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Quality control standardization of the leaves and root of *Landolphia owariensis* (Apocynaceae)

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ABSTRACT

The leaves and roots of *Landolphia owariensis* have received much attention for their incredible ethno-medicinal uses and are widely commercialized as aphrodisiac, anti-infective and anti-inflammatory agents in West Africa. A systematic evaluation of the leaves and roots was carried out in order to set standards for its identification and quality control. The organoleptic, macro-morphology and micromorphology of whole and powdered leaves and roots were carried out. Physicochemical, phytochemical, fluorescence analysis and preliminary HR-LC/MS analysis were also performed.From organoleptic and macro-morphological studies, the leaf was identified to be ovate in shape with an acuminate apex, symmetrical base, entire margin and reticulate venation. Characteristic microscopic features of the leaf lamina, petiole and midrib include the presence of hypostomatic anomocytic stomata, wavy walled epidermal cells, a concentric vascular bundle displaying xylem and phloem cells, unicellular clothing trichomes and secretory cells. Prismatic calcium oxalate crystals, broken stomata, stone cells, pitted vessels, fibers, cork cells and unicellular clothing trichomes are found in the leaf and root powder. Various solvent soluble extractives, ash content, moisture content, pH and fluorescence characteristics were determined. Tannins, saponins, triterpenes and alkaloids were the major phytoconstituents identified. The essential diagnostic attributes of *L. owariensis* established in this study are useful for the authentication and quality control of the plant.

Keywords: *Landolphia owariensis*, pharmacognostic, physicochemical, phytochemical, morphology, HR-LC/MS.

INTRODUCTION

Over the years, the commercialization of medicinal plant products by pharmaceutical, cosmeceutical and neutraceutical industries has gained global interest and popularity due to the wide acceptance and perceived safety of herbal medicines. In spite of this, the lack of standard quality control parameters for the authentication of useful plant materials is a great hurdle in popularizing time-tested herbal-based medicines [1]. Standardization of crude drugs of plant origin is therefore important as this will fulfill the basic requirements of having products with reproducible safety and efficacy [2].

Landolphia owariensis (P. Beauv) of the family Apocynaceae is an edible woody climber usually found in the tropics of Africa. It takes the form of a bush when growing in open savannah but becomes a vine when growing in forested areas reaching heights of about 70 m [3]. The roots and leaves are used traditionally to treat gonorrhea, sexual dysfunction, arthritis, lumbago, fever, malaria, intestinal worms and stomach aches [4]. Previous scientific studies have shown that various parts of *L. owariensis* possess anti-inflammatory, anti-nociceptive, antioxidant, antimicrobial and hepato-protective properties [4-6]. Its latex has also been shown to enhance colon specific controlled drug release when applied as a secondary coating upon hard gelatine capsules [7]. Flavonoids and phenolic acids have been identified as the major constituents of the edible seed pulp [8, 9].

On the Ghanaian market, *Landolphia* species have received much attention for their incredible ethno-medicinal uses and are widely commercialized as aphrodisiacs. For such a widely commercialized herb with huge prospects for exploitation and utilization in the herbal industry, the unavailability of standardization parameters promotes the unethical practice of altering or substituting the authentic plant material with inferior products as a result of carelessness, ignorance or for financial gain [2]. Moreover, the processes of preparation, storage and transport of plant materials may result in the decomposition, contamination and variation in composition of bioactive constituents, leading to little or no therapeutic efficacy [10]. Thus said, this study examines the pharmacognostic, phytochemical and physicochemical properties of the leaves and roots of *L. owariensis* in order to develop standard parameters for its

authentication and quality control.

MATERIALS AND METHODS

Plant collection and processing

The fresh leaves and roots of *L. owariensis* were harvested from the Botanical Gardens of Kwame Nkrumah University of Science and Technology (KNUST) in January, 2018. The samples were authenticated by Dr. George Henry Sam of the Herbal Medicine Department, KNUST and herbarium specimens with the voucher numbers KNUST/HM1/2018/L011 and KNUST/HM1/2018/R021 for the leaf and root respectively were deposited at the Faculty of Pharmacy and Pharmaceutical Sciences herbarium. Fresh leaves and roots were used for macroscopic and microscopic analysis. About 400 g of leaves and roots were shade dried for a week, milled into a coarse powder and kept in at ambient temperature until ready for use.

Extraction of plant materials

A methanol extract of the root, which is the most commercialized part, was prepared as follows. Fifty grams (50 g) of the dried powdered roots of *L. owariensis* was cold macerated with 150 mL of methanol for 24 h. The filtrate obtained was concentrated on a rotary evaporator at low temperature. This afforded a light yellow oily (1.45 g) extract labeled LO-MeOH.

Organoleptic and macroscopic evaluation

For organoleptic studies, various sensory parameters of the fresh leaves and roots such as texture, colour, taste, shape, odour and size were evaluated. The type of leaf, size, presence or absence of petiole and characteristic features of the leaf lamina including the shape, apex, base, margin and venation were recorded in macroscopic studies. To determine the average length and width of the leaf, fifty fresh leaves were randomly selected measured. The fresh root was studied for its type, fracture, curvature and surface features [11, 12].

Qualitative microscopy and histological studies

Free-hand thin sections of the leaf lamina and transverse sections of the midrib and petiole were made with a razor and cleared by boiling in 80% chloral hydrate for about an hour until the green pigment was lost. The sections were observed under a microscope (Leica light microscope DM 1000 LED, Wetzlar, Germany) fitted with a camera (Leica ICC50 HD, Jos Hansen and Soehne Gmbh, Germany). Sections were mounted unstained in 5% glycerin or stained with some reagents in order to identify specific cell types. The reagents used included 0.1% phloroglucinol plus a drop of concentrated hydrochloric acid for the observation of lignified cells, Sudan III for the observation of fat in oil cells and N/50 iodine solution for the observation of starch granules. Characteristic histological or morphological features of the leaf such as types of stomata, trichomes and epidermal cells and other cell types were studied at various magnifications. From the fresh root, thin sections were obtained and studied microscopically [10, 11].

Quantitative microscopy

With the aid of a camera lucida and a stage micrometer, quantitative leaf surface parameters including the stomatal number, stomatal index, palisade ratio, veinlet terminations and vein-islets numbers were determined from sections of the fresh cleared leaf lamina following a previous methods as described by Amponsah *et al.* (2014) [13]. Six replicates were obtained from different fields of view and the results presented as a range from the lower to the upper limits.

Powder microscopy

Different staining reagents such as N/50 iodine, Sudan III and 0.1% phloroglucinol plus a drop of concentrated hydrochloric acid were added to the dried powdered leaves and roots and studied microscopically. Characteristic structures and cell components (ergastic cell contents) were observed and photomicrographs were taken at various magnifications [12].

Phytochemical screening

The major classes of phytochemical constituents in the leaf and roots were evaluated according to established methods [12].

Physicochemical analysis

The physicochemical features including the total ash, water-soluble ash and acid-insoluble ash, pH of 1% solvent extracts, solvent soluble extractives and moisture content (loss on drying) were analyzed for the leaves and roots following standard pharmacognostic methods for analysis of herbal drugs as described in the European Pharmacopoeia [2, 14]. The experiments were carried out in triplicate and the results expressed as the mean ± standard deviation.

Fluorescence analysis

Fluorescence analysis of the samples was carried out by treating the powdered plant samples with various solvents and chemical reagents bearing acidic and basic groups on clean non-fluorescence glass slides and the fluorescence pattern of the solution was monitored in visible light as well as under short and long ultraviolet (UV) light [2].

HR-LC/MS analysis of methanol extract of the root of *L. owariensis*

HR-LC/MS analysis of the methanol root extract was carried out on a LTQ Orbitrap spectrometer (Thermo Fisher, USA) equipped with a HESI-II source. The spectrometer was equipped with an Agilent 1200 (Santa Clara, USA) HPLC system consisting of a pump, PDA detector, column oven (30 °C) and an auto-sampler. The spectrometer was operated with nominal mass resolving power of 60,000 at *m/z* 400 with a scan rate of 1 Hz. N_2 served as sheath gas and Ar was used as collision gas. The following parameters were used for experiments: sheath gas 55 arbitrary units, auxiliary gas 8 arbitrary units, spray voltage 5 kV, capillary temperature 300 °C, capillary voltage 20 V and tube lens 100 V. The analytical HPLC separations were performed according to our previously described method [15].

RESULTS AND DISCUSSION

The standardization of a crude drug involves the process of prescribing a set of innate characteristics, constant parameters as well as definitive quantitative values that can assure consumers of the quality, efficacy and safety of herbal products [1]. In this study, the leaves and roots of *L. owariensis*, a popularly known commercialized herb in West Africa have been standardized based on anatomical, pharmacognostic, physicochemical and phytochemical properties and the results are discussed as follows.

Organoleptic and macro-morphological description of leaf and root

Organoleptic and macro-morphological studies make available the simplest and quickest means to establish the identity of a plant in its natural habitat based on sensory perception. These features give a preliminary indication about any quality variations or substitution of

closely related species [16]. Fig. 1 displays the upper and lower leaf surfaces as well as the inner and outer root of *L. owariensis*. The detailed organoleptic and macro-morphological features are outlined on Table 1.

Table 1: Organoleptic and macro-morphology of the leaf and root of *L. owariensis*

Parameter	Leaf	Root
Taste	Bitter	Sweet
Odour	Characteristic	Aromatic
Colour		
Upper /Outer	Deep green	Yellowish brown
Lower/Inner	Green	Buff
Texture of powder	Coarse	Smooth
Type	Simple, petiolated	Tap
Leaf arrangement	Opposite	
Shape	Oblong-ovate	Cylinderical
Margin	Entire	-
Apex	Acuminate	-
Base	Asymmetric	-
Venation	Reticulate	-
Length	6-10 cm	10-13 cm
Width	2-4 cm	1-3 cm
Thickness	-	1.-1.5 cm
Surface	Glabrous	Rough
Fracture	-	Fibrous

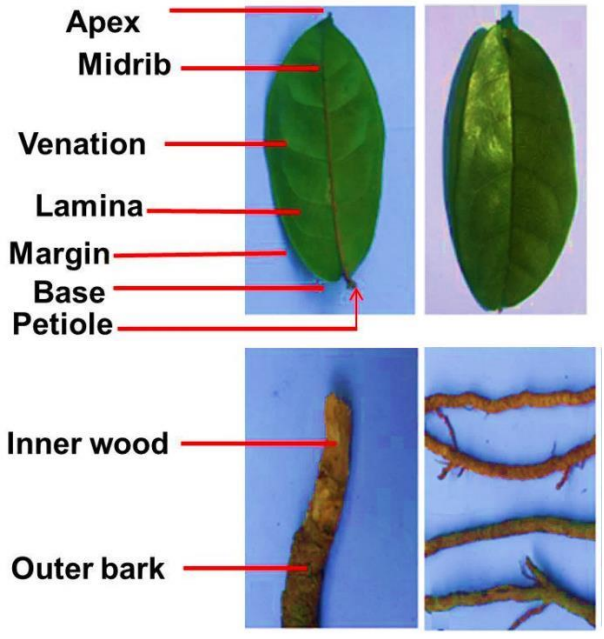

Figure 1: A- upper surface; **B**- lower surface; **C**, **D**- roots of *L. owariensis*

Microscopy of the fresh leaf lamina

Plant species belonging to the same genus may be macro-morphologically similar, hence may require further studies of the internal anatomy for correct identification and differentiation. The microscopy of fresh cleared leaf of *L. owariensis* revealed that, the upper and lower epidermises are composed of wavy walled epidermal cells (Fig. 2A). Anomocytic stomata, which are a type of stomata with three or four equally sized subsidiary cells are found on the upper epidermis but absent on the lower epidermis (Fig. 2B). Thus the leaf is said to be epistomatous. Vein islets and vein terminals are also identified on the upper surface (Fig. 2C).

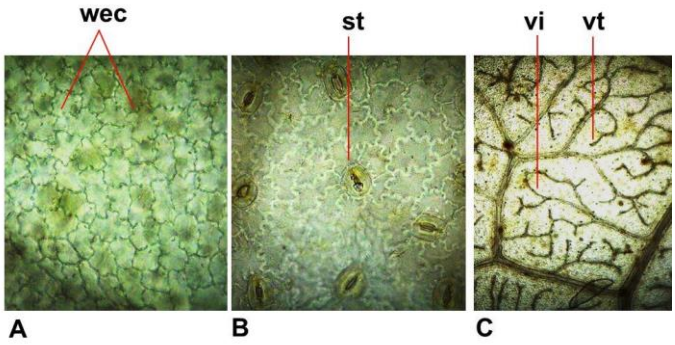

Figure 2: Microscopy of *L. owariensis* leaf lamina: **wec**- wavy walled epidermal cells, **st**- anomocytic stomata **vi**- vein islet, **vt**- veinlet terminations (×100)

T/S of the midrib and petiole

The transverse section (T/S) of the midrib displays a cup-shaped midrib base (dorsal surface) with a double layered upper epidermal cell enveloped in a thick cuticle (Fig. 3). An array of parenchymatous cells (Fig. 3A) bulges in the slight protrusion on the ventral surface of the midrib and create a bridge between the orthogonal lying palisade cells on its left and right side. A long set of vascular bundle is observed on the dorsal surface whiles a shorter set is observed on the ventral surface. The vascular bundle is concentric, which is a type of conjoint vascular bundle with one vascular element completely surrounding the other. In this case, the phloem completely surrounds the xylem. After staining with 0.1% phloroglucinol-HCl, the lignified xylem vessels stained red whiles the phloem remained clear (Fig. 3B). At the centre of the vascular bundle lays a cluster of secretory cells observed as sacs filled with yellowish substance (Fig. 3C).

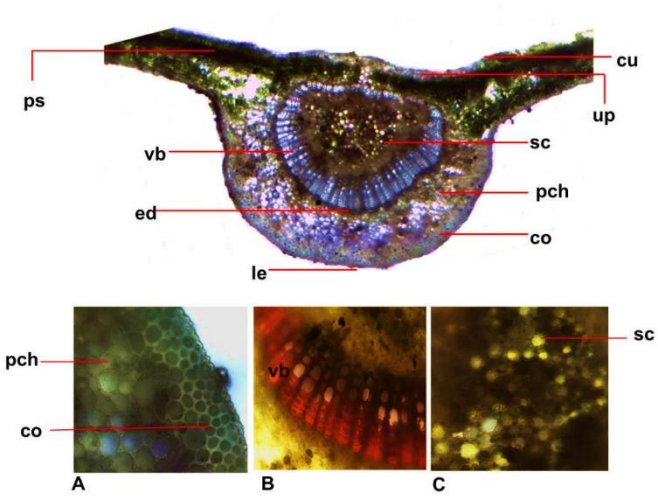

Figure 3: T/S of *L. owariensis* leaf midrib (×10); **cu**-cuticle, **ps**- palisade cell, **pch**- parenchyma cells, **up**- upper epidermis, **ed**- endodermis, **le**- lower epidermis, **co**- collenchyma, **vb**- vascular bundle, **sc**- secretory cells (×40)

Microscopy of the T/S of the petiole revealed an epidermis composed of a single layer of epidermal cells surrounded by a thin cuticle on the exterior surface. Beneath the epidermal cells lie several layers of collenchyma which appear as angled cells with thickened corners. An array of multicellular uniseriate clothing (non-glandular) trichomes is notably present on the exterior surface of the cuticle (Fig. 4).

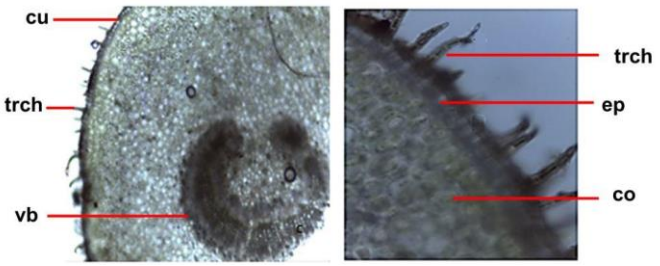

Figure 4: T/S of *L. owariensis* leaf petiole: **cu**- cuticle, **trch**- multicellular uniseriate clothing trichomes, **co**- collenchyma, **vb**- vascular bundle, **ep**- epidermal cells (×10, ×40)

Leaf surface constants

Leaf surface constants including the epidermal number, palisade ratio, stomatal number, stomatal index, vein islet number and vein termination number were determined and results presented in Table 2. These are unique diagnostic features of the leaf that enable the easy identification of closely related plant species and detect adulterants. The stomatal index especially remains constant regardless of the age of plant or geographical location and is mostly used in many monographs.

Table 2: Leaf surface constants for *L. owariensis* leaf

Parameter	Ranges
Palisade ratio	3-(3.5)-4
Epidermal number /mm²	173-(193)-212
Stomatal number /mm²	6.4-(9.0)-11.2
Stomatal index	13-(16.4)-19
Vein islet number /mm²	7-(9)-11
Vein termination number /mm²	15-(23)-31

Values are presented as a range from the lower to upper limit [lower limit — (average) — upper limit] obtained from five (5) replicate experiments

T/S of fresh roots

The T/S of the root displayed a thick outer layer of cork cells beneath which two layers of epidermal cells are found. About three to five layers of collenchyma cells are located directly below the epidermal cells. Several parenchyma cells are found dispersed in the cortex among which single and clusters of starch grains and prismatic calcium oxalate crystals are distributed. Lignified medullary rays consisting of about twenty to twenty-five cells each are found arranged in parallel from the cortical region towards the central pith (Fig. 5).

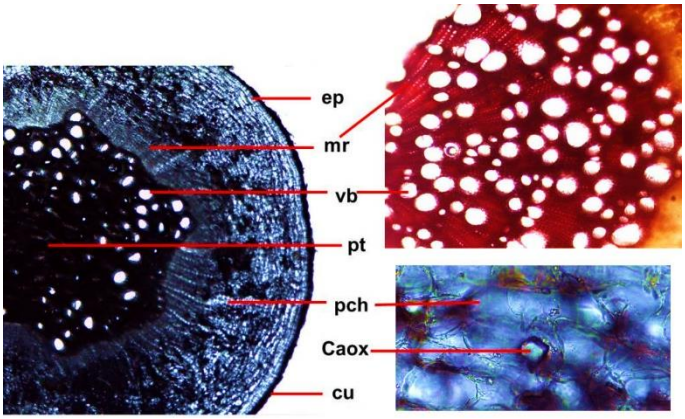

Figure 5: T/S of *L. owariensis* root: **ep**- epidermal cells, m**r**-medullary rays, **vb**-vascular bundle, **pt**- pith, **pch**-parenchyma cells, **CaOx**- calcium oxalate crystals, **cu**- cuticle (×40)

Powder Microscopy

Reduction of whole plants to the powdered form destroys the macro-morphology making it impossible to use macro-morphological features as a basis for authentication. Microscopy of the powdered crude drugs is hence important in order to determine the unique internal features of a powdered drug for easy identification and detection of adulteration.

The powdered leaf microscopy showed fibres, stomatal cells, several pitted vessel elements and starch grains (Fig. 6). Microscopy of the powdered roots revealed the presence of prismatic calcium oxalate crystals, pitted vessels, sclereids or stone cells, fibers and cork cells (Fig. 7). The calcium oxalate crystals observed are prismatic in form and very abundant in the root powder. The abundance of prismatic calcium oxalate crystals in the roots of the plant is a unique characteristic feature of the plant that can be used in the identification. The types of stone cells identified are asterosclerieds which are heavily thickened, have a large lumen and are mostly stratified. Uniseriate clothing trichomes are also observed. The trichomes however, cannot be used for standardization as they show a continuous variation depending on the environmental conditions they grow in.

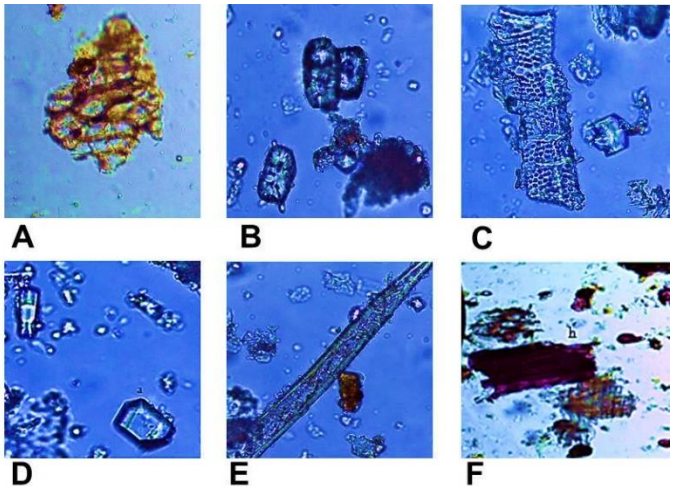

Figure 6: Microscopy of powdered leaves: **A**-fibres, **B**- stomata, **C**- pitted vessel elements, **D**- starch grain (×100)

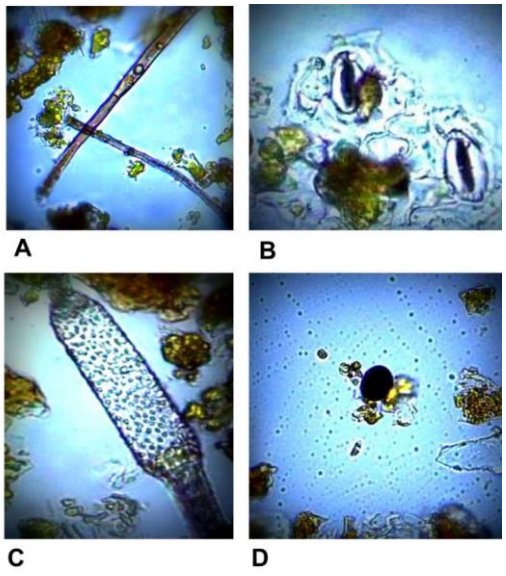

Figure 7: Microscopy of powdered roots – **A**-cork cells, **B**- stone cells, **C**- pitted vessels, **D**- prismatic calcium oalate crystal, **E**-clothing trichome, **F**- stained clustered fibres (×100)

Physicochemical properties

The physical and chemical properties of powdered crude drugs are useful parameters for identification and quality control. The solvent soluble extractive value is indicative of the weights of the extractable chemical constituents using a particular solvent. It suggests the suitable solvents required for maximum extraction of phytoconstituents from a plant drug. It also provides a clue on the solubility nature of constituents present in the plant material [1]. From our results polar solvents like ethanol, water and methanol have a high extractive power for the leaf; hence are the best solvents to extract the phytoconstituents of the leaves whiles non-polar and semi-polar solvents such as pet-ether, ethyl acetate and chloroform are the best for extraction of the roots (Table 3).

Table 3: Physicochemical properties of *L. owariensis* leaf and root

Parameter (%$^{w}/_{w}$)	**Leaves**	**Roots**
Loss on drying	57.81 ± 1.44	68.08 ± 1.66
Total ash	5.69 ± 0.12	8.42 ± 0.60
Water soluble ash	9.24 ± 0.68	0.57 ± 0.05
Acid-insoluble ash	0.78 ± 0.09	1.86 ± 0.09
Extractive values (mg/g^{-1})		
Water	19.2 ± 1.17	6.7 ± 0.55
Ethanol 95%	21.6 ± 1.17	7.2 ± 0.55
Methanol	16.3 ± 2.25	5.0 ± 0.90
Ethyl acetate	13.0 ± 0.17	9.1 ± 0.95
Chloroform	8.0± 0.15	10.6 ± 1.12
Pet. ether	0.9 ± 0.50	9.9 ± 0.87

Values are presented as the mean ± standard deviation for three (3) replicates in each experiment

Analysis of the pH of edible solutions of plant extracts is important to determine whether or not crude preparations may cause irritations to the gastrointestinal tract (GIT). Water is the solvent of choice for oral preparations of herbal medicines. The pH of the aqueous extracts of both leaves and roots were weakly acidic ranging between 5.82 and 5.94 respectively. This implies that traditional aqueous preparations of *L. owariensis* are less likely to cause GIT irritation when consumed.

Moisture content determination is a vital parameter for assessing the effectiveness of a drying process and guides the manufacturer to assess the possibility of deterioration during storage [17]. According to the European Pharmacopoeia, the limit for moisture content of herbal or vegetable drugs should range between 10-12% [14]. The moisture content of the fresh leaves and roots of *L. owariensis* ranged between 57-68% $^{w}/_{w}$ (Table 3). This shows that *L. owariensis* in its natural habitat stores a high amount of moisture. There is therefore the need for immediate drying after harvesting to prevent the deterioration of secondary metabolites if the material has to be stored for a long period. This is because high moisture content can activate certain enzymes that may result the decomposition secondary metabolites or generate compounds with less activity. Moreover at suitable temperatures, high moisture content may aid the growth of microorganisms such as mycotoxins producing fungi [1, 14].

The total ash is the inorganic ash that remains after a vegetable drug or herbal material is incinerated at a controlled temperature. It usually consists of carbonates, phosphates, silicates and silica and it measures the purity of a crude drug. Water soluble ash is a good indicator of either past extraction of the water soluble salts in the materials or shows incorrect preparation [18]. The total ash, acid insoluble ash and water soluble ash were determined and presented in Table 3. The leaves and root had varying ash contents indicating varying contents of mineral compounds that may naturally occur with the plant material. Thus any values beyond this may indicate contamination or adulteration with inorganic material such as siliceous material.

Fluorescence analysis

Fluorescence analysis complements other evaluation parameters to determine the authenticity of a crude drug based on the colors emitted by fluorescing constituents in visible light and ultraviolet (UV) light. Addition of specific acidic or basic reagents converts non-fluorescing compounds into fluorescing ones for easy identification [16]. Table 4 presents the results of fluorescence characteristics of various solvent extracts of the leaves and roots of *L. owariensis* and upon treatment with different acidic and basic chemical reagents in visible, short (254 nm) and long (365 nm) UV light.

Table 4: Fluorescence analysis of the leaves and roots of *L. owariensis*

L. owariensis leaves			
Powder +Reagent /Solvent	**Visible light**	**UV-254 nm**	**UV- 365 nm**
Powder + Water	Dark green	Greyish-brown	Red
Powder + Ethanol 95%	Dark green	Greyish-brown	Red
Powder + Methanol	Dark green	Greyish-brown	Red
Powder + Ethyl acetate	Brownish green	Brown	Red
Powder + Chloroform	Yellowish green	Brown	Red
Powder + Petroleum ether	Yellowish green	Brown	Red
Powder +Acetic acid	Light green	NF	Cream
Powder +Conc. H_2SO_4	Olive green	NF	Cream
Powder +Conc. HCl	Army green	NF	Yellow
Powder + $FeCl_3$ (5%)	Greenish black	NF	NF
Powder +Alcoholic KOH	Reddish brown	NF	NF
Powder +Iodine solution (0.5M)	Reddish brown	NF	NF
Powder +NH_3 solution (25%)	Brick red	NF	Light green
L. owariensis roots			
Powder +Reagent /Solvent	Visible light	UV-254 nm	UV- 365 nm
Powder + Water	Pale yellow	Orange	Greenish blue
Powder + Ethanol 95%	Pale yellow	Orange	Blue
Powder + Methanol	Pale yellow	Light yellow	Blue
Powder + Ethyl acetate	Light yellow	Light yellow	Yellow
Powder + Chloroform	Light yellow	Yellow	Yellow
Powder + Petroleum ether	Light yellow	Yellow	Light yellow
Powder +Acetic acid	Brown	NF	Cream
Powder +Conc. H_2SO_4	Caramel	NF	Lime
Powder +Conc. HCl	Light brown	NF	Mint green
Powder + $FeCl_3$ (5%)	Brown	NF	NF
Powder +Alcoholic KOH	Reddish brown	NF	NF
Powder +Iodine solution (0.5M)	Brownish black	NF	NF
Powder +NH_3 solution (25%)	Light brown	NF	Mint green

NF- No fluorescence

Preliminary phytochemical screening

The results of the phytochemical analysis are presented on Table 5. These phytoconstituents may contribute to the various folkloric medicinal uses of the plant.

Table 5: Phytochemical constituents of the leaves and roots of *L. owariensis*

Constituents	Leaves	Roots
Tannins	+	-
Reducing Sugars	+	+
Alkaloids	-	+
Saponins	+	+
Flavonoids	+	+
Coumarins	-	-
Phytosterols	-	-
Triterpenoids	+	+

Key; + Detected, - Not detected

Comparison of LC/MS of *L. owariensis* and *L. heudelotti*

Landolphia heudelotti is a closely related species which is common in Ghana. We previously reported the presence of lignans, neolignans, sesquilignans and similar derivatives as major constituents from *L. heudelotti* [19]. In the present study, the LC/MS of MeOH extracts of *L. heudelotti* stem bark (LH-MeOH) and *L. owariensis* roots (LO-MeOH) were analysed and compared based on retention times and mass analysis (Fig. 8). The results suggested the occurrence of some known bioactive compounds in *L. owariensis*. These were guaiacylglycerol-8-O-4'-coniferyl alcohol ether (1), guaiacylglycerol-8-O-4'-coniferyl aldehyde ether (2) [20], guaiacylglycerol-8-O-4′-pinoresinol ether (3) [21] balanophonin (4) [22], buddlenol E (5) [23], picrasmalignan A (6) [24] and pinoresinol (7) [25] (Table 6).

Table 6: Identified compounds in the methanol extract of *L. owariensis* from HR-LC/MS

No.	Molecular weight (*m/z*)	Molecular formula	Retention time/min	Compound Name
1	399.14 $[M+Na]^+$	$C_{20}H_{24}O_7$	7.61	Guaiacylglycerol-8-O-4'-coniferyl alcohol ether
2	397.14 $[M+Na]^+$	$C_{20}H_{22}O_7$	8.89	Guaiacylglycerol-8-*O*-4'-coniferyl aldehyde ether
3	577.20 $[M+Na]^+$	$C_{30}H_{34}O_{10}$	9.44	Guaiacylglycerol-8-*O*-4′-pinoresinol ether
4	357.13 $[M+H]^+$	$C_{20}H_{20}O_6$	9.59	Balanophonin
5	607.21 $[M+Na]^+$	$C_{31}H_{36}O_{11}$	9.89	Buddlenol E
6	535.19 $[M+H]^+$	$C_{30}H_{30}O_9$	10.19	Picrasmalignan A
7	359.14 $[M+H]^+$	$C_{20}H_{22}O_6$	11.34	Pinoresinol

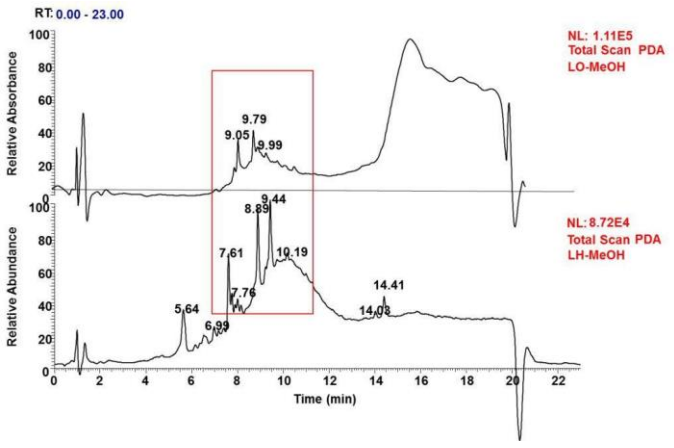

Figure 8: HR-LC/MS (in positive mode) of *L. heudelotti* stem bark (LH-MeOH) and *L. owariensis* roots (LO-MeOH) extracts

CONCLUSION

The present study concentrated on providing standards for the identification and authentication of *L. owariensis*. The study has made available the first report on detailed description of the pharmacognostic, physicochemical and phytochemical properties of the leaves and roots of *L. owariensis*. The essential diagnostic attributes of *L. owariensis* established in this study may be useful for authentication and quality control of the plant.

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REFERENCES

1. Folashade O, Omoregie H, Ochogu P. Standardization of herbal medicines-A review. Int J Biodivers Conserv. 2012; 4:101-112.
2. Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacogn Phytochem. 2014; 2:69-73.
3. Burkill HM. The useful plants of West Tropical Africa, Families A–D. 2nd ed. Royal Botanic Gardens: Kew, United Kingdom, 1985.
4. Nwaogu L, Alisi C, Ibegbulem C, Igwe C. Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf. Afr J Biotechnol. 2010; 6:890-893.
5. Owoyele B, Olaleye S, Oke J, Elegbe R. Anti-inflammatory and analgesic activities of leaf extracts of *Landolphia owariensis*. Afr J Biomed Res. 2001; 4:131-133.
6. Awah FM, Uzoegwu PN, Ifeonu P, Oyugi JO, Rutherford J, Yao X, *et al*. Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants. Food Chem. 2012; 131:1279-1286.
7. Obitte N, Chukwu A. The synergistic effect of *Landolphia owariensis* latex and Eudragit® L-100-coated capsules on the in vitro controlled release of metronidazole for possible colon targeting. Asian J Pharm. 2011; 5:75.
8. Okonkwo TJ, Osadebe PO, Proksch P. Bioactive phenylpropanoids, phenolic acid and phytosterol from *Landolphia owariensis* P. Beauv stringy seed pulp. Phytother. Res. 2016; 30:78-83.
9. Okonkwo TJ, Osadebe P. Isolation and characterization of potential bioactive compounds from *Landolphia owariensis* P. Beauv stringy seed pulp. Int J Appl Res Nat Pro. 2013; 6:28-38.
10. Baidoo MF, Asante-Kwatia 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.
11. Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. Wright-Scientechnica: Bristol, 1975.
12. Evans WC. Trease and Evans' Pharmacognosy E-Book. 15th ed. Saunders WB, Elsevier Health Sciences. 2009.
13. Amponsah IK, Mensah AY, Otoo A, Mensah MLK, Jonathan J. Pharmacognostic standardisation of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). Asian Pac J Trop Biomed. 2014; 4:941-946.
14. Duțu L. Pharmacognostic Methods for Analysis of Herbal Drugs, According to European Pharmacopoeia. Promising Pharmaceuticals. Bucharest, Romania. 2012; 38-62.
15. Mireku EA, Kusari S, Eckelmann D, Mensah AY, Talontsi FM, Spiteller M. Anti-inflammatory tirucallane triterpenoids from *Anopyxis klaineana* Pierre (Engl.), (Rhizophoraceae). Fitoterapia. 2015; 106:84-91.
16. Mukhi S, Bose A, Panda P, Rao M. Pharmacognostic, physicochemical and chromatographic characterization of Samasharkara churna. Journal of Ayurveda and integrative medicine. 2016; 7:88-99.
17. Laloo D, Kumar M, Prasad SK, Hemalatha S. Quality control standardization of the roots of *Potentilla fulgens* Wall.: A potent medicinal plant of the Western Himalayas and North-eastern India. Pharmacognosy Journal. 2013; 5:97-103.
18. Kabra A, Sharma R, Singla S, Kabra R, Baghel US. Pharmacognostic characterization of *Myrica esculenta* leaves. Journal of Ayurveda and integrative medicine. 2019; 10:18-24.
19. Mireku E, Mensah A, Mensah M, Amponsah I, Mintah D. Phytochemical constituents and anti-oxidative properties of *Landolphia heudelotti* roots. Int J Pharm Sci Res. 2017; 8:2862-2866.
20. Woo KW, Suh WS, Subedi L, Kim SY, Kim A, Lee KR. Bioactive lignan derivatives from the stems of *Firmiana simplex*. Bioorg Med Chem Lett. 2016; 26:730-733.

21. Willför S, Reunanen M, Eklund P, Sjöholm R, Kronberg L, Fardim P *et al*. Oligolignans in Norway spruce and Scots pine knots and Norway spruce stemwood. Holzforschung. 2004; 58:345-354.
22. Zhan R, Zhang Y, Chen L, Chen Y. A new (propylphenyl) bibenzyl from *Eria bambusifolia*. Nat. Prod. Res. 2016; 30:1740-1745.
23. Houghton PJ. Lignans and neolignans from *Buddleja davidii*. Phytochemistry. 1985; 24:819-826.
24. Zhao F, Chen L, Bi C, Zhang M, Jiao W, Yao X. In vitro anti-inflammatory effect of picrasmalignan A by the inhibition of iNOS and COX-2 expression in LPS-activated macrophage RAW 264.7 cells. Mol Med Rep. 2013; 8:1575-1579.
25. During A, Debouche C, Raas T, Larondelle Y. Among plant lignans, pinoresinol has the strongest antiinflammatory properties in human intestinal Caco-2 cells. J Nutr. 2012; 142:1798-1805.

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