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Pharmacognostic and physicochemical studies of the leaves of *Hoslundia opposita* Vahl (Lamiaceae)

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Abstract

Hoslundia opposita is an important herb of ethnomedical importance in Ghana. Infusions of the leaves are widely used in traditional medicine as a purgative, diuretic, febrifuge, antibiotic, and antiseptic. The plant is known to have antibacterial, antifungal and antiviral activities. Due to commercialisation of this important herb, there is the need to develop standardization parameters for the authentication and quality control of the leaves of *Hoslundia opposita*. The macromorphological, qualitative and quantitative features, physicochemical and phytochemical features of the medicinally used parts were evaluated using standard methods. The plant is a glabrous herb with simple leaves. The leaves are elliptic, with an acuminate apex. It is amphistomatic with anomocytic stomata. The quantitative indices of the leaf and physicochemical parameters have also been established. The pharmacognostic features established in this study may be used as part of the pharmacopoeial standards for the correct identification and quality control of *Hoslundia opposita*.

Keywords: *Hoslundia opposita*, Pharmacognostic studies, physicochemical properties, monograph, microscopy, standardisation

Introduction

Plant-derived substances have recently been of great interest owing to their versatile applications^[1]. There is a renewed interest in herbal medicines due to its many advantages including cost effectiveness, easy accessibility and availability. Herbal products are now extensively accepted as therapeutic agents for several diseases, with over 80% of the world's developing population now dependent on them for healthy living^[2].

These products are increasingly being sought out globally as medicinal products, nutraceuticals and cosmetics^[3]. Some of these plants have been used traditionally for centuries and modern scientific studies have shown a strong justification for their traditional or folkloric applications. This lends support for the search for pharmacologically active compounds from these plants to serve as leads for drug discovery^[4]. One of such plants is *Hoslundia opposita*, a perennial herb of the Lamiaceae family.

Hoslundia opposita is locally known in Ghana as 'aberewa anisu' (Akan) or 'asifuaka' (Fante). It is also commonly called orange bird berry plant. It is an herbaceous perennial or sometimes soft shrubs growing up to 1.2 m high. It has a widespread natural distribution and occur in countries such as Nigeria, Ivory Coast Cameroon, and Ghana^[5,6]

In Ghana, the roots and leaves of *H. opposita* are used ethnomedically in treating stomach ulcers, chronic and deep wounds, dermatitis, sore throats, venereal infections and as insect repellents^[5,7,8]. Elsewhere, in other African countries, infusion of the leaves is used as purgative, antimalarial, febrifuge, antiseptic and in treating cough, chest pain and hookworm infection^[6].

The acaricidal^[9], larvicidal^[10], insecticidal^[11], antimalarial^[12], antimicrobial^[13], hepatoprotective^[14], antidiabetic^[15], antioxidant^[8] and CNS depressant^[16,6] activities of various parts of the plant have been reported by several authors. The plant is used in a number of herbal preparations in Ghana.

The commercialisation of the plant has given rise to varying degree of substitution and adulteration of the products containing this invaluable herb thereby leading to low or no therapeutic effect and in some instances adverse drug reactions. This study was therefore designed to establish quality parameters for the correct identification and quality control of *Hoslundia*-derived medicinal products.

Materials and Methods

Chemicals, reagents and equipment

All the chemicals and reagents used in this study were of analytical grade. These include; petroleum ether, ethyl acetate, methanol, chloroform, ethanol, acetic acid, concentrated sulphuric acid, bismuth nitrate, potassium iodide, lead acetate, ferric chloride, hydrochloric acid, sodium hydroxide, Fehling's solutions A and B, acetic anhydride, ammonia and anisaldehyde (All from BDH laboratory Ltd. Poole, England), aluminium precoated silica gel plates GF254 (0.25mm thick) (Alpha laboratories, UK), Leica ICC50 HD microscope (Jos Hansen and Soehne Gmh Germany). Jenway UV-VIS spectrophotometer (Germany), Bruker Fourier transform infrared (FT-IR) spectrometer (Hansen and Soehne GmbH (Hamburg, Germany), Mettler Toledo AG pH meter (8603 Schwerzenbach, Switzerland).

Plant collection and processing

The leaves of *Hoslundia opposita* was harvested from the Physic garden of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST) in the month of September, 2017. The leaves were air dried for 24 hours and then milled to coarse powder. The powder was then kept in paper bags and stored at ambient temperature until ready for use.

Organoleptic evaluation

Organoleptic evaluation was carried out by observing the leaves, taking note of the colour, odour and taste of the leaves and other diagnostic parameters.

Macromorphological studies

Different macroscopic parameters of the leaf's lamina were noted. Leaf parameters included the type of leaf, shape, arrangement, apex margin, venation, base, texture and colour. Dimensions of the leaves and petiole of were also determined.

Microscopic and histologic techniques

Study of transverse sections

To obtain transverse sections of the midrib and petiole of the leaf, free hand using razor blade sections were made for qualitative microscopic analysis. Cellulosic and other identifying features were studied staining with phloroglucinol in concentrated HCl and N/50 iodine. Microscopic evaluation of the tissues was supplemented with photomicrography of different magnifications taken with DM700 light microscope fitted with a camera, Leica ICC50 HD (Jos Hansen and Soehne Gmh Germany). Standard procedures were followed according to Kokate *et al* [17].

Powder microscopy

The powdered leaves of *H. opposita* were studied using a light microscope. Minute quantities of the leaves were mounted on a slide using water, phloroglucinol in concentrated HCl and iodine solution as mountants. Photomicrographs of the distinctive cellular structures and inclusions were taken with DM700 light microscope fitted with a camera.

Quantitative microscopy

Features that were not easily characterized using general microscopy were calculated and these included stomatal number, stomatal index, palisade ratio, vein islet and veinlet termination numbers. They were evaluated according to the method described by Evans [17].

Physicochemical parameters

Physicochemical analysis such as total ash, water soluble ash, acid insoluble ash, petroleum ether, alcohol and water-soluble extractives were determined according to standard methods described by Vermani and Chauhan [18].

Extractive value determination

The extractive values of the leaves of *Hoslundia opposita* were evaluated according to standard methods [17]. About 4 g of the air-dried leaves were weighed into three labelled conical flasks respectively. Each sample was macerated with 100 mL each of 95% ethanol and water separately for 24 hours at room temperature. The flasks were agitated gently at 20 minutes intervals during the first 2 hours of extraction and allowed to stand for the rest of the 24 hours. The extracts were filtered rapidly, taking precautions against loss of solvent. 25 mL of the filtrate was evaporated to dryness in a porcelain dish on a water-bath and at 100°C for 3 hours for the water extracts, 60°C for 1 hour for the ethanol. The porcelain dishes and petri dishes were then weighed. The average extractive value and standard error of the mean was determined.

pH of extracts

About 1% extracts were prepared by method of cold maceration. About 1 g of the powdered leaves was extracted using 100 mL of water and 95% ethanol for 2 hours and then filtered using a Whatman number 1 filter paper. The pH of the filtrate was determined using a pH meter.

Ash value determination

Total ash content

About 2 g of the powdered leaves were weighed into a dry porcelain dish. The porcelain dish was then placed in a muffle furnace at 550 °C for 4 hours. It was then cooled in a desiccator and the ash obtained was weighed. The total ash was then calculated as the percentage of the sample [17].

Acid Insoluble Ash

The total ash residue was transferred into a beaker containing 25 mL of HCl. The resulting mixture or acid solution was filtered through an ash-less filter paper. The beaker was rinsed several times with hot water into the filter. The filter was washed with hot water until the washes were acid free to litmus. The filtrate was allowed to drain and the filter paper was gently taken out of the funnel, which was then folded into a small triangle and placed in a crucible. It was dried in an oven and ignited in the furnace at 600 °C. It was cooled and weighed [17].

Phytochemical investigation

The presence of secondary metabolites such as tannins, alkaloids, glycosides, triterpenoids and phytosterols were determined according to standard methods [17].

Thin layer chromatography (TLC)

About 5 g of the powdered leaves were cold macerated using 80 mL of ethyl acetate for 24 hours. The filtrate was concentrated to 5 mL at room temperature and used for the analysis. Analytical TLC on silica gel G60 F₂₅₄, 0.25 mm layer developed with petroleum ether/ethyl acetate [7:3]. Separated compounds were detected with anisaldehyde / H₂SO₄ spray reagent.

Ultra-violet spectroscopy

About 4 g of the powdered leaves were cold macerated using 100 mL of 70% ethanol. The extract was filtered and the

ethanolic extract analysed using a Jenway UV-VIS spectrophotometer at a wavelength range of 200–700 nm with a scan speed of 480 nm/min.

Results

Macromorphological and organoleptic features of the leaves

The leaf is green on the adaxial surface and light green on the

abaxial surface (Figure 1c&d). It has a characteristic odour. The leaf powder has a bland taste. The lamina is elliptic with an acuminate apex. It has a serrate margin with an asymmetrical base (Figure 1). The venation is reticulate and has a glabrous surface.

The length and width of the leaf lamina were found to be 10.43 ± 0.31 cm and 4.61 ± 0.12 cm respectively with a petiole length of 1.59 ± 0.08 cm.

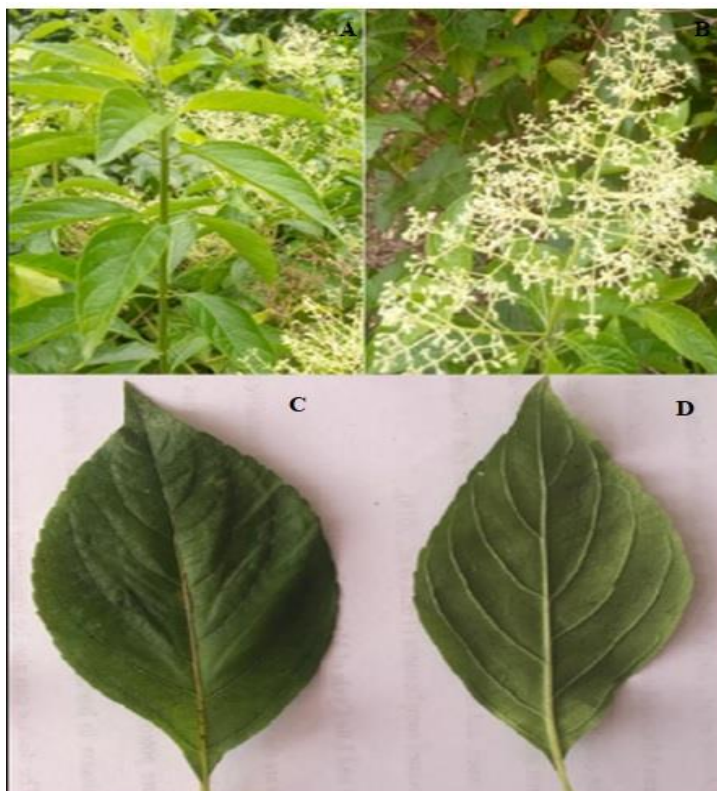


Fig 1: Whole plant (a) Flower (b) Upper (c) and Lower (d) leaf surfaces *H. opposita* respectively

Micromorphology

The leaf is amphistomatic with anomocytic stomata on both abaxial and adaxial surfaces. The lateral leaf surface displayed multicellular clothing trichomes ($124.0 - 333.3\mu\text{m}$) in length. The stomatal number and index were determined to

be 104 - 130 - 156 and 13.10 - 18.36 - 24.40% respectively. The vein islet and veinlet termination numbers for the upper surface ranged 12-14-15 and 14-17-19 respectively. The palisade cells are conspicuous with a palisade ratio of 6 - 8 - 11 (Figure 2).

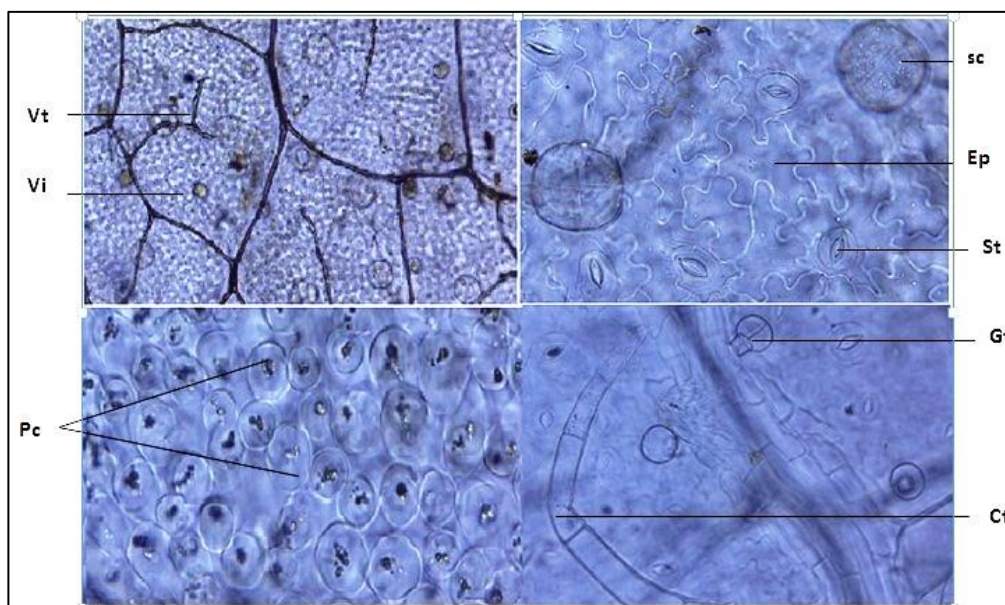


Fig 2: Leaf surface features of the lamina of *H. opposita*. **St:** stomata **Gt:** glandular trichomes **Ct:** clothing trichome **Pc:** palisade cells **Vt:** Veinlet termination **Vi:** Vein islet **Ep:** Epidermal cells **Sc:** Secretory cavity

Transverse section (T/S) of midrib

T/S of the midrib showed an undulating surface with a slight depression on the ventral surface. The ventral surface was

lined with pentagonal shaped epidermal cells. It contained both glandular and clothing trichomes as well as collenchyma and parenchyma cells (Figure 3).

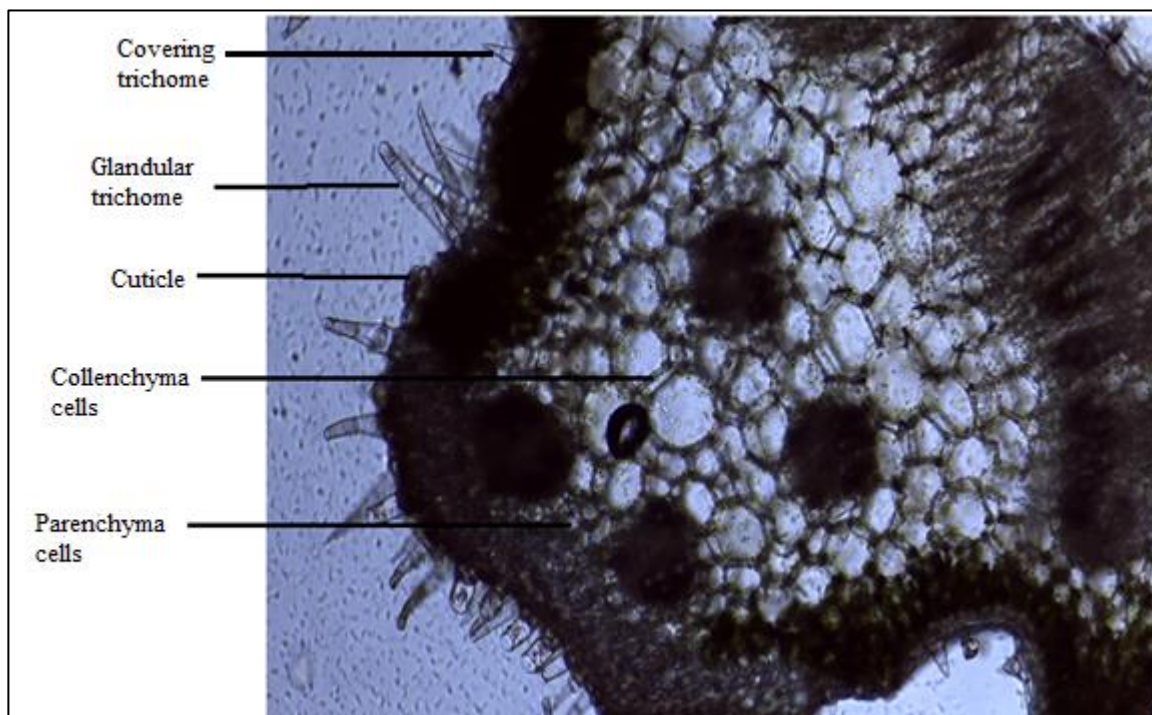


Fig 3: Transverse section of midrib of *H. opposita*

Powder microscopy

Powder microscopy of the leaf of *H. opposita* showed the presence of broken trichomes, crystals, fibres, oil cells, epidermal cells and stomata (Figure 4)

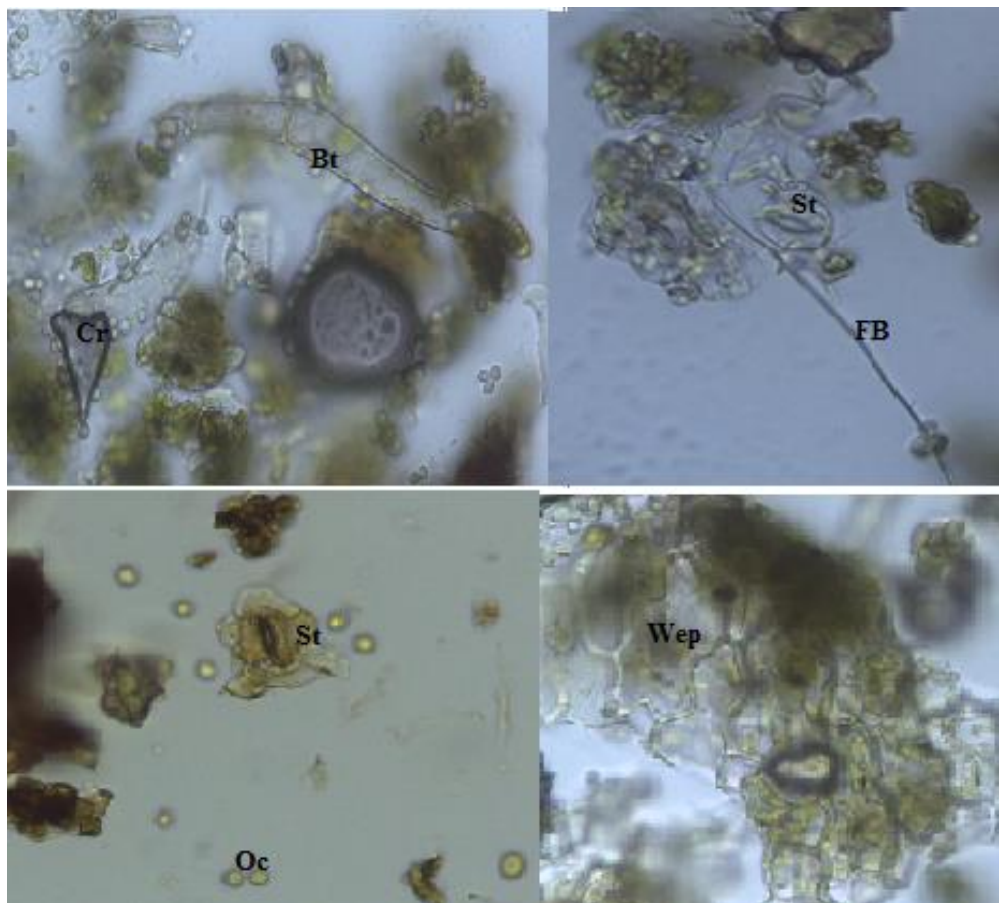


Fig 4: Leaf powder microscopy of *H. opposita* **Bt:** Broken trichomes **Cr:** Crystal, **St:** Stomata, **FB:** Fibre, **Wep:** Wavy epidermal cells, **Oc:** Oil cells

Physicochemical parameters

The physicochemical parameters of the leaves, such as the total and acid insoluble ash, water and alcohol soluble extractives are documented (Table 1).

Table 1: Physicochemical parameters

Physical parameter	Leaves
Total Ash (%)	10.114±0.124
Acid Insoluble Ash (%)	0.609±0.433
Water soluble extractive (% ^{w/w})	42.86±0.333
Alcohol soluble extractive (% ^{w/w})	6.46±0.667
pH (1% aqueous extract)	6.76±
pH (1% ethanol extract)	6.81±

Phytochemical analysis

Phytochemical screening of the powdered leaf revealed the presence of all major plant phytoconstituents with the exception of alkaloids (Table 2).

Table 2: Phytoconstituents of powdered leaf

Test	Results
Tannins	+
Glycosides	+
Alkaloids	-
Saponins	+
Coumarins	+
Flavonoids	+
Sterols	+
Triterpenoids	+

Key (+) detected (-) not detected

Thin layer chromatography (TLC)

The TLC chromatogram of the leaves showed six prominent spots labelled L1 to L6 with R_f values 0.19, 0.34, 0.47, 0.72, 0.79, and 0.87 respectively (Figure 5).

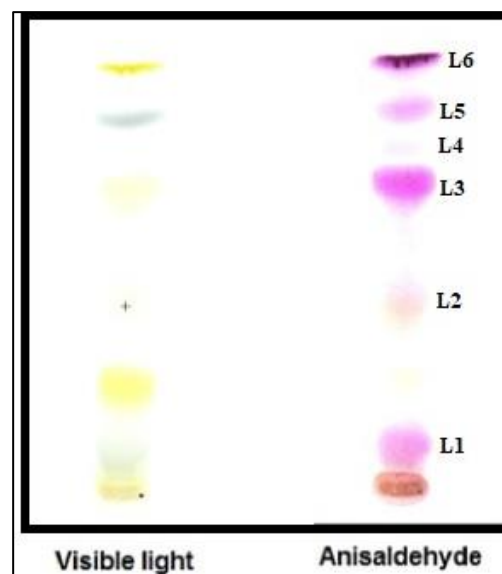


Fig 5: TLC chromatogram of the leaves of *Hoslundia opposita* under visible light and sprayed with anisaldehyde reagent.

Ultra-violet spectroscopy

The ultra-violet (uv) analysis showed different characteristic peaks with the maximum absorption at 279 nm (Figure 6).

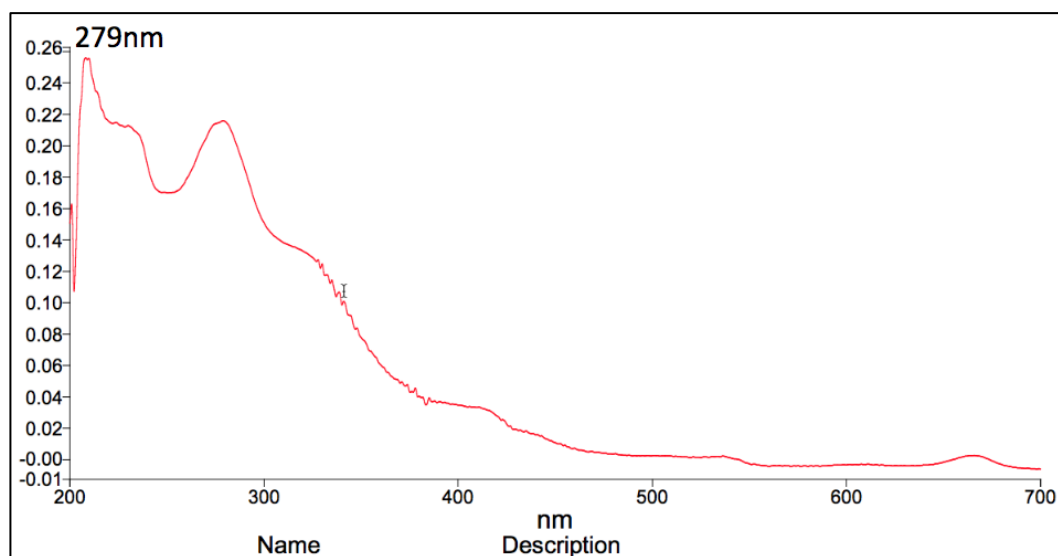


Fig 6: UV spectrum of *Hoslundia opposita*

Discussion

Medicinal plants have certain signatures, which are specific to them and can be used as diagnostic tools for their identification and evaluation. These features are established by pharmacognostic studies, a study which is indispensable in establishing quality control parameters for the determination of the identity, purity, consistency, efficacy and safety of herbal products. This helps researchers and manufacturers to be confident on the quality of herbal raw materials and products they are dealing with. *Hoslundia opposita* is an herbaceous perennial. The macro and micro-morphological studies carried out revealed characteristic features that could be useful in the correct identification and authentication of *H. opposita* (Figure 1-4).

Transverse section of the midrib showed a bulging undulating ventral surface lined with straight walled epidermal cells. The ventral surface was covered with both clothing and glandular trichomes. The presence of broken trichomes, fibres, crystals, oil cells, stomata and epidermal cells in the powdered leaves is characteristic hence will help in its identification. Extractive value determination as a means of evaluating crude drugs serves to detect adulteration with a previously extracted material [17]. This is of importance again as it informs the nature of the chemical constituents of the plant. From the results, the plant is more soluble in water as compared to ethanol, thus it can easily be formulated into dosage forms for human consumption. The pH of the extracts of the leaves was found to be 6.76 and 6.81 for water and ethanol respectively.

This could imply that oral dosage forms of the plant can be produced, as it is not likely to affect the gastrointestinal tract. The percentage of total ash and acid insoluble ash of the leaves give an indication of earthy matter or the inorganic composition and other impurities present with it. The dry ash of the leaves after incineration was fine and grey.

Glycosides, tannins, flavonoids, saponins, coumarins, sterols and triterpenoids revealed during phytochemical screening are responsible for the reported therapeutic activity of the part of the plant. TLC profile of the leaves showed prominent spots relevant as a fingerprint for further identification of the plant. The UV spectra also lends support in the identity and quality control of the leaf. Chemical profiling involving chromatographic fingerprinting has come into the spotlight as an invaluable quality control tool for herbal products due to the complex array of chemical principles [19]. Thus, the characteristic spots on the TLC chromatogram of *H. opposita* would be indispensable in the assurance of the authenticity and quality of the plant or its derived products.

Conclusion

The pharmacognostic features established in this study may serve as a means for assessing the quality and purity of *Hoslundia opposita*. These characteristic features may be used as standards for setting up a monograph for the correct identification and quality control of the plant.

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