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Pharmacognostic standardisation of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae)

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ABSTRACT

Objective: To establish the pharmacognostic characters for the correct identification and quality control of *Hilleria latifolia* (*H. latifolia*), an important herb in Ghanaian folklore medicine, for the treatment of infections, pain and inflammation.

Methods: The macro-morphological, qualitative and quantitative microscopic features, physicochemical and phytochemical features of the medicinally used parts of *H. latifolia* were evaluated using standard methods.

Results: The plant has simple, alternate leaves with entire margin. The lamina is ovate to broadly lanceolate with an acuminate apex. It is hypostomatic with anomocytic stomata. The plant contains abundant prismatic crystals in all parts. Starch grains abound in the roots. The quantitative indices of the leaf and physicochemical parameters have also been established.

Conclusions: The pharmacognostic features established in this study may be used as part of the pharmacopoeial standard for the correct identification and quality control of *H. latifolia*.

1. Introduction

Herbal medicines are an important part of therapy throughout the world. They have been widely utilized as effective remedies for the prevention and treatment of variety of disease conditions for millennia by almost every known culture[1]. It is reported that 80% of the population use herbs in developing countries[2]. In Ghana, the use of herbal medicines is widespread in both the rural and urban areas. There is integration of alternative and complementary medicine and orthodox medicine in some selected public hospitals throughout the country by the Ghana Health Service. Thus, natural remedies contribute significantly to healthcare delivery in Ghana.

The more effective a natural drug, the greater its exploitation and the chances of its depletion. To meet the growing demand, the natural drug is easily adulterated with low grade material and in some cases completely substituted. Thus, it is important to establish standards for authentication of efficacious medicinal plants used in the treatment of diseases. Sometimes the same vernacular names are given to closely related species and therefore these plants are easily substituted. Pharmacognostic studies will ensure plant identity and lay down standardization parameters which will help prevent adulteration[3]. Such study does not only help in authentication but also ensures reproducible quality of herbal products in commerce.

Hilleria latifolia (Lam.) H. Walt. (*H. latifolia*) (Phytolaccaceae)

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is a perennial herb that is common in the forest regions of Ghana. It also occurs in other parts of tropical Africa as well as South America. In Ghana, it is commonly known as *Avegboma* (Ewe) and *Anafranaku* (Akan)[4]. In Ghanaian culture, different parts of the plant are used for treatment of a variety of diseases. The leaves are effective in treating rheumatism, otalgia and boils; whereas the flowers are used for asthma[5,6]. In La Cote d'Ivoire a leaf decoction is administered by enema to treat ascites and food poisoning, feverish pains and violent headaches[6]. However, there is no pharmacognostic standard for the plant; hence the present investigation was under taken to evaluate various pharmacognostical parameters such as macroscopic, microscopic, physicochemical and phytochemical features, and fluorescence of the leaf, stem and root extracts of *H. latifolia*.

2. Materials and methods

2.1. Chemicals

Toluidine blue was purchased from Surechem Products Ltd., Suffolk, England. All other analytical grade chemicals were purchased from Sigma Aldrich Co Ltd. Irvine, UK and BDH Laboratory Supplies (England). Aluminium precoated silica gel plates 60 F_{254} (0.25 mm thick), used for the analytical thin layer chromatography (TLC) was purchased from Merck Germany.

2.2. Plant collection and processing

The whole plant of *H. latifolia* was harvested from the campus of Kwame Nkrumah University of Science and Technology (KNUST) in the month of January, 2014 and authenticated at the Department of Herbal Medicine, KNUST, where a herbarium specimen has been deposited (KNUST/HM 2/2014/S14). The plant material was washed with water to remove foreign objects and the different parts (*i.e.* leaves, stem and roots) separated and shade dried. It was then milled to coarse powder, kept in paper bags and stored at ambient temperature until ready for use.

2.3. Organoleptic evaluation

Organoleptic evaluation was done by observing the leaves with the naked eyes and taking note of the colour, size, odour and other diagnostic parameters.

2.4. Macroscopic evaluation

Different macroscopic parameters of the leaves, stem and root were noted. Evaluation of the leaves included the observation of type of leaf, shape, arrangement, apex, margin, venation, base, texture *etc*.

2.5. Microscopic and histological analysis

2.5.1. Study of transverse sections

For qualitative microscopic analysis, freehand transverse sections of the midrib and petiole of the leaf were made using razor blade. Lignified, cellulosic and other identifying features were studied by staining the sections with toluidine blue, phloroglucinol in concentrated HCl and N/50 iodine[7]. Microscopic evaluation of the tissues was supplemented with photomicrography of different magnifications taken with Leica light microscope DM 1000 LED (Wetzlar, Germany).

2.5.2. Powder microscopy

The coarsely powdered leaf, stem and root of *H. latifolia* were studied under the microscope. Small quantities of the various plant parts were mounted on a slide using chloral hydrate, phloroglucinol in concentrated HCl and iodine solution. Photomicrographs of the different cellular structures and inclusions were taken.

2.5.3. Quantitative microscopy

The different leaf parameters like stomatal number, stomatal index, palisade ratio, vein islet and veinlet termination numbers were evaluated according to the method described by Kumar *et al*[8].

2.6. Physicochemical parameters

Physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, petroleum ether, alcohol and water soluble extractives as well as loss on drying of various plant parts were determined according to standard methods[9].

2.7. Fluorescence analysis

The water, petroleum ether and ethanol extracts were observed for characteristic fluorescent colours under visible light, short and long UV wave length regions[10,11].

2.8. Phytochemical screening

The presence of secondary metabolites such as tannins, alkaloids, glycosides, terpenoids, sterols *etc*. were determined according to standard methods[12].

2.9. TLC profile

Five grams each of the powdered plant materials were cold-extracted with 30 mL of chloroform for 24 h. The filtrate was concentrated under vacuum to 5 mL and used for the experiment. Analytical TLC on silica gel G60 F_{254}, 0.25 mm layer was developed

using petroleum ether/chloroform (2:8) as the mobile phase. Separated compounds were detected with anisaldehyde/H_2SO_4 and also characteristic fluorescence was investigated under visible light and UV light at 365 nm.

3. Results

3.1. Organoleptic features of leaves

H. latifolia is a slender perennial herb with more or less woody stem (Figure 1). The leaf is green on the adaxial and light green on the abaxial surfaces with a characteristic pungent odour. The lamina is ovate to lanceolate with an oblique base, entire margin and a short to long acuminate apex. The leaf is leathery with a glabrous surface and a reticulate venation.

Figure 1. Macromorphological features of *H. latifolia.*

3.2. Microscopy of leaf and transverse sections of midrib and Petiole

The leaf is dorsiventral with a flat lamina. It is hypostomatic with anomocytic stomata on the abaxial surface (Figure 2). Leaf surface microscopy showed the presence of abundant prismatic crystals (3.9-110.5 μm in length) and uniseriate clothing trichomes (94.7-347.2 μm in length) (Figure 2). The stomatal number and index ranged between 170-218 and 21-27 respectively. The vein islet number was in the range of 10-21 and the vein termination number ranged 28-40. The palisade cells are very conspicuous (Figure 3) with a palisade ratio of 4.5-8.3.

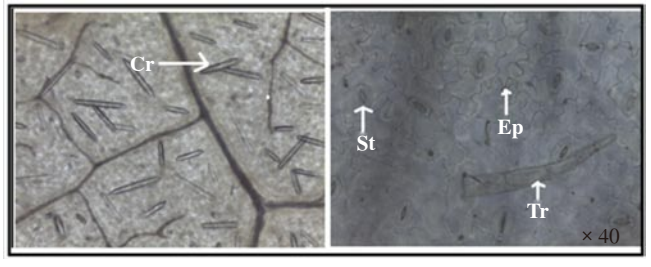

Figure 2. Microscopic features of leaf with crystals seen under palisade cells.
Cr: Crystals; St: Stomata; Ep: Epidermal cells with wavy anticlinal walls.

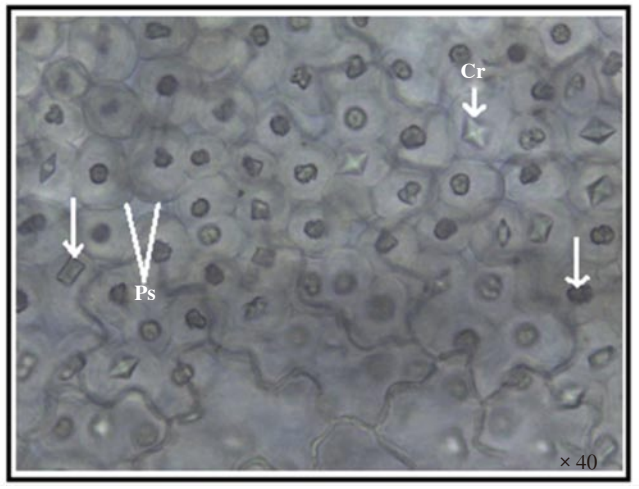

Figure 3. Microscopic features of adaxial leaf surface showing palisade cells.
Ps: Palisade cells; Cr: Tetrahedral prismatic and rosette crystals (arrowed) embedded in palisade cells.

Transverse sections of the midrib showed a bulge on the ventral side and a depression on the dorsal surface (Figure 4). Both surfaces were lined with elongated epidermal cells (with no conspicuous cuticle). A large number of uniseriate, multicellular covering trichomes (3-8 celled) arised from both lower and upper epidermal surfaces with very few glandular trichomes (unicellular head and unicellular stalk) on the abaxial surface. Cortical parenchyma cells contain a large number of prismatic crystals. The vascular bundle is collateral with lignified xylem elements towards the adaxial surface and phloem towards the abaxial side. There is a prominent vascular cambium between the xylem and phloem.

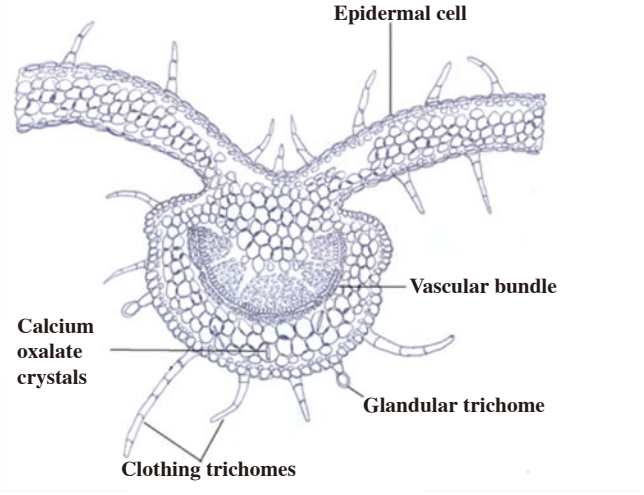

Figure 4. Transverse section of the midrib of *H. latifolia* (free hand sketch).

In transverse section, the petiole is not circular but bulges towards the ventral surface with a depression on both upper lateral sides (Figure 5). All other features were similar to that of the midrib (Figure 4).

Figure 5. Transverse sections of the petiole of *H. latifolia*.

3.3. Powder microscopy

Powder microscopy of *H. latifolia* showed the presence of abundant tetragonal prismatic calcium oxalate crystals in all parts of the plant (Figure 6). There is a large number of broken pieces of multicellular clothing trichomes in the leaves and stem. The leaf powder displayed the presence of stomata (Figure 6A); whereas that of the root showed abundant starch grains (Figure 6B). Also the lignified vessels with annular and scalariform thickening were present in the powdered stem (Figure 7).

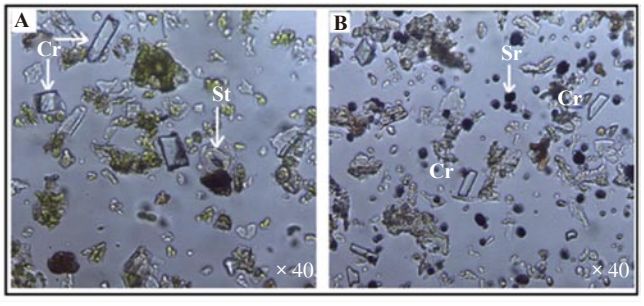

Figure 6. Powder microscopy characteristics of different parts of the plant.
A: Leaf; B: Root.
Cr: Calcium oxalate crystals; Sr: Starch grains; St: Stomata.

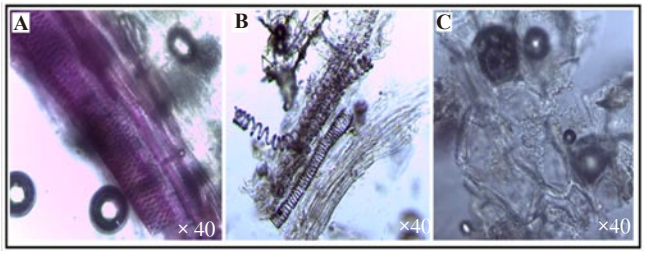

Figure 7. Microscopic characteristics of the stem powder.
A: Scalariform vessels stained with phloroglucinol/HCl; B: Annular vessels; C: Epidermal cells.

3.4. Physicochemical parameters of H. latifolia

The extractive values and ash values of the leaves, stem and roots of *H. latifolia* are shown in Table 1. The pH values of the petroleum ether, ethanol and water soluble extractives are shown in Figure 8. The petroleum ether soluble extracts of all plant parts was alkaline (pH>7) whereas the ethanol and water soluble extracts were weakly acidic to neutral.

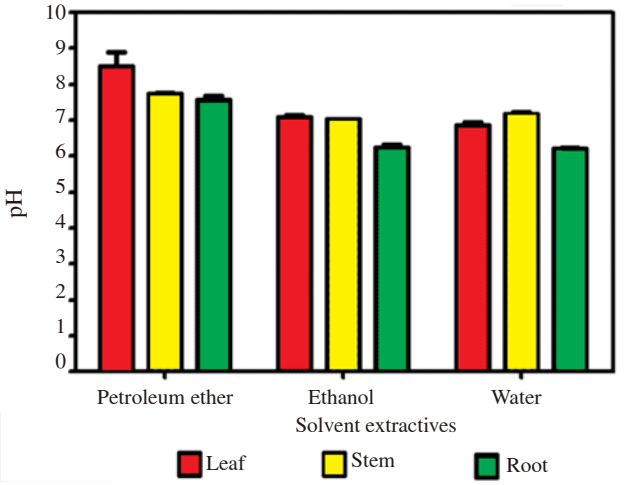

Figure 8. pH of petroleum, ethanol and water extracts of *H. latifoloa*.

Table 1
Physicochemical parameters (%W/W) of *H. latifolia*.

Physical parameter	Leaves	Stems	Roots
Petroleum ether soluble extractive	3.092±0.428	5.579±0.888	4.156±0.157
Alcohol soluble extractive	24.048±0.208	10.012±1.652	12.036±0.564
Water soluble extractive	27.060±1.060	10.170±0.670	15.800±0.871
Total Ash	16.667±0.286	7.389±0.246	5.424±0.200
Acid-insoluble Ash	0.490±0.002	0.985±0.000	0.493±0.004
Water soluble Ash	16.028±0.434	6.404±0.246	4.907±0.204
Moisture content	80.650±1.190	74.610±0.790	66.140±0.860

3.5. Fluorescence analysis of extracts

The petroleum ether, ethanol and water soluble extracts were observed under visible light and UV light (254 and 365 nm wavelengths) for their characteristic fluorescence colours. The characteristic fluorescence colours of the leaf, stem and root extracts are stated in Tables 2–4.

Table 2
Fluorescence characteristics of leaf extracts.

Plant extract (leaf)	Visible light	Long wavelength (365 nm)	Short wavelength (254 nm)
Petroleum ether	Green	Red	Yellow
Ethanol	Dark green	Red	Black
Water	Olive yellow	Light green	Dark brown

Table 3
Fluorescence characteristics of stem extracts.

Plant extract (root)	Visible light	Long wavelength (365 nm)	Short wavelength (254 nm)
Petroleum ether	Light yellow	Pink	Light yellow
Ethanol	Light green	Rose-pink	Dark brown
Water	Straw	Light green	Dark brown

Table 4
Fluorescence characteristics of root extracts.

Plant extract (root)	Visible light	Long wavelength (365 nm)	Short wavelength (254 nm)
Petroleum ether	Pale yellow	Red	Pale yellow
Ethanol	Pale yellow	Light blue	Pale yellow
Water	Straw	Light green	Pale yellow

3.6. Phytochemical screening of plant parts

Phytochemical analysis showed the presence of triterpenoids, tannins and reducing sugar in all the plant parts. Alkaloids were absent in all plant parts (Table 5).

Table 5
Chemical constituents of different plant parts.

Phytochemicals	Leaves	Stems	Roots
Triterpenoids	+	+	+
Phytosterol	+	-	+
Alkaloids	-	-	-
Flavonoids	-	-	+
Saponins	-	-	+
Glycoside (reducing sugars)	+	+	+
Tannins	+	+	+

+: Present; -: Absent.

3.7. TLC analysis

The TLC chromatogram showed two prominent pink spots (S_1 and S_2) in all three extracts with R_f values of 0.7 and 0.6 respectively (Figure 9). The leaf extract showed three additional pink spots with R_f values of 0.93, 0.88 and 0.83. The stem and root extracts contained two compounds with characteristic blue and light blue florescence under UV 365 nm.

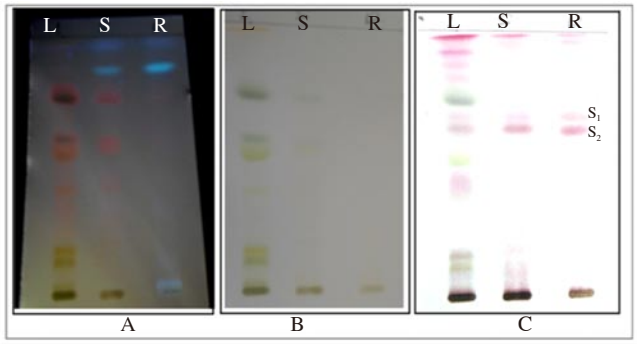

Figure 9. TLC chromatogram of different parts of *H. latifolia.*
A: Viewed under UV 365 nm; B: Viewed in visible light; C: Sprayed with anisaldehyde in concentrated H_2SO_4.
S_1 and S_2: Common prominent compounds in all extracts.

4. Discussion

H. latifolia is used extensively in traditional medicine for the treatment of diseases, especially as an anti-infective, anti-inflammatory and analgesic agent[13]. The anti-nociceptive, anti-inflammatory and antioxidant activity of the ethanol extracts of the aerial parts of the plant has been reported[4,14]. However there are no pharmacopoeial standards for the correct identification of the plant. Thus in this study, the pharmacognostic features of *H. latifolia* is being reported for the first time.

H. latifolia, as a natural drug, can be described as a shrubby herb up to 2 m tall, with some weak bristly hairs on young branches. Leaves alternate, simple and entire; stipules absent; petiole 1–7 cm long; blade ovate or elliptical to broadly lanceolate, 8–20 cm×3.5–9 cm; base rounded to cuneate and often unequal; apex long-acuminate. Inflorescence axillary, sometimes terminal, raceme 4–10 cm long, up to 30 cm in fruit, many-flowered; axis hairy; bracts 1–2 mm long, caudate, caduceus[13]. There are no petals; the sepals and stamens are 4 each with one very short style. It produces smooth reddish fruits at maturity, about 2 mm in diameter[15]. The plant has a disagreeable odour, especially in the roots.

The histological examination and powder microscopy showed that all parts of the plant contain prismatic crystals with the rosette and tetrahedral prisms in the palisade cells, a very important diagnostic feature. Uniseriate clothing trichomes were present in both the leaf and stem.

The moisture content of the fresh leaves, stem and roots ranged between 66%-80% w/w. Thus *H. latifolia* has a high moisture content and therefore there is need for immediate drying after harvesting to check the possible deterioration of glycosidic compounds. This is because the moisture content of a crude drug is responsible for its decomposition due to microbial attack or chemical changes. Excess moisture in a crude drug, at relatively high temperature, will lead to activation of enzymes and provide suitable conditions for the proliferation of microorganisms[16].

The ash values established in this study may be useful in the determination of the purity and quality of *H. latifolia*. The total ash for the leaves was higher than that of the stem and root, indicating a high amount of inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. The acid insoluble ash was very low. The acid-insoluble ash value shows that a very small amount of the inorganic component is insoluble in acid and this is of diagnostic importance. The extract values gives an idea about the nature of the chemical constituents present in the plant and is useful for the estimation of specific constituents soluble in that particular solvent used for the extraction as well as the determination of exhausted materials. The water and ethanol soluble extractives

were higher than that of petroleum ether. Thus water and ethanol are a good choice of solvent for formulation of *H. latifolia*. The water, ethanol and petroleum ether soluble extractives showed characteristic fluorescence under visible light and UV light, and this may be useful in the detection of adulterants.

Phytochemical evaluation and chemo-profiling are useful for the quality assessment of plant materials. Preliminary phytochemical analysis of the plant ascertained the presence of triterpenoids, phytosterols, reducing sugars and tannins. The TLC chromatogram of *H. latifolia* may be useful in the identification of various parts of the plant and the detection of adulterants.

The present study focused on establishing pharmacognostic standards for the identification and authentication of *H. latifolia* as well as detection of adulterants. The important diagnostic features of *H. latifolia* established in this study may be useful as a partial monograph.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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