Authentication and quality control of *Uapaca heudelotii* Baill. - An investigation of pharmacognostic, phytochemical and physicochemical properties of its leaves and stem bark

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Abstract

*Uapaca heudelotii* Baill. is well known in various African cultures for its application in the treatment of infections and inflammatory conditions. This study was focused on providing standard identification parameters for authentication and quality assurance of *U. heudelotii* through morphological observations, screening of phytochemical constituents, fluorescence, spectroscopic and physicochemical analysis. *U. heudelotii* leaves are simple, elliptic and arranged in whorls. The bark is greyish-brown with longitudinal striations on the outer surface and pale red on the inner surface. Leaf lamina microscopy displayed anticlinal polygonal straight-walled epidermal cells, with anisocytic stomata found only on the abaxial surface. Leaf surface constants were determined. Microscopy of powdered leaves and barks revealed the presence of epidermal cells, starch grains, calcium oxalate, sclereids and pitted vessels. Alkaloids, flavonoids, coumarins, saponins, triterpenoids, phytosterols and tannins were identified in both stem bark and leaves. The total phenolic content for the leaf and bark were 219.2 ± 10.013 and 153.9 ± 1.602 mg/g gallic acid equivalent respectively. The total flavonoid contents were recorded as 1036 ± 33.37 and 310.2 ± 79.00 mg/g quercetin equivalent for the leaf and bark respectively. The total ash for the aqueous and alcoholic extracts were slightly acidic (3.5). In elemental analysis, lead (Pb) was detected within the acceptable limit (0.0019-0.0025 mg/kg). In conclusion, the current results have provided standard parameters for the correct identification and quality assessment of *U. heudelotii*.

Keywords

*Uapaca heudelotii*, macro-morphology, micro-morphology, fluorescence, mineral content

Introduction

Phyotherapy has for the past decades seen an increased usage and wide acceptability in both developing and industrialized countries due its effectiveness and safety. This upsurge in the practice and commercialization of herbal drugs is however faced with the challenge of misidentification of closely related species and adulteration of crude materials for financial gains (1). In facing this challenge, it is fundamental that conditions to ensure correct identification and quality of samples be laid down (2). Establishing standards for proper identification, quality and safety assurance through pharmacognostic, phytochemical and physicochemical studies is thus very vital (3).
Plant description, habit and distribution
The genus *Uapaca* (Euphorbiaceae) consists of about 50 species with strikingly similar morphological features distributed across most of tropical Africa and Madagascar (4, 5). *Uapaca heudelotii*, commonly called the “sugar plum”, is a well-known species recognized for its medicinal uses in traditional medicine. It is an evergreen dioecious small to medium-sized tree with a condensed low-branching crown which bears sweet edible fruits (4, 6). The plant is usually found growing at river banks in riverine forests.

Ethnomedical uses
In folk medicine, decoctions of the stem bark are used to treat cough and cold (4, 7), fever (4, 7), headaches (4, 7), tooth-ache (7), gastrointestinal infections (7), female infertility (7), skin infections (4, 7), rheumatism (4, 7) and as enema for constipation or haemorrhoids (4, 6, 7). The leaves are pulped with palm-oil for external application to furuncles and to relieve migraines (7). The plant is locally called “kuntan” by the Akans in Ghana and is highly commercialized as a massage to aid toddlers late in walking (7).

Biological activity and phytochemistry
In a previous study, extracts from *U. heudelotii* demonstrated potent anti-sickling and antibacterial activity attributed to anthocyanins and organic acids (8). Various solvent fractions and flavonoid glycosides from the stem bark also showed broad spectrum antibacterial activity as well as antioxidant effect (9-11).

Pharmacognostic studies
The misapplication of herbs or natural products usually begins with wrong identification of species which look similar to the naked eye. Such challenges can be resolved by pharmacognostic studies. Pharmacognostic studies deal with the study of the morphological, phytochemical and physicochemical properties of a plant drug. Unlike taxonomic identification, a pharmacognostic study includes parameters which help in identifying adulteration in dry powder form also. In spite of the medicinal importance of *U. heudelotii*, a search in literature reveals no data on its pharmacognostic study. SI was calculated by the equation:

\[
SI = \frac{\text{Traview for each sample}}{\text{Field magnification}} 
\]

Physical and organoleptic evaluation
For organoleptic evaluation, the texture, colour, odour and taste of whole and powdered samples were determined. The type of leaf, arrangement pattern, petiole and surface characteristics of the lamina such as shape of leaf, apex, venation, base and margin were recorded. Thirty (30) fresh leaves were selected randomly and measured for their average length and width. Pieces of the stem bark were observed for peculiar characteristics on the inner and outer stem bark, fracture and curvature types (13).

Microscopic evaluation
The various microscopic studies were carried out using the Leica DM 750 microscope (Jos Hansen and Soehn GmbH, Hamburg, Germany), employing a stage micrometre and a camera lucida. The fresh transverse sections of the midrib, petiole and the cleared sections of the leaf lamina were observed under the microscope mounted either in a solution of glycerine or stained with phloroglucinol (0.1%w/v) with drop of concentrated hydrochloric acid. Cell types including epidermal cells, stomata, venation etc. were observed. Photomicrographs at x10 and x40 magnifications were taken (14).

Materials and Methods

Chemicals and reagents
All chemicals and reference drugs (chloral hydrate, glycerine, phloroglucinol, hydrochloric acid, iodine solution, ethanol, chloroform, petroleum ether, ethyl acetate, aluminium chloride and Folins Ciocalteu reagent, ammonia (NH₃), sulphuric acid (H₂SO₄), hydrochloric acid (HCl), ferric chloride (FeCl₃) and potassium hydroxide (KOH) were obtained from Sigma-Aldrich Co Ltd Irvine, UK. All organic solvents (ethanol (EtOH), petroleum ether (pet-ether), ethyl acetate (EtOAc), chloroform (CHCl₃) and methanol (MeOH)) were of analytical grade and obtained from BDH, Laboratory Supplies (Merck Ltd, Lutterworth, UK).

Harvesting and processing of plant material
Fresh disease-free stem barks and leaves of *U. heudelotii* were collected near the Asuobone River in the Afram plains district of the Eastern Region of Ghana in October, 2019. The identity of the sample was confirmed by Dr George Henry Sam of the Herbal Medicine Department, KNUST. Herbarium specimens, KNUST/HM1/2020/L003 and KNUST/HM1/2020/5B004 were placed at the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST.

The plant materials were cleaned to remove all foreign materials. Fresh samples were observed for gross morphological features. For microscopy, thin sections of the fresh leaf midrib, petiole and lamina were obtained using a sharp blade. The sections were cleared of all green pigments by boiling in 80% chloral hydrate for about 4 hr and stored in glycerine until investigation. About 200 g of the stem bark and leaves were cleaned, air dried at room temperature for two weeks and pulverized to obtain dried coarse powder. The powdered materials were stored in air tight containers at room temperature.

Macroscopic and organoleptic evaluation
For organoleptic evaluation, the taste, colour, odour and texture of whole and powdered samples were determined. The type of leaf, arrangement pattern, petiole and surface characteristics of the lamina such as shape of leaf, apex, venation, base and margin were recorded. Thirty (30) fresh leaves were selected randomly and measured for their average length and width. Pieces of the stem bark were observed for peculiar characteristics on the inner and outer stem bark, fracture and curvature types (13).

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Subsequently, quantitative leaf surface data including the stomatal index, palisade ratio, veinlet termination and vein islet numbers were determined using three (5) different samples of cleared leaf lamina. The number of the cell types per square millimetre (mm²) of epidermis was noted. Determinations were made from five fields of view for each sample and the results expressed as the mean ± standard deviation (SD) (15). The stomatal index remains constant regardless of age of plant and is usually used for authentication purposes. SI was calculated by the equation:
$SI = \left[ \frac{S}{S+E} \right] \times 100$

where, $S$ and $E$ are the number of stomata and epidermal cells respectively in microscopic view field.

**Physicochemical parameters**

The total, water-soluble and acid-insoluble ashes were determined. The pH of 1% aqueous and ethanolic extracts was also determined. The mineral content and extractive values (determined using cold maceration with ethanol, water, petroleum ether and ethyl acetate) were determined for the stem bark and leaves according to previously established methods (16, 17).

**Phytochemical screening**

Preliminary phytochemical screening were carried out to identify the presence of the major classes of secondary metabolites in the plant samples (13). The total flavonoid and total phenolic contents were determined using the aluminium chloride colorimetric and Folin Ciocalteu methods respectively (18, 19). Five samples each of the stem bark and leaves were used for this assay and the results calculated as the mean ± SD.

**Fluorescence analysis**

A small quantity of the powdered plant sample was mixed with a few millilitres of solvent (water, 95% ethanol, ethyl acetate, chloroform, petroleum ether) or reagent (conc. $\text{NH}_3$, conc $\text{H}_2\text{SO}_4$, conc $\text{HCl}$, $\text{FeCl}_3$, alcoholic KOH, iodine solution or $\text{NH}_3$ solution). The fluorescence colours displayed by the mixtures were observed under long and short wave UV lights as well as in day light and noted (20).

**Elemental Content Analysis**

The presence and quantities of selected minerals and metals in the leaf and stem bark of *U. heudelotii* was assessed by the Energy Dispersive X-ray Fluorescence (17).

**Ultraviolet (UV-Vis) and Infrared (IR) Spectrometry**

At a wavelength range of 200-800 nm and a scan speed of 50 nm/s (PerkinElmer UV spectrophotometer), characteristic UV fingerprints were developed for the methanol stem bark and leaf extracts. Their IR spectra were also obtained from a PerkinElmer (model 1600) Fourier Transform-IR spectrophotometer.

**Results**

**Organoleptic and Macroscopic description**

*U. heudelotii* is a small to medium-sized tree with a dense low-branching crown. It bears simple leaves arranged in whorls of 7 to 8 on a single petiole. Matured leaves were dark green on the upper surface and light green on the lower surface. The leaf laminar was elliptic in shape with an acuminate to obtuse apex, entire margin, cuneate base and pinnate reticulate venation. The leaf was papery in texture and had a glabrous surface (Fig. 1. AB). The powdered leaf was coarse with a characteristic odour and a bitter taste. The stem bark appeared greyish-brown on the outer surface with longitudinal striations, mosses and cracks. It was pale-red on the inside and exuded a reddish sap when bruised (Fig. 1. CD). The stem bark broke with a short or fibrous fracture. Powdered stem bark felt rough, had a characteristic odour and bitter taste. Table 1 summarizes the organoleptic and macroscopic characteristics of the stem bark and leaf.

**Table 1.** Organoleptic and macroscopic features of the leaf and stem bark of *U. heudelotii*

| Parameter       | Leaf                              | Stem bark                              |
|-----------------|-----------------------------------|----------------------------------------|
| Odour           | Characteristic                    | Characteristic                         |
| Colour          | Deep green (adaxial)/Light green (abaxial) | Greyish-brown (outer) Pale red (inner) |
| Texture of powder | Coarse                           | Sandy                                  |
| Origin          | -                                 | Trunk                                  |
| Type            | Simple                            | -                                      |
| Arrangement     | Spiral/whorl                      | -                                      |
| Shape           | Elliptic                          | -                                      |
| Margin          | Entire                            | -                                      |
| Apex            | Acuminate-obtuse                  | -                                      |
| Base            | Cuneate                           | -                                      |
| Venation        | Pinnate                           | -                                      |
| Surface         | Glabrous                          | Moss, Cracks Longitudinally striated    |
Microscopic description

The leaf lamina displayed anticlinal polygonal straight-walled epidermal cells on the adaxial and abaxial surfaces. The leaf was hypostomatous with anisocytic stomata distributed only on the lower surface (Fig. 2. AB). Both surfaces were glabrous with no trichomes. Reticulate venation pattern with generally four-sided vein-islets and few branched free ending ultimate endings (veinlet terminations) were observed (Fig. 2. C). The transverse section (T/S) of the leaf midrib had an almost circular outline and a slightly protruded adaxial surface forming a pear-like shape (Fig. 2. D). Below the cuticle was a row of irregularly shaped epidermal cells. About four to five rows of closely packed polygonal collenchyma cells, followed by loosely packed isodiametric parenchyma cells were dispersed in the cortex. Tanniferous cells were found among parenchyma in the cortex. The vascular bundle was arranged in a circular system surrounded by a sheath of ruffled lignified sclerenchyma in the core. An arch-shaped lignified xylem tissue was observed at the centre (Fig. 2. DE). The T/S of the petiole showed a similar cellular arrangement as that of the midrib. Epidermal cells in a single row were followed by about six to eight rows of tightly packed collenchyma cells. Loosely packed parenchyma cells were observed in the cortex. Lignified sclerenchyma

| Texture | Papery | Rough |
|---------|--------|-------|
| Fracture | -       | Short (outer surface) Fibrous (inner surface) |
| Petiole | Petiolate | -   |
| Average length of leaf/cm | 21.83 ± 3.04 | - |
| Average width of leaf/cm | 7.11 ± 1.51 | - |

average length is presented as the mean ±SD [n=30]
cells arranged in a circular pattern surrounded a central vascular bundle. Several parenchyma cells occupied the core around the vascular bundles (Fig. 2F). Microscopy of the powdered leaf showed the presence of epidermal cells and starch grains, while the stem bark powder had prismatic-shaped calcium oxalate crystals, stone cells, pitted vessels and fibres (Fig. 2G).

**Quantitative microscopy**
The leaf surface constants evaluated in this study included the stomatal number, stomatal index, vein-islet number, vein termination number and palisade ratio. The result is presented as the mean ± standard deviation (Table 2).

**Physicochemical studies**

| Leaf surface parameter | Results |
|------------------------|---------|
| Stomatal number /mm²   | 6.4 ± 1.67 |
| Stomatal index/%       | 22.1 ± 2.36 |
| Vein islet number / mm²| 6.0 ± 2.20 |
| Vein termination number/ mm² | 7.8 ± 2.58 |
| Palisade ratio/ mm²    | 5.6 ± 0.89 |

values are presented as the mean ± SD [n=5]

The parameters evaluated in physicochemical evaluations of the stem bark and leaf included the solvent extractive values, ash values, pH and elemental content. Results obtained are presented in Table 3.

| Parameter | Leaves | Stem Bark |
|-----------|--------|-----------|
| Extractive values (% w/w) | | |
| Water extract | 25.00 ± 0.338 | 28.00 ± 0.129 |
| Ethanol extract | 28.31 ± 0.318 | 37.31 ± 0.125 |
| Ethyl acetate extract | 35.04 ± 0.464 | 10.24 ± 0.113 |
| Petroleum ether extract | 32.80 ± 0.194 | 6.40 ± 0.191 |
| Ash values (% w/w) | | |
| Total ash | 6.41 ± 0.208 | 5.01 ± 0.258 |
| Acid insoluble ash | 1.10 ± 0.115 | 3.10 ± 0.238 |
| Water soluble ash | 1.08 ± 0.044 | 0.28 ± 0.018 |
| pH determinations | | |
| Water extract | 3.79 ± 0.175 | 3.43 ± 0.070 |
| Ethanol extract | 5.23 ± 0.133 | 4.46 ± 0.095 |
| Elemental analysis (%) | | |
| Calcium (Ca) | 0.480 ± 0.013 | 0.600 ± 0.030 |
| Potassium (K) | 0.924 ± 0.075 | 1.602 ± 0.162 |
| Magnesium (Mg) | 0.264 ± 0.020 | 0.336 ± 0.043 |
| Phosphorous (P) | 0.050 ± 0.001 | 0.117 ± 0.008 |
| Metal content (mg/kg) | | |
| Iron (Fe) | 266.84 ±19.170 | 245.22 ± 0.175 |
| Lead (Pb) | 0.0019 ± 0.0003 | 0.0025 ± 0.0002 |
| Copper (Cu) | 15.54 ± 2.170 | 13.23 ± 1.910 |
| Zinc (Zn) | 23.21 ± 3.075 | 19.19 ± 1.448 |

values are presented as the mean ± SD [n=3]

**Fluorescence analysis**

Characteristic fluorescence emissions by the stem bark and leaf powders in various solvents and reagents under visible and UV light is presented on Table 4.

**Fluorescence analysis of the leaf and stem bark of U. heudelotii**

| Sample + solvent / reagent | Visible light | UV (254nm) | UV (365nm) |
|---------------------------|--------------|------------|------------|
| Powder + Water            | Cream        | Light green | NF         |
| Powder + Ethanol (95%)    | Green        | Pink       | Brown      |
| Powder + Ethyl acetate    | Green        | Pink       | Brown      |
| Powder + Chloroform       | Cream        | Light green | Cream      |
| Powder + Petroleum ether  | Light green  | Pink       | Brown      |
| Powder + Concentrated ammonia | Brown    | Pink       | NF         |
| Powder + Concentrated sulphuric acid | Brown  | NF         | NF         |
| Powder + Ferric chloride  | Green        | Deep green | NF         |
| Powder + Alcoholic Potassium hydroxide | Green    | Green      | NF         |
| Powder + Iodine solution  | Green        | NF         | NF         |
| Powder + Ammonia solution (25%) | Brown    | Green      | NF         |
| Powder + Concentrated hydrochloric acid | Brown | NF         | NF         |
| Powder + Ferric chloride  | Brown        | NF         | NF         |
| Powder + Alcoholic potassium hydroxide | Brown  | Green      | NF         |
| Powder + Iodine solution  | Brown        | Green      | NF         |
| Powder + Ammonia solution (25%) | Brown    | Purple     | NF         |

NF- no fluorescence

**Phytochemical screening**

Qualitative phytochemical screening showed the occurrence of alkaloids, flavonoids, saponins, phytosterols, tannins, coumarins, reducing sugars and triterpenoids in both the leaves and stem bark (Table 5). The total phenolics and total flavonoid contents of the leaf and stem bark were determined using gallic acid (100, 50, 25, 12.5, 6.25, 3.12 µg/mL) and quercetin (100, 50, 25, 12.5, 6.25, 3.12 µg/mL) as
The total phenolic content of the leaf and stem bark were respectively determined to be 219.2 ± 10.013 and 153.9 ± 1.602 mg/g gallic acid equivalent (GAE). The total flavonoid content of the leaf was 1036 ± 33.3 while the stem bark had 310.2 ± 7.9 mg/g quercetin equivalent (QE).

**UV and IR analysis**

The UV spectra (Fig. 4) showed two $\lambda_{\text{max}}$ each at 203/279 nm and 203/281 nm respectively for the stem bark and leaf of *U. heudeilotii*. Prominent absorption was observed at 203 nm. Similar UV absorption patterns were observed for the stem bark and leaf suggesting the presence of similar constituents with extensive conjugated ring systems. Similarly, the IR spectra of both leaf and stem bark extracts showed similar fingerprint with absorption bands mainly at 2900-3300 cm$^{-1}$ (broad) for hydroxyl groups (-OH stretch) and around 1600 cm$^{-1}$ for alkene groups (C=C stretch).

**Discussion**

Pharmacognostic studies comprise various qualitative and quantitative tests performed on crude herbal drugs in order to authenticate or establish their identity, purity and quality (21). In this study, the leaves and stem bark of *U. heudeilotii* were evaluated for pharmacognostic, phytochemical and physicochemical characteristics.

The study of a plant’s morphology through macroscopic and microscopic evaluations are crucial as they serve as the simplest, quickest and easiest means of identi-
fying the plant in its natural habitat and helps in differentiating it from other related species (22). From the results, the presence of hypostomatic leaves with anticalinal polygonal straight-walled epidermal cells are consistent generic features previously observed in the foliar morphology of *Uapaca* species (5). It was reported that pericytic stomata occur in *U. heudelotii* (12). However, in the present report anisocytic stomata having guard cells between two larger subsidiary cells and one distinctly small one were generally observed for this species as was also reported (5). Other studies also report the presence of tetracytic stomata in *U. vanhouottei*, trichomes in *U. togensis*, *U. vanhouitteii* and *U. sansibarica* (12). Apart from the generic anticalinal straight-walled polygonal epidermal cells, the presence of large tan-niferous epidermal cells and absence of trichomes are also distinguishing features observed *U. heudelotii*.

Among the surface constants studied for the leaf, the stomatal index remains relatively constant regardless of the age and habitat of a specific plant species and is very useful for distinguishing species of the same genus. A stomatal index (SI) range of 22.4-34% was previously reported for *Uapaca* species (12). In this study, the SI recorded was 22.1%, which falls in range with this previous report.

For easy transportation and commercial purposes, most crude plants are processed into their powdered forms. Establishing standards for powdered plant materials through microscopy and physicochemical analysis is therefore important as it aids in the detection of adulterants in powdered crude drugs (23). Fibres, epidermal cells, stone cells, calcium oxalate crystals and starch grains were observed in powdered samples. The presence of calcium oxalate crystals was also reported in *U. staudtii* and *U. togensis* (12).

The estimation of extractive values determines the extractive power of specific solvents i.e. the amount of chemical constituents extractable by a particular solvent under specified conditions (17). From the results obtained, ethyl acetate and petroleum ether had the highest extractive power for the leaves while ethanol and water afforded high extractive yields for the stem bark. This implies that the constituents of the leaves may be less/mid-polar in nature while the stem bark has more polar constituents.

The ash content of a crude drug is the amount of insoluble inorganic matter naturally occurring with the plant or added to it deliberately to adulterate it for financial gains (24). The acid-insoluble ash particularly specifies adulteration with siliceous materials while the water-soluble ash gives information on the possibility of previous extraction with water (25). The leaves and stem bark had a total ash of about 5% and 6% respectively, implying that for 2 g of dried powdered leaf or stem bark of *U. heudelotii* the residual matter including non-volatile impurities must be approximately less than 10%. The acid-insoluble ash obtained was between 1-3% which is quite favourable as it indicates low amounts of inorganic matter naturally adhering to the crude drug. The values would be useful in determining the quality of crude samples of *U. heudelotii*.

The average pH values obtained from aqueous and ethanolic extracts were relatively acidic (pH = 3.7 - 5.2). The aqueous extracts being more acidic than the ethanol extracts.

Qualitative phytochemical screening gives an indication of the major classes of phytoconstituents in a plant sample which also contribute to its biological effect. The current results were consistent with previous reports on the stem bark and leaf of *U. heudelotii* (8, 10). The presence of polyphenols in the plant may contribute significantly to the plant’s therapeutic effect in traditional medicine such as its use in the treatment of skin, respiratory, urinary and gastrointestinal infections. In a previous study, flavonoids from the stem bark of *U. heudelottii* were shown to possess significant antibacterial activity (10). Polyphenols such as flavonoids, tannins and coumarins have been shown to complex bacterial cell membrane proteins, interfere with bacterial adhesion and cause enzyme deactivation leading to the death of bacteria (26, 27).

Foods or herbs containing minerals such calcium (Ca), phosphorous (P), magnesium (Mg), potassium (K), zinc (Zn), copper (Cu) and iron (Fe) are essential for the proper functioning of the human body and aids in the prevention and treatment of several diseases (28, 29). Fe is required for the normal production of oxygen-carrying red blood cells (haemoglobin), needed for energy supply, normal immune function and to prevent anaemia. Cu is required for the maintenance of healthy bones, nerves and blood vessels and also aids in iron utilization and immune function (30). Heavy metals such as lead (Pb) on the other hand, are environmental pollutants and pose a great threat to human health (31). Ingestion of large amounts of Pb is detrimental to the immune, nervous, skeletal, renal, cardiovascular and reproductive systems resulting in severe damage to the heart, kidneys, brain and reproductive organs (30). Exposures to lead both prenatally and at early childhood is linked to lower intelligence, learning deficits and impaired motor function in children (30). The highest permissible limit of lead (Pb) in medicinal herbs is 10 mg/kg according to the Food and Agriculture Organization (FAO) (3). The content of lead detected in the leaf and stem bark of *U. heudelotii* were 0.0019 and 0.0025 mg/kg respectively which falls in range of permissible limit. Nevertheless, the elemental content of any plant drug may vary according to its geographical location, mineral composition of the soil, climatic conditions as well as human activities (30). It must however be noted that risk of danger associated with the consumption of heavy metals depends on the average daily dietary intake.

**Conclusion**

The present pharmacognostic studies on the leaves and stem bark of *U. heudelotii* has provided standard macro and micro-morphological features and physicochemical properties that can aid in authentication of both whole and powdered samples of the plant. Major classes of secondary metabolites including phenolic compounds, saponins, alkaloids, triterpenes and sterols were identified. Future aspects of this research may consider investigation of the
pharmacognostic and physicochemical features of other closely related species in order to make a clear distinction among common species in the region. Further phytochemical analysis shall consider identification of specific constituents in the plant.

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Authors contributions

KS and AYM conceptualized, designed and coordinated the study. Material collection macroscopic and microscopic analysis were conducted by AKO and LG. Phytochemical, physicochemical and fluorescence analysis were done by EAK and AYM. All authors contributed to writing the manuscript, reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Khan MS, Ahmad I. Chapter 1- Herbal medicine: current trends and future prospects. In: Mohd SAK, Iqbal A, Debprasad C. Editors. New Look to phytomedicine [e-book]. Academic Press; 2019 [cited 2021 Aug 3]; 3-13. https://doi.org/10.1016/B978-0-12-814619-4.00001-X

2. Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacogn Phytochem. 2014;2(5):69-73.

3. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues [Internet]. Geneva: World Health Organization; 2007 [cited 2021 Aug 10]. Available from: https://apps.who.int/iris/handle/10665/43510

4. Lemmens, RHMJ. Uapaca heudelotii Baill. In: Lemmens, RHMJ, Louppe, D, Oteng-Amoako, AA. Editors. Plant Resources of Tropical Africa / Ressources végétales de l’Afrique tropicale [Internet]. Netherlands: Wageningen; 2012 [cited 2021 Aug 3]. Available from: http://www.prota4u.org/search.asp

5. Levin GA. Systematic foliar morphology of Phyllanthoideae (Euphorbiaceae). I. Conspicuous. In: Annals of the Missouri Botanical Garden [e-book]. Vol. 73. St. Louis: Missouri Botanic Gardens Press; 1986 [cited 2021 Aug 10]; 29-85. https://doi.org/10.2307/2399139

6. Breteler F.J. Uapaca (Phyllanthaceae) in the Guineo-Congolian forest region: a synoptic revision. Plant Ecol Evol. 2013;146(1):75-94. https://doi.org/10.5091/plecevo.2013.770

7. Burkhill H. Amphimas pterocarpoides Harms (Family: Leguminosae -Papilionoideae). In: The useful plants of west tropical Africa [e-book]. Vol. 3. Kew: Royal Botanical Gardens; 1985. [cited 2021 Aug 3] Available from: http://plants.jstor.org/stable/10.5555/al.ap/upwta.3_383

8. Ngbolua K, Tshibangu D, Mpiana P, Mihigo S, Mavakala B, Ashande M et al. Anti-sickling and antibacterial activities of some extracts from Gardenia ternifolia subsp. jovis-tonantis (Welw.) Verdc.(Rubiacaeae) and Uapaca heudelotii Bail.(Phyllanthaceae). J Adv Med Pharm Sci. 2015;2(1):10-19. https://doi.org/10.9734/JAMP/2015/13427

9. Achika JI, Ayo RG, Oyewale AO, Habila JD. Antibacterial activity of fractions of Uapaca heudelotii Bail. Synergistic effect with ciprofloxacin. Albanian J Pharm Sci. [Internet]. 2018 [cited 2021 Aug 14]; 11:1-7.

10. Achika JI, Ayo RG, Oyewale AO, Habila JD. Flavonoids with antibacterial and antioxidant potentials from the stem bark of Uapaca heudelotii. Helyon. [Internet]. 2020 [cited 2021 Aug 14]; 6(2):e03381. https://doi.org/10.1155/2018/1920198

11. Kambale JK, Ngolua KN, Mpiana PT, Mudogo V, Tshibangu DST, Wumba DMR et al. Evaluation in vitro de l’activité antifalcémiant et effet antioxydant des extraits d’Uapaca heudelotii Bail. (Euphorbiaceae). Int J Biol Chem Sci. 2017;7(2):523-34. https://doi.org/10.4313/ijbcs.v7i2.9

12. Kadiri A, Ayodele A, Olowokudejo J, Uchemunefia DJ. Comparative leaf epidermal morphology of five West African species of Uapaca Bail (Phyllanthaceae Pro Forma Euphorbiaceae). Niger J Bot. [Internet]. 2013 [cited 2021 Aug 14]; 7:54-60. Available from: https://plantsbotomast.wordpress.com/2015/10/19/stomatina-uapaca/

13. Evans WC. Trease and Evans Pharmacognosy. 15th ed. London: Elsevier limited; 2002

14. Baidoo MF, Asante-Kwiatia E, Mensah Ay, Sam GH, Amponsah IK. Pharmacognostic characterization and development of standardization parameters for the quality control of Entada africana Guill. & Perr. J Appl Res Med Aromat Plants. 2019;12:36-42. https://doi.org/10.1016/j.jarmap.2018.11.003

15. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines-A review. Int J Biodivers Conserv. 2012;4(3):101-12. https://doi.org/10.5897/JBCC11.163

16. Adjei S, Entsua-Mensah P, Amponsah IK, Baah MK, Kwakye NAA, Addae-Kyereme NYK. Pharmacognostic and physicochemical studies of the leaves of Holsolegia opposita Vahl (Lamiaceae). J Pharmacogn Phytochem. 2020;9(5):2996-3001. Available from: https://www.phytojournal.com/archives/2020/vol9issue5/PartAP/9-5-S047-475.pdf

17. Kitcher C, Mireku-Gyimah NA, Bekoe EO, Sarkodie JA, Frimpong-Manso S, Tattah G et al. Crude drug analysis and elemental content of the leaves and stem bark of Adansonia digitata L. (Malvaceae), an indigenous Ghanaian medicinal plant. Plant Sci Today. 2021;8(2):264-72. https://doi.org/10.14719/pst.2021.8.2.1027

18. Blainski A, Lopes GC, De Mello JCP. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limuminium brasilense L. Molecules. 2013;18(6):6852-65. https://doi.org/10.3390/molecules18066852

19. Chang C-C, Yang M-H, Wen H-M, Chern J-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-82. https://doi.org/10.38212/2224-6614.2748

20. Muthukrishnan S, Sivakkumar T. Physicochemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of Schleichera oleosa (Lour.) Oken. Ind J Pharm Sci. 2018;80(3):525-32. https://doi.org/10.4172/pharmaceutical-sciences.1000387

21. Dutu L. Pharmacognostic methods for analysis of herbal drugs, according to European Pharmacopoeia. In: Purusotam B. Editor. Promising Pharmaceuticals [e-book]. Romania: Bucharest; 2012 [cited 2021 Aug 10]: 38-62.

https://plantscience.today.online
22. Asante-Kwafie E, Mensah AY, Baidoo FM, Asomaning AG. Quality control standardization of the leaves and root of *Landolphia owariensis* (Apocynaceae). *J Phytopharmacol.* 2019;8(4):185-91. https://doi.org/10.31254/phyto.2019.8407

23. Vignesh RM, Sumitha VR. Macro and microscopic evaluation of *Gmelina arborea* Roxb.–A botanical pharmacognostic approach for quality control of raw drug material. *Plant Sci Today*. 2020;7(1):55-60. https://doi.org/10.14719/pst.2020.7.1.648

24. Mandal AK, Sujith T, Rajesh A, Divya KG, Sunilkumar KN, Shakila R. Powder microscopic, physicochemical and chromatographic approach for the quality control of anti-hypertensive drug *Rattha Piththathirku Kudinir Chooranam*. *Plant Sci Today*. 2021;8(3):604-49. https://doi.org/10.14719/pst.2021.8.3.1137

25. Bhusnure OG, Suryawanshi S, Swamy SV, Gholve SB, Girm PS, Birajdar MJ Standardization and quality evaluation of herbal drugs. *J Drug Deliv Ther.* 2019;9(3)-s:1058-63. https://doi.org/10.22270/jddt.v9i3-s.2941

26. Daglia M. Polyphenols as antimicrobial agents. *Curr Opin Biotechnol.* 2012;23(2):174-81. https://doi.org/10.1016/j.copbio.2011.08.007

27. Coppo E, Marchese A. Antibacterial activity of polyphenols. *Curr Pharm Biotechnol.* 2014;15(4):380-90. https://doi.org/10.22270/jddt.v9i3-s.2941

28. Mahood HE. Estimation of essential elements and mineral in *Catharanthus roseus* and its biological importance as a medicinal plant. *Plant Cell Biotechnol Mol Biol*. 2021;22(25):1-7.

29. Bhat R, Kiran K, Arun A, Karim AJ. Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. *Food Anal Methods*. 2010;3(3):181-87. https://doi.org/10.1007/s12161-009-9107-y

30. Abadin H, Ashizawa A, Stevens YW, Llados F, Diamond G, Sage G et al. Toxicological profile for lead. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2007. [cited 2021 Aug 3] Available from: https://www.ncbi.nlm.nih.gov/books/NBK158762/

31. Dghaim R, Al Khatib S, Rasool H, Ali Khan MJ. Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. *J Environ Public Health [Internet]*. 2015. https://doi.org/10.1155/2015/973878