Science, Uburu, Ebonyi State, Nigeria.
[3]Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

ABSTRACT

Vitex doniana and *Milletia aboensis* are used in traditional medicine to treat a wide range of diseases. The study aims to determine the pharmacognostic qualities of these plants. Organoleptic, microscopy, histochemical, physicochemical, and phytochemical profiling were all examined. *V. doniana* fruit powder revealed a reddish-black powder with an odor and a sweet flavor, but *M. aboensis* root powder revealed a tasteless milky color powder with an uncharacteristic odor. Microscopic examination of *V. doniana* fruit powder revealed a lignified heart-shaped, elongated stone cell, secretory gland attached to lignified vessel element, strands of fiber cells, and oil cells, whereas *M. aboensis* root powder revealed prisms of calcium oxalate crystals, cork cells, lignified spiral vessels, and fiber elements. Histochemical analysis revealed that both plants contained cellulose, lignin, and starch. Calcium oxalate crystal was seen in *M. aboensis* while protein bodies, fats and oil, gum, and mucilage were seen in only *V. doniana.* Physicochemical analysis of *V. doniana* fruit and *M. aboensis* root indicated moisture content (14.68% and 8.80%), total ash (11.75% and 9.00%), acid insoluble ash (2.00% and 2.50%), water-soluble ash (2.60% and 6.00%), water-soluble extractive (10.02% and 6.60%), and alcohol soluble extractive (9.80% and 3.20%), respectively. The phytochemical study revealed that both plant extracts have a high concentration of phytocompounds. Ethyl acetate fractions of both plants contained the most phenolic phytoconstituents. This research will aid in the development of drug pharmacopeia standards, verifying authenticity, and preventing adulteration.

Key Words: *Pharmacognostic, V. doniana, M. aboensis, Phytochemical, Microscopic*

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INTRODUCTION

Plants have long been utilized as medicine and are, therefore, the foundation for medical care. The World Health Organization [1] reported that a sizable portion of the population still uses plant medicine in these modern times, particularly in underdeveloped nations. Herbal medicine plays an important role in restoring the health of individuals and communities, therefore, safety and scientific evidence of the efficacy of these remedies are vital to improve the standards The primary reasons why plant medicines are so popular in underdeveloped nations are that they are easily obtainable, inexpensive, and seen as "safer" than conventional treatments. The demand for medicinal plants and their products is high despite the lack of scientific proof of the potential mechanisms of action and the many problems with the development of traditional medicine [2]. This has led to the necessity of appropriate research to support the traditional uses of herbal medicines, clarify their mechanisms of action, and standardize these medications to ensure their efficacy, safety, and quality. Pharmacognostic studies not only guarantee the consistency of quality and purity in crude drugs but also provide a cost-effective and dependable method for

Corresponding author: Ajaghaku Amara Anwuchaepe
Address: Department of Pharmacognosy and Traditional Medicine, David Umahi Federal University of Health Science, Uburu, Ebonyi State, Nigeria.
E-mail: ✉ ajaghakuaa@dufuhs.edu.ng
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accuracy [3]. Emphasis has been placed on the necessity of conducting both macroscopic and microscopic examinations on medicinal plants as a foundational step for accurate identification, and these examinations should precede any other testing procedures, according to the WHO's directive [1].

Vitex doniana (Verbanaceae) is a deciduous tree widespread in tropical Africa [4]. Several studies have reported that the bark, leaves, roots, and fruits of *V. doniana* have medicinal value. In traditional medicine, it is used to treat a litany of ailments, cure infertility, cancer, epilepsy, eye problems, and pain management. The fruit is eaten to improve fertility. Women drink a decoction of the root for backaches. It is also used to treat anemia, gonorrhea, jaundice, leprosy, and dysentery [5]. In Nigeria, the hot water extract of the dried stem bark is used as a stimulant also; the dried leaves are used to treat dizziness [6].

Millettia aboensis (Fabaceae) is an evergreen, non-climbing leguminous shrub [7, 8]. Most of the various parts of *M. aboensis* are useful in traditional medicine. According to Ugwueze, the leaves are used for the treatment of ulcers, as laxatives, and for general healing. The root has been reported to cure gastrointestinal disorders and liver problems. The leaves, stem, and root in combination with other herbs are used for venereal diseases such as syphilis, and gonorrhea [9]. In Nigeria, it is used to treat colic and respiratory difficulties; the leaves are chewed and applied on the painful spot. Also, maceration of the root in alcohol is used to treat hernias [7]. This study aims to evaluate the pharmacognostic parameters of these plants for proper identification, and standardization and confirm its authenticity to prevent adulteration of the drugs.

MATERIALS AND METHODS

Plant materials

V. doniana fruits and *M. aboensis* roots were collected in the wild from Agulu and Abagana respectively in Anambra State, Nigeria. They were authenticated by a trained taxonomist, Mr Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. They were deposited in the Herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka with voucher numbers PCG 474/A/035 and PCG 474/A/021, respectively.

Preparation and extraction

The plant materials were subsequently cleaned, the fruit pulp separated from the seeds, the roots were shredded, then air-dried at room temperature for 3 weeks, and pulverized with a mechanical grinding machine. A 2.2 kg of *V. doniana* fruit and 6 kg of *M. aboensis* root powder were extracted in 7.5 L and 10 L of methanol, respectively, using a Soxhlet extractor. Using a rotary evaporator, the resulting solutions were filtered using Whatman filter paper and the filtrates concentrated to dryness *in vacuo* at 40 °C.

Fractionation of the extracts

A 400 g and 70 g of the *V. Doniana* and *M. Aboensis* extracts respectively containing water (200 mL in 100 g of extract) were subjected to liquid-liquid partition successively with n-hexane (1000 mL), ethyl acetate (1000 mL), and n-butanol (500 mL) in increasing order of polarity to obtain n-hexane, ethyl acetate, and butanol fractions. The fractions were filtered and concentrated at 40 °C *in vacuo* using a rotary evaporator.

Pharmacognostic parameters

Organoleptic examinations of the powdered plants

An organoleptic evaluation was carried out using standard methods [10]. Sensory organs were used to evaluate the color, odor, taste, and texture of the powdered plant.

Microscopic and histochemical examination

The microscopical and histochemical parameters of the powdered plant materials were carried out using standard methods [10].

Examination of starch

A 0.1 g of the powdered samples were mounted in an iodine solution and a blue-black coloration confirmed the presence of starch. The slides were covered and observed under a microscope.

Examination of epidermal trichomes and calcium oxalate crystals

A 0.1 g of the powdered samples were gently boiled in a chloral hydrate solution. The resulting mixtures were placed on clean glass slides (with chloral hydrate as a mountant) and covered. The slides were examined under a microscope for the presence of epidermal trichomes and calcium oxalate crystals.

Examination of lignin

The plant powders (0.1 g) were stained with phloroglucinol and allowed to stand until dry. After drying, concentrated hydrochloric acid and glycerin were added. The slide was covered with a cover slip and examined under a microscope. Lignified vessels, fibers, parenchyma, sclereids, or hairs are stained pink/red.

Examination of gum and mucilage, fats and oil, and cellulose

To 0.1 g of the powdered samples, ruthenium red was added and the appearance of a stain confirmed the presence of gum and mucilage. Sudan IV reagent was added and the appearance of stain confirmed the presence of fats and oil. Chlor-zinc-iodine solution was added and blue or violet colouration confirmed the presence of cellulose.

Phytochemical analysis

The qualitative phytochemical analysis of the extracts and fractions was carried out using standard methods described by Odoh *et al.* [11]. A quantity of 0.2 g of the plant extracts and fractions was dissolved in methanol individually and the resulting solutions were used for the analysis.

Test for alkaloids

The plant extracts and fractions (0.2 g) were heated in 20 mL of 2% acid solution (HCL) individually in a water bath for about 2 min. The resulting solutions were allowed to cool and then filtered then 5 mL of the filtrates used for the following tests:

- *Dragendorff's test:* To each labeled test tube, 5 mL of the sample was added, followed by 1 mL of Dragendorff's reagent. The formation of orange or red precipitates indicated the presence of alkaloids.
- *Hager's test:* The samples (5 mL) were placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. The formation of yellow precipitate confirmed the presence of alkaloids.
- *Wagner's test:* The samples (5 mL) were placed in labeled test tubes and a few drops of Wagner's reagent (solution of iodine and potassium iodide) were added. A reddish-brown precipitate indicated the presence of alkaloids.
- *Mayer's test:* A quantity of 5 mL of each of the samples was placed in labeled test tubes and a few drops of Mayer's reagent (potassium mercuric iodide solution) were added. The formation of a cream color precipitate indicated the presence of alkaloids.

Test for saponins

- *Frothing test:* The samples (5 mL) were placed in labeled test tubes. 5 mL of distilled water was added and shaken vigorously. The test tubes were observed for the presence of persistent froth.
- *Emulsion test:* To the frothing solution, 3 drops of olive oil were added and shaken vigorously. The solution was observed for the formation of emulsion.

Test for tannins

To 3 mL of each of the samples, a few drops of 0.1% Ferric chloride were added and observed for brownish green or a blue-black coloration.

Test for flavonoids

- *Ammonium hydroxide test:* A quantity of 2 mL of 10% ammonia solution was added to a portion of each of the samples and allowed to stand for 2 minutes. Yellow coloration at the lower ammoniacal layer indicated the presence of flavonoids.
- *Sodium hydroxide solution test:* A quantity of 10 mL of 10% sodium hydroxide solution was added to a portion of each of the samples and observed for color changes in the lower alkaline layer: yellow color (flavones), blue to violet color (anthocyanins), yellow to orange color (flavonones).

Test for steroids and terpenoids

- *Liebermann-Burchard test:* Acetic anhydride (2 mL) was added to 0.5 g of each of the fractions and methanol extracts. Concentrated H_2SO_4 (2 mL) was carefully added to the resulting mixture and observed for color change from violet to blue or green.
- *Salkowski test:* The plant extracts and fractions were dissolved in methanol individually and the resulting solutions were used for the test. A 5 mL of each of the samples was mixed in 2 mL of chloroform and concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for cardiac glycosides

- *Keller-Killani test:* A 0.2 g of the methanol extracts and fractions were dissolved in 5 mL of methanol individually and was treated with 2 mL glacial acetic acid containing one drop of ferric chloride solution. Concentrated H_2SO_4 (3 mL) was carefully poured into the inclined test tube containing the mixture. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides.

Physicochemical analysis

The analysis of the plant was carried out on the ash value, moisture content, and extractive values using standard methods [11, 12].

Total ash

The nickel crucible was placed in a furnace for 15 min at 35 ºC in triplicate, cooled in a desiccator for one hour, and weighed as W_1. A 2 g (W_0) of the powdered material was added to each nickel crucible and evenly spread by tapping the crucibles. The contents were heated gently to eliminate moisture and char the plant materials completely. The temperature was gradually increased until the carbon had vaporized and the residue was devoid of carbon at 450 ºC indicated by a whitish ash colour. The crucibles were removed, cooled in a desiccator, and reweighed (W_3). The

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heating, cooling, and weighing were repeated until a constant weight was achieved. The percentage ash content was determined by the relationship below:

$$\% \text{ Total Ash} = \frac{W_3 - W_1}{W_0} \times 10 \quad (1)$$

The average of the three total ash values was calculated.

Acid -insoluble ash

Concentrated hydrochloric acid (25 mL) was added to the crucibles containing total ash and the mixture was gently boiled for 5 min under a watch–glass cover. After cooling, the contents were filtered using an ash-less filter paper of known weight (W_1). The residue on the filter paper was washed with hot water until the filtrate became neutral to blue litmus paper. Subsequently, the filter paper with the insoluble matter was dried to a constant weight (W_2) at 105 ºC. The percentage of acid-insoluble ash was determined by the relationship below:

$$\% \text{ Acid} - \text{Insoluble Acid} = \frac{W_2 - W_1}{W_0} \times 100 \quad (2)$$

The averages of three acid-insoluble ash values were calculated.

Water–soluble ash

To the crucibles containing total ash, 25 mL of water was added and boiled for 5 min. The contents were filtered using ash-less filter paper of known weight (W_1). After washing the residue with hot water, the filter paper was dried in an oven at 105 ºC until a constant weight (W_2) was obtained. The percentage Water – insoluble ash was determined by the relationship:

$$\text{Weight of Residue} = W_2 - W_1 \quad (3)$$

The average of three water-soluble ash values was calculated.

Extractive value

Water-Soluble extractive value

Preheated evaporating dishes were weighed (W_1) in triplicate. Then, 5 g (W_0) of the powdered drug sample was accurately weighed into each of the dishes. To this, 100 mL of water was added and the mixture was macerated for 24 h. The flask and its content were shaken mechanically for 6 h and were allowed to stand for 18 h thereafter, the mixture was filtered and the filtrate was evaporated to dryness on an electric hot plate using an evaporating dish. It was further dried to constant weight at 105 ºC. The evaporating dish (W_2) was weighed after cooling. The percentage water-soluble extractive value was calculated using the relationship;

$$\% \text{ Water} - \text{Soluble Extractive} = \frac{W_2 - W_1}{W_0} \times 100 \quad (4)$$

The average of the three water-soluble extractives was calculated.

Alcohol soluble extractives

Preheated evaporating dishes were weighed (W_1) in triplicate. A 100mL of 99% ethanol was added to the 5 g (W_0) of the powdered sample and the mixture was macerated for 24 h. The flask and its content were shaken mechanically for 6 h and were allowed to stand for 18 h. The mixture was filtered and the filtrate was evaporated to dryness on an electric hot plate using an evaporating dish. It was further dried to constant weight at 105 ºC. The evaporating dish (W_2) was weighed after cooling. The percentage alcohol soluble extractive value was calculated using the relationship:

$$\% \text{ Alcohol} - \text{Soluble Extractive} = \frac{W_2 - W_1}{W_0} \times 100 \quad (5)$$

The average of three alcohol-soluble extractives was calculated.

Moisture content

A quantity of 3 g (W_0) of the powdered drug sample was weighed into a pre-heated evaporating dish (W_1) and placed in an oven at 100-150 ºC to dry for 5 h and weighed. The drying and weighing were continued at 1 h intervals till the difference between two successive weighings corresponds to not more than 0.25% (constant weight is reached when two consecutive weighing after drying for 30 min and cooling for 30 min in a desiccator, shows not more than 0.01 g difference. The moisture content was calculated as;

$$\text{Moisture Content} = \frac{\text{The difference in weight from the sample}}{3} \times 100 \quad (6)$$

$$\text{The difference in weight from the sample} = W_0 - \text{Final weight}$$

$$\text{Final weight} = \text{Constant weight} - W_1$$

RESULTS AND DISCUSSION

Organoleptic analysis

The primary characteristics of standardizing and identifying crude natural pharmaceuticals are organoleptic (sensory) evaluations, which a parameters that don't require the use of scientific tools or incur any costs [13].

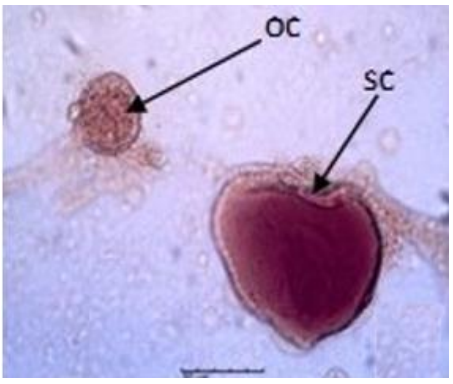
The established sensory characteristics of *V. doniana* and *M. aboensis* **(Table 1)** are crucial for ensuring the purity and quality of these drugs.

Table 1. Organoleptic evaluation of *Vitex doniana* fruit and *M. aboensis* root

Features	Observation	
	V. doniana **fruit pericarp**	***M. aboensis*** **root**
Texture	Coarse powder	Coarse powder
Odor	Characteristic	Non-characteristic
Taste	Sweet	Tasteless
Color	Reddish-black	Milky

Microscopic analysis

The importance of macroscopic and microscopic characters in general is widely recognized in taxonomic considerations and is successfully used in the identification of plants at genus and as well as at species levels [14]. The microscopic analysis of *V. doniana* fruit pulp and *M. aboensis* root **(Figure 1)** revealed important diagnostic features that will help in the identification and authentication of the raw drug materials to ensure the quality and purity of the drugs. The transverse section of *V. doniana* **(Figure 2)** revealed a thick-walled epicarp, a mesocarp, and an endocarp. Stone cells were scattered within the fruit mesocarp. The endocarp had elongated parenchymatous tissues 20-25 µm in length. The transverse section of *M. aboensis* root **(Figure 2)** revealed a cell arrangement typical of a dicot showing the epidermis, cortex, vascular bundles (xylem and phloem), and pith. Heart-shaped stone cells (sclereids) and lignified tissues were observed in the powder microscopy of *V. doniana*. *M. aboensis* root revealed typical root features (woody plant part) which include the fragment of cork cells, lignified spiral vessels, and fibers. The tissue arrangement in the transverse section of the root is typical of a dicotyledonous root.



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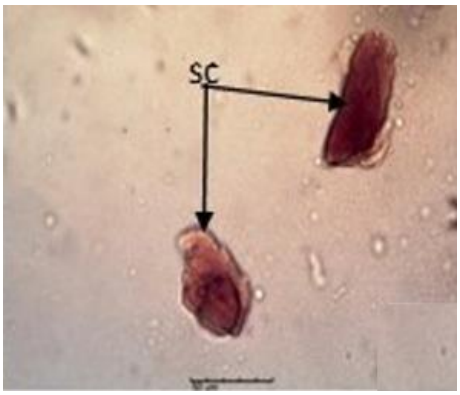

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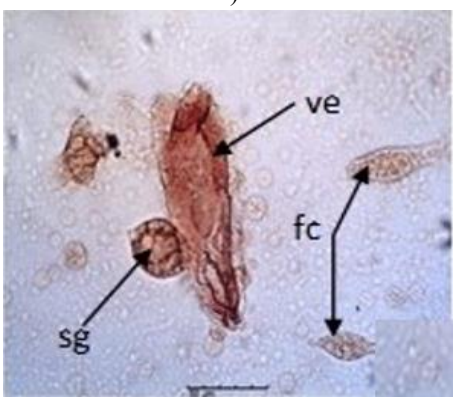

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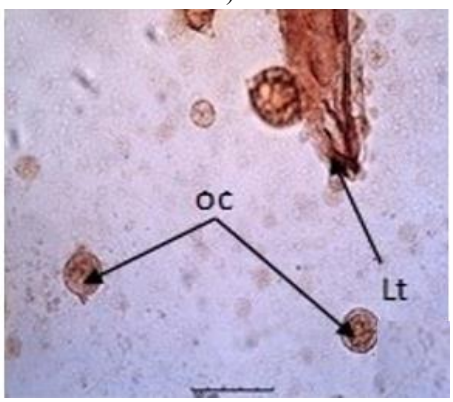

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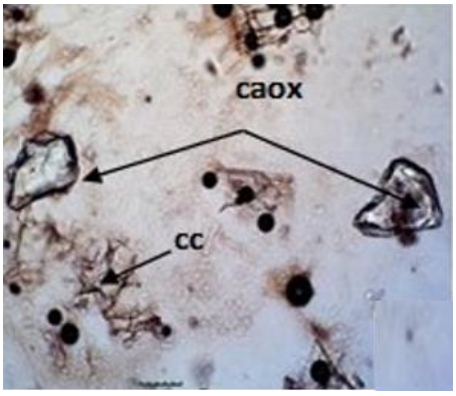

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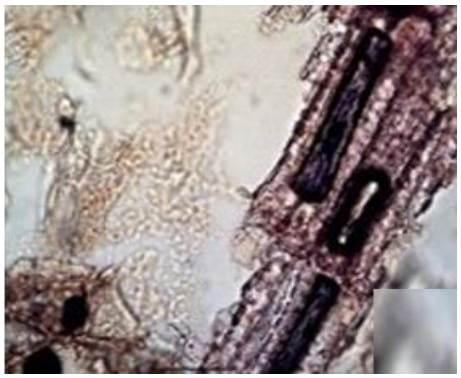
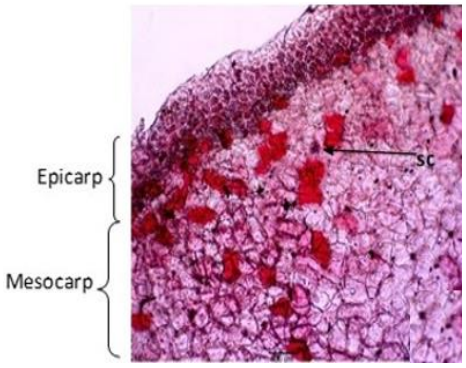


f)

Figure 1. Microscopic characters of *V. doniana* fruit and *M. aboensis* root powders; a) Chemomicroscopy of the fruit powder showing an oil cell (oc) and a large heart-shaped stone cell (sc) X400, b) Chemomicroscopy of the fruit powder showing elongated stone cells (sc), c) Chemomicroscopy of the fruit powder showing a secretory gland (sg) attached to the vessel element (ve) and strands of fibre cells (fc), d) Chemomicroscopy of the fruit powder showing oil cells (oc) and lignified tissues (Lt), e) Chemomicroscopy of the powder of *M. aboensis* showing prisms of calcium oxalate crystals (coax) and cork cells (cc), and f) Chemomicroscopy of the powder of *M. aboensis* showing spiral vessels and fibre elements that are lignified



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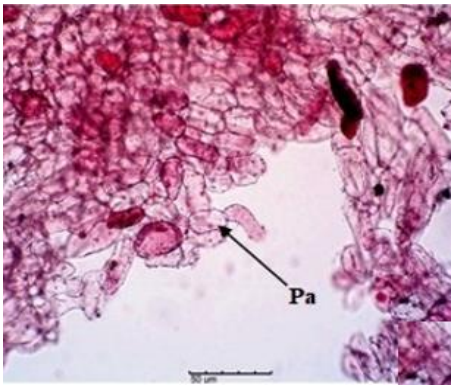

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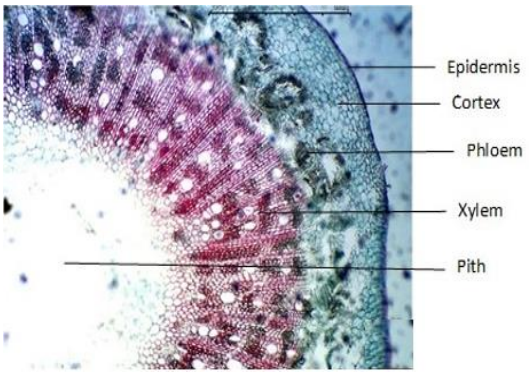

c)

Figure 2. Transverse section of *V. doniana* fruit and *M. aboensis* root
a) Transverse section of *V. doniana* fruit pericarp showing the thick-walled epicarp and the mesocarp. Stone cells (sc) are seen scattered within the mesocarp, b) Transverse section of *V. doniana* showing part of the endocarp with elongated parenchymatous tissues (pa) (20–25 µm in length), and c) Transverse section of the root of *M. aboensis* root

Histochemical examination

The result of the histochemical analysis revealed the presence of starch, lignin, calcium oxalate crystals, and cellulose in *M. aboensis* root while only lignin is absent in *V. doniana* fruit as presented in **Table 2**.

Table 2. Histochemical analysis of *Vitex doniana* and *M. aboensis*

Features	Observation	
	***V. doniana* fruit**	***M. aboensis* root**
Starch	Present	Present
Lignins	Present	Present
Calcium oxalate	Absent	Present
Cellulose	Present	Present
Gum and mucilage	Present	Absent
Fats and oil	Present	Absent
Protein bodies	Present	Absent

Phytochemical analysis

Phytochemical analysis of *V. doniana* fruit pulp and *M. aboensis* root **(Tables 3 and 4)** revealed the presence of flavonoids and other phenolic constituents like tannins and anthraquinones in the plant extracts and ethyl acetate fractions. Phytochemicals are not only limited to their ecophysiological functions but also have potential medicinal effects on humans [15]. Several flavonoids (e.g. isoflavones) are referred to as phytoestrogens based on their ability to mimic estrogen in mammals and they reportedly have various human health effects including, antioxidant, anticancer, oestrogenic [16], anti-allergy, anti-inflammatory, anti-viral and antimicrobial properties [17].

Saponins are characterized by their soapy qualities, a distinctive category of glycosides. Studies indicate that saponins exhibit effective antifungal properties. Tannins, on the other hand, have been observed to hinder the growth of microorganisms by causing the precipitation of microbial proteins, rendering nutritional protein inaccessible to them [18].

Table 3. Phytochemical constituents of methanol extract and fractions of *V. doniana* fruit

Phytochemical	ME	nHF	EF	BF	WF
Alkaloids	+	-	+	-	-
Tannins	+	-	+	+	+
Flavonoids	+	-	+	+	+
Steroids	+	+	-	+	+
Terpenoids	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+
Saponins	+	-	+	+	+
Anthraquinones	+	-	+	+	+

Key: + = Present; - = Absent
ME = Methanol extract, nHF = n-hexane fraction, EF = ethyl acetate fraction, BF = butanol fraction, and WF = water fraction

Table 4. Phytochemical constituents of methanol extract and fractions of *M. aboensis* root

Phytochemical	ME	nHF	EF	BF	WF
Alkaloids	+	-	-	-	-
Tannins	+	-	+	+	+
Flavonoids	+	-	+	+	+
Steroids	+	+	-	+	+
Terpenoids	+	+	+	+	+
Cardiac glycosides	+	-	+	+	+
Saponins	+	-	+	+	+
Anthraquinones	+	-	+	+	+

Key: + = Present; - = Absent
ME = Methanol extract, nHF = n-hexane fraction, EF = ethyl acetate fraction, BF = butanol fraction, and WF = water fraction

Physicochemical analysis

Physicochemical evaluation of crude drugs is important in detecting adulteration and improper handling of drugs. Physicochemical parameters of *V. doniana* fruit pulp and *M. aboensis* root are presented in **Table 5**. Moisture content is a measure of the water content in a sample. *V. doniana* fruit pulp and *M. aboensis* root revealed a moderate moisture content which is an indication that they can be stored for a long time without microbial degradation. This agrees with the required range of safe storage limits for plant food materials as reported by Vunchi *et al*. [19]. Aiwonegbe *et al*. [20]. reported that the moisture content of *V. doniana* fruit pulp was found to be higher than 10.00%. This is lower than the report by Vunchi *et al*. [19] of 16.6% which could be as a result of the drying method or differences in environmental conditions. The total ash of crude drugs is used to determine the presence or absence of foreign inorganic matter such as metallic salts and or silica [20]. A low amount of total ash, acid-insoluble, and water-soluble ash was found in *V. doniana* fruit pulp and *M. aboensis* root which indicated little inorganic and non-physiological matters such as silica. A higher acid insoluble ash value was found in *M. aboensis* root compared to *V. doniana* fruit pulp. This could be a result of little remnants of earthy material since it's a root drug acid insoluble ash that is generally sand and other siliceous matter [11]. The extractive value of a crude drug is the solubility of the chemical constituents present in the drug when extracted with a particular solvent. *V. doniana* fruit pulp and *M. aboensis* root expressed higher water-soluble extractive than alcohol-soluble extractive indicating that the constituents of these plants are more soluble in water than alcohol.

Table 5. Physicochemical analysis of *V. doniana* fruit and *M. aboensis* root

PARAMETER	*V. doniana*	*M. aboensis*
Moisture content	14. 68 ± 0.25	8.80 ± 0.69
Total ash	11.75 ± 0.07	9.00 ± 0.10
Acid insoluble ash	[a]2.00 ± 0.05	[a]2.50 ± 0.02
Water soluble ash	[a]2.60 ± 0.02	[a]6.00 ± 0.04
Water soluble extractive	10.02 ± 0.03	6.60 ± 0.02
Alcohol soluble extractive	9.80 ± 0.02	3.20 ± 0.01

[a] Calculated from the initial weight of the sample (2 g)
yield (% w/w)

CONCLUSION

The study revealed important diagnostic features of *V. doniana* and *M. aboensis*. Consequently, the findings of this study will greatly help in the identification and authentication of these plants; as well as serve as a reference for the compilation of a suitable monograph for the standardization of these plants.

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Conflict of interest: None

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Ethics statement: None

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