



RESEARCH ARTICLE

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 leaf and bark was 6.41 ± 0.208 and 5.01 ± 0.258 respectively. The pH values 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

Phytotherapy 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.

Ethnomedicinal 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 standard parameters for its quality control. Previous studies focused on investigating the foliar morphology of some *Uapaca* species for taxonomical purposes (5, 12). In this study, the pharmacognostic and physicochemical characteristics, elemental content, phytochemical, spectroscopic and fluorescence patterns of the stem bark and leaves of *U. heudelotii* were evaluated.

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 Folin 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 (CH₃Cl) 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/SB004 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 fresh 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).

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 \pm 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. NH_3 , conc H_2SO_4 conc, HCl, FeCl_3 , alcoholic KOH, iodine solution or 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. heudelottii* 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. heudelottii 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

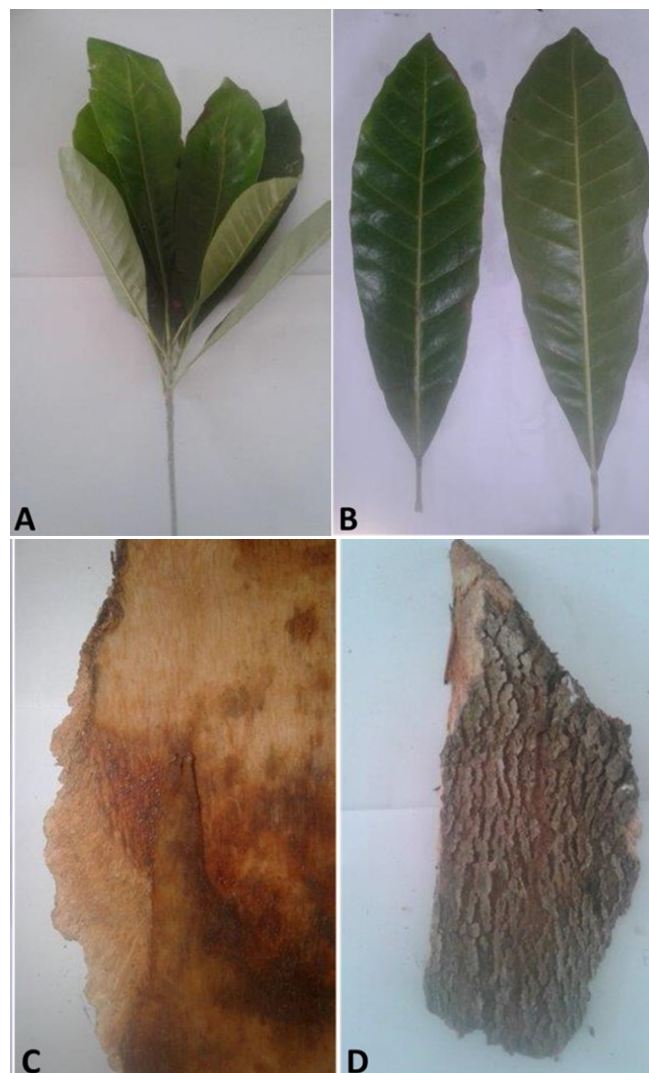


Fig. 1. Leaf and stem bark of *U. heudelottii* [A- leaf whorl, B- upper and lower surfaces of leaf, C- inner bark, D- outer bark].

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. heudelottii*

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

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]

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

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

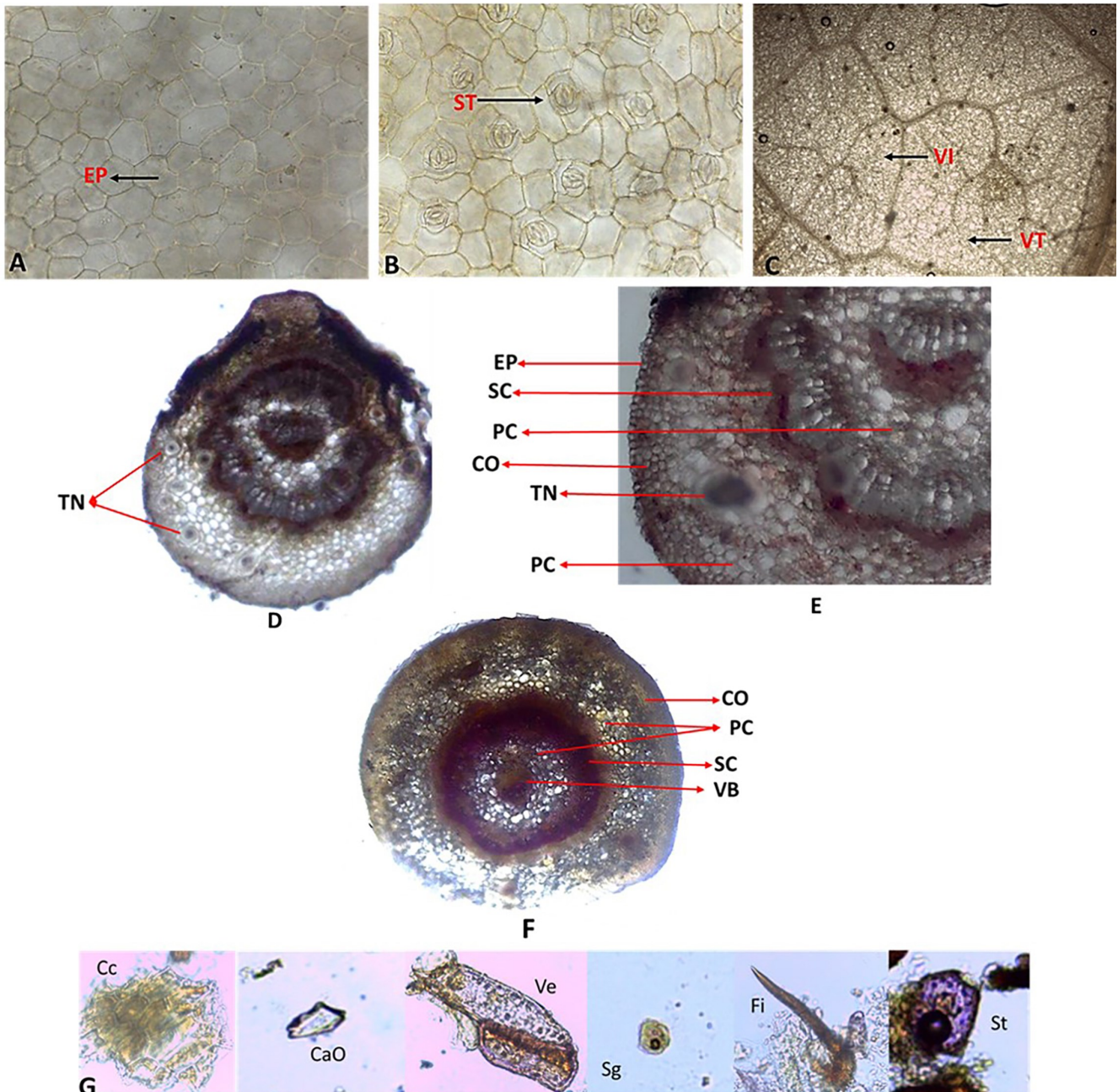


Fig. 2. Microscopy of the leaf and stem bark of *U. heudelotii* [A- upper surface of fresh leaf with straight-walled epidermal cells; B- lower surface of fresh leaf with anisocytic stomata; C- veinlet terminations and vein islets; D, E- transverse section (T/S) of the midrib; F- T/S of the petiole; G- microscopy of powdered leaf and stem bark; [EP- epidermal cells, PC- parenchyma cells, CO- collenchyma cells, SC- sclerenchyma cells, TN- tanniferous cells, ST- stomata, VI- vein islet, VT- veinlet termination, VB- vascular bundle, Sg- starch grain, Stc- stone cells, CaO- Calcium oxalate crystal, Ve- pitted vessel, Fi- fibre].

were glabrous with no trichomes. Reticulate venation pattern with generally four-sided vein-islets and few branched

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

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 \pm standard deviation (Table 2).

Physicochemical studies

Table 2. Leaf surface constants for the fresh matured leaf of *U. heudelotii*

Leaf surface parameter	Results
Stomatal number /mm ²	6.4 \pm 1.67
Stomatal index/%	22.1 \pm 2.36
Vein islet number / mm ²	6.0 \pm 2.20
Vein termination number/ mm ²	7.8 \pm 2.58
Palisade ratio/ mm ²	5.6 \pm 0.89

values are presented as the mean \pm 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.

Table 3. Physicochemical evaluation of the leaf and stem bark of *U. heudelotii*

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

values are presented as the mean \pm 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.

Table 4. Fluorescence analysis of the leaf and stem bark of *U. heudelotii*

Sample + solvent / reagent	Visible light	UV (254nm)	UV (365nm)
<i>U. heudelotii</i> Leaves			
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 + Concentrated hydrochloric acid	Green	Deep green	NF
Powder + Ferric chloride	Green	Green	NF
Powder + Alcoholic Potassium hydroxide	Green	Green	NF
Powder + Iodine solution	Green	NF	NF
Powder + Ammonia solution (25%)	Brown	Green	NF
<i>U. heudelotii</i> Stem bark			
Powder + Water	Reddish brown	Brown	Brown
Powder + Ethanol (95%)	Brown	Pink	NF
Powder + Ethyl acetate	Light green	Pink	Brown
Powder + Chloroform	Cream	Light	Cream
Powder + Petroleum ether	Light green	Pink	Light pink
Powder + Concentrated ammonia	Reddish brown	NF	NF
Powder + Concentrated sulphuric acid	Brown	NF	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

Table 5. Phytochemical constituents of the leaf and stem bark of *U. heudelotii*

Phytochemical	Test	Leaves	Stem bark
Reducing sugars	Fehling's test	+	+
Alkaloids	Dragendorff's test	+	+
Coumarins	Fluorescence test	+	+
Flavonoids	Alkaline reagent test	+	+
Saponins	Frothing test	+	+
Phytosterols	Lieberman Buchard's test	+	+
Tannins	Ferric chloride test	+	+
Terpenoids	Salkowski's test	+	+

key: (+): detected

total flavonoid contents of the leaf and stem bark were determined using gallic acid (100, 50, 25, 12.5, 6.25, 3.12 μ g/mL) and quercetin (100, 50, 25, 12.5, 6.25, 3.12 μ g/mL) as

reference substances respectively. Thus, the TPC was calculated as milligram of gallic acid equivalent and TFC as milligram quercetin equivalent/gram of dried extract based on their respective standard calibration curves (Fig. 3. AB). The

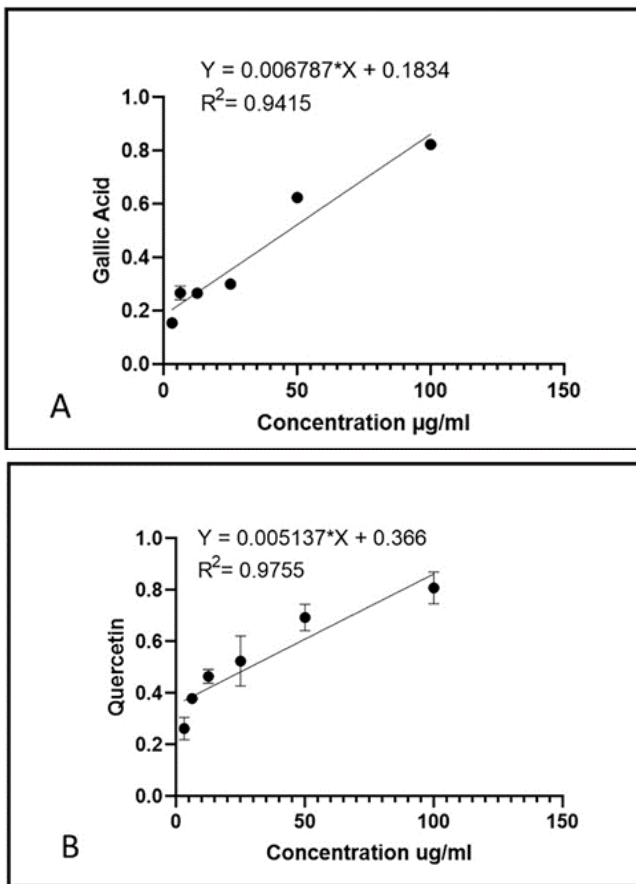


Fig. 3. Calibration curves for gallic acid (A) and quercetin (B) for determination of total phenolic and total flavonoid contents [concentrations used: 3.12, 6.25, 12.5, 25, 50, 100 $\mu\text{g/mL}$].

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 λ_{max} each at 203/279 nm and 203/281 nm respectively for the stem bark and leaf of *U. heudelotii*. Prominent absorption was observed at 203 nm. Similar UV absorption patterns were observed for the stem

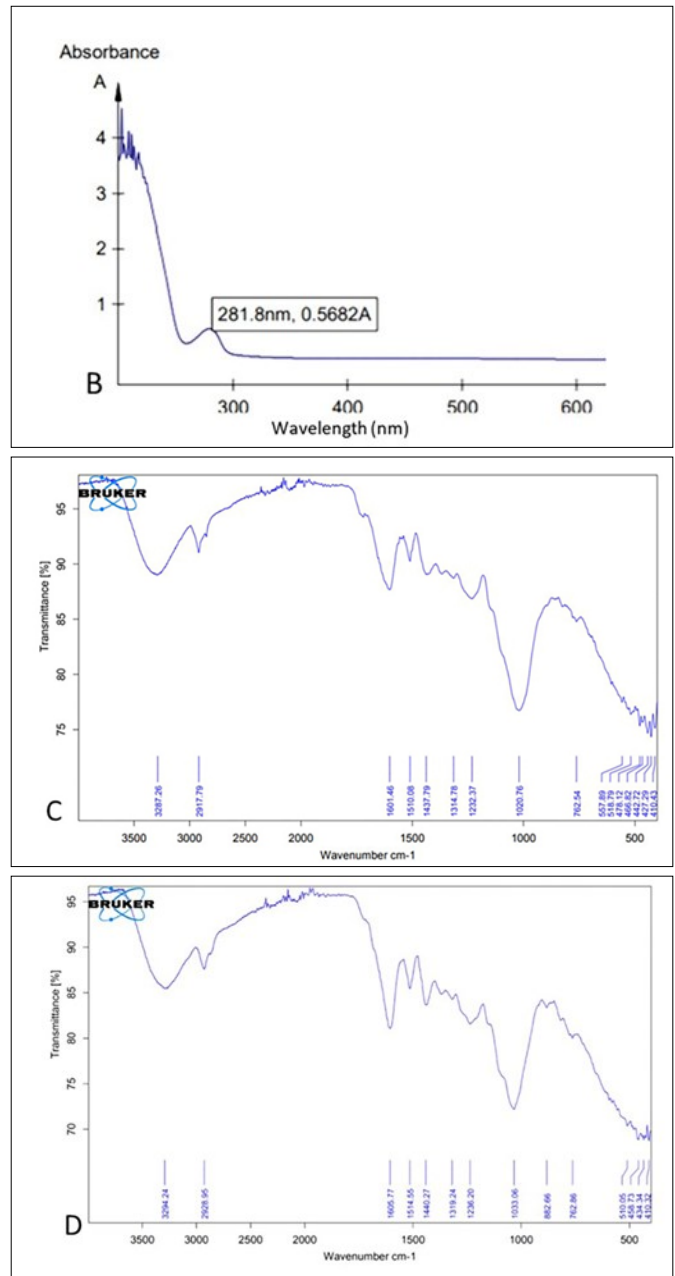
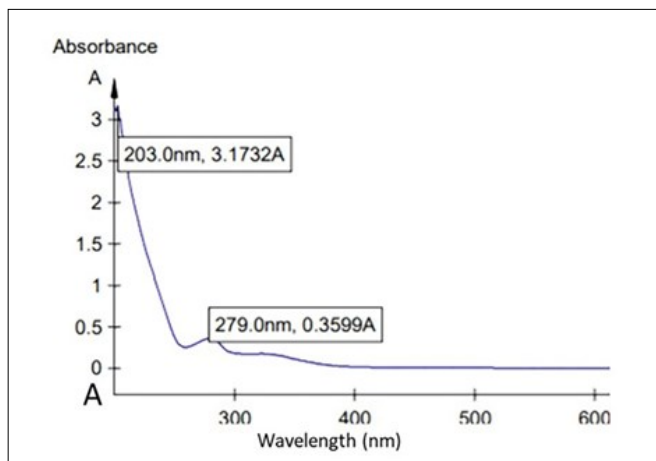


Fig. 4. UV spectra of the of the leaf (A) and stem bark (B) MeOH extracts; IR spectra of the leaf (C) and stem bark (D) MeOH extracts

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. heudelotii* 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-

ifying 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 anticlinal 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. heudelottii* (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. vanhouttei*, trichomes in *U. togoensis*, *U. vanhoutteii* and *U. sansibarica* (12). Apart from the generic anticlinal straight-walled polygonal epidermal cells, the presence of large tanniferous epidermal cells and absence of trichomes are also distinguishing features observed *U. heudelottii*.

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. togoensis* (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. heudelottii* 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. heudelottii*.

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. heudelottii* (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. heudelottii* 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. heudelottii* 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

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