Pharmacognostical and phytochemical standardization of *Houttuynia cordata* Thunb.: A potent medicinal herb of North–Eastern India and China

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ABSTRACT

Aim: *Houttuynia cordata* Thunb. (Saururaceae) is one of the perennial herb indigenous to North-East India and China. Despite the popular utilization of this herb as medicine, still no study has been reported so far regarding the pharmacognostical standardization. Thus, the aim of the present study was to scientifically establish a standard monograph on the basis of pharmacognostical and phytochemical aspects. **Methods:** The quality control standardization of *H. cordata* was done as per the methods described in the World Health Organization guidelines (2002). **Results:** The diagnostic characters of the *H. Cordata* leaf and rhizome portion were evaluated based on the macroscopical and microscopical characters. Determination of various physicochemical parameters such as water soluble ash (1.12% w/w), acid insoluble ash (4.02% w/w), sulphated ash (3.15% w/w), alcohol soluble extractive (12.8% w/w), water soluble extractive (14.9% w/w), loss on drying (3.42% w/w) and crude fibres content (13.10% w/w) was ascertained. Heavy metal, microbial load, fluorescence drug analysis, and preliminary phytochemical screening of different fractions were also carried out. Total phenols (45.74 mg/g tannic acid equivalent, TAE), tannins (33.29mg/g TAE), flavonoids (104.55 mg/g rutin equivalent, RE), and flavonols (17.16mg/g RE) were quantified from the ethanolic extract of the whole plant. Quantification of quercetin in the ethanolic extract was assessed by HPTLC analysis and was found to contain 4.39%, w/w. **Conclusion:** The obtained qualitative and quantitative standards will provide referential information for correct identification and standardization of this medicinal plant.

KEYWORDS: *Houttuynia cordata*, pharmacognosy, quercetin, HPTLC

INTRODUCTION

The relevance of pharmacognosy in standardization of herbal drugs was long been stressed. Many monographs based on pharmacognostical studies have emerged as an aid in the taxonomical and botanical identifications.[1] The process of standardization can be achieved by stepwise pharmacognostic studies. These studies will probably helps in the identification and authentication of the plant material.

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Houttuynia cordata Thumb. is a perennial herb with heart–like leaf and stoloniferous rhizome native to Japan, South–East Asia, and the Himalayas. It is a single species of the genus *Houttuynia* belonging to Saururaceae family and is restricted only to specialized moist habitats. *H. cordata* has been identified as one of the most potential medicinal and edible wild plant resources in China. Its young plants, including the aerial stems, leaves and underground stems are consumed as wild vegetable and its mature plants are commonly used as a kind of traditional medicinal herb in China, Korea, India, Vietnam and Thailand. In North–East India, this plant is eaten raw as a medicinal salad for lowering the blood sugar level and is commonly known by the name "Jamyrdoh" (Khasi and Jaintia tribes of Meghalaya). *H. cordata* contains six major classes of phytochemicals *viz*. essential oils, flavonoids, alkaloids, fatty acids, sterols and polyphenolic acids and these compounds exhibit a variety of pharmacological activities like anti–cancer,[2] anti–oxidant,[3] anti–diabetic,[4] anti–hypertension,[5] anti–inflammatory,[6] anti–mutagenic[7]

and anti–bacterial.[8] It is effective in treating pneumonia, severe acute respiratory syndrome, human immunodeficiency virus, influenza virus and refractory haemoptysis.[3,9,10] In view of the importance of *H. cordata* in traditional and modern system of medicine, it was thought worthwhile to develop quality standard for the same. As far as chemistry and pharmacology of this plant is concerned, large number of scientific data is available but a systematic standardization study is still lacking. Hence, in the present investigation, an attempt has been made to standardize the *H.cordata* herb which would help in the identification as well as in checking possible chances of adulteration, if any. Further, the study will also help in quality assurance of finished herbal products from this plant.

EXPERIMENTAL METHODS

Plant material

Whole plant of *H. cordata* was collected during the month of June–September (2012) from various areas of the West and East Jaintia's Hills district (*viz.* Jowai, Mihmyntdu, Khliehriat, Ladrymbai) of Meghalaya, North–East, India. Voucher herbarium specimen (COG/HC/011-2012), was prepared and preserved along with sample of crude drug in the Pharmacognosy research laboratory of Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi (U.P), India. The plant material was identified and authenticated by Dr. B.K. Sinha (Scientist In–charge), Botanical Survey of India, Shillong, Meghalaya.

Pharmacognostical studies

For pharmacognostical studies, samples of plant in fresh condition were preserved in 70% alcohol. Pharmacognostical study including morphological, histochemical and powder studies were carried out by taking the sections with the help of rotary microtome (York Scientific Industries Pvt. Ltd.).[11] Safranin (1%), fast green and toludine blue (0.2%) were used as staining reagents. Reagents like ferric chloride, ruthenium red, iodine solution and mixture of concentrated HCl and alcoholic pholoroglucinol solution (1:1, v/v) were used for histochemical tests. Photographs of different magnification were taken with Nikon trinocular microscopic unit, Model E–200, Japan. For normal observation bright field was used whereas for the study of crystal, fibers and lignified cells, polarized light was employed. Since many plant characters have bi–refringent property as seen under polarized light, hence they appear bright against dark background.

Physicochemical evaluation

Physicochemical constants such as foreign matter, loss on drying, extractive values, microbial count, crude fibre content, heavy metal analysis, total ash, acid–insoluble ash, water–soluble ash and sulphated ash values were carried out on shade–dried powdered drug following methods described in WHO guidelines and Indian Herbal Pharmacopoeia.[12,13] Fluorescence analysis of powdered drug was carried out under visible/daylight and UV light (254nm & 365nm) as per the standard procedures.[14]

Phytochemical evaluation

Preliminary phytochemical screening

The coarsely powdered plant material of *H. cordata* was exhaustively extracted for 24h by soxhlation using 95% ethanol (3L v/v) as solvent for extraction. The resulting extract was filtered and concentrated under reduced pressure to obtain the crude ethanolic extract of *H. cordata* (EHC). The ethanol extract (EHC) was then subjected to successive fractionation by suspending in aqueous media and then partitioning with solvents of varying polarity such as hexane, chloroform and ethyl acetate in order of their ascending polarity. Further, the extract (EHC) and its successive fractions were subjected to preliminary phytochemical tests to check the presence of various phytochemical classes.[15]

Quantitative estimation of various classes of phytoconstituents

Total phenolic and tannin contents were estimated spectrophotometrically in EHC according to the method described by Makkar *et al.*, 2000 using tannic acid as standard.[16] Estimation of total flavonoid and flavonol content were also ascertained using rutin as the external standard.[17] Total alkaloid content in the plant material was estimated using the usual gravimetric analysis in which the plant material was first extracted with H_2SO_4 and was further given successive washes with chloroform and diethyl ether.[18]

Thin layer chromatography (TLC) and High performance thin layer chromatography (HPTLC) analysis

Thin layer chromatography of EHC was analysed on pre–coated aluminium silica gel plates 60 F–254 as stationary phase. Mobile phases used for developing the chromatogram were composed with mixtures of solvents having varying polarity. Different reagents were sprayed

to confirm the presence of various phytoconstituents such as Dragendroff's reagent for alkaloids, vanillin sulphuric acid for the presence of terpenoidal class and sodium metaperiodate followed by benzidine for glycosides or glycoside containing sugars.

HPTLC is a sophisticated and automated technique, which is useful in separation of phytochemical mixtures present in the sample. Pre–coated plates and auto sampler were used for precision and to achieve significant separation. A stock solution of both EHC and standard quercetin in methanol was prepared in concentration of 5mg/mL and 0.2mg/mL respectively. Mobile phase for developing the chromatogram was composed of chloroform, methanol and formic acid mixture in the ratio 7.5:1.5:1 (v/v/v). The study was carried out using Camag–HPTLC instrumentation equipped with Linomat V sample applicator, Camag TLC scanner 3, Camag TLC visualizer and WINCATS 4 software for data interpretation. The R_f values were recorded and the developed plate was screened and photo–documented at ultra violet range with wavelength (λ_{max}) of 254nm.

RESULTS

Morphological, histological and powder evaluation

The leaves of the plant are green, 4–8cm in length, 3–4cm in width, simple and are broad with a long petiole, ovate–cordate with shortly acuminate apex bearing entire margin and are pubescent due to presence of trichomes. The leaves are slightly pungent in taste with a characteristic aromatic odour (Fig. 1).

The Petiole, in transverse sections through the distal end, exhibits a single U-shaped vascular strand with incurved ends. Histologically, the section also showed the presence of single layered epidermis followed by a wide zone (3–4 layers) of cortex comprised of parenchymatous cells. It is followed by continues ring of pericycle composed of sclerenchymatous cells. There are 4–6 vascular bundles arranged in ring form just below the pericycle. The central region shows the presence of large pith composed of parenchymatous cells with abundant simple and compound starch grains and raphides of calcium oxalate crystals (Fig. 2).

The transverse section of the leaf showed the epidermal layer (upper and lower) which is made up of single layer of parenchymatous cells (10–15µm in length and 5–10µm in width) covered with a thick cuticle. The leaf is of isobilateral nature bearing a single layered

Figure 1. *Houttuynia cordata* thunb.

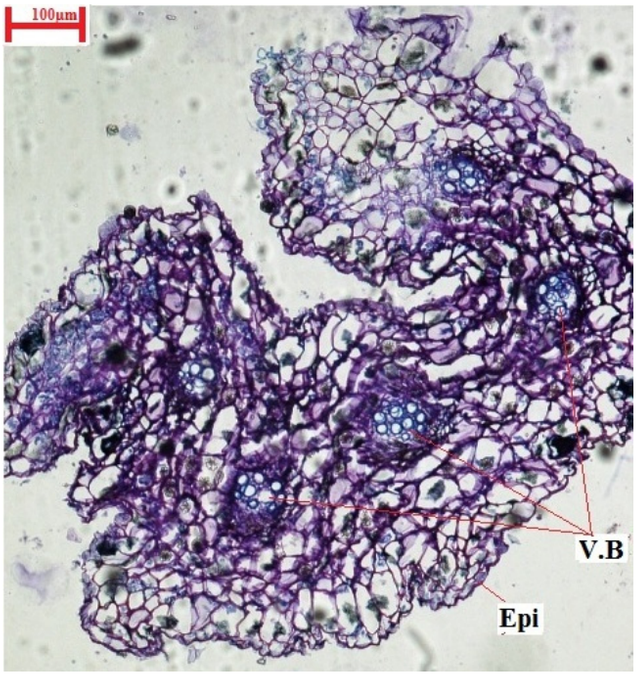


Figure 2. Histological study through petiole of *H. cordata*.

of wide palisade cells (arm palisade type) in both upper and lower epidermis continued over the midrib region. The lower epidermal layer of the lamina region showed the presence of two distinct types of trichomes *viz.* multicellular covering trichomes (2–4 celled) bearing pointed or blunted ends (90–150µm in length and 10–15µm in width) and glandular trichomes (3–4 celled glandular head) bearing unicellular stalk (70–120µm in length and 25–40µm in width). The spongy parenchymatous cells at mesophyll of the lamina when viewed under polarised light showed the presence of raphides of calcium oxalate crystals. The central region of the midrib

contains the vascular bundle bearing phloem and lignified xylem vessels with spiral thickenings (Fig. 3). In addition, the leaf at several places of the lamina bear the presence of ranunculaceous type of stomata on lower surface of the leaf and the values of stomatal number and stomatal index were found to be 104–119.4–132/mm^2 and 11–17 respectively.

The rhizomes are hairy, cylindrical in shape, 2–6mm in diameter with prominent ridges and are green in color having characteristics odour and slight pungent in taste. The transverse section of rhizome shows the presence of single layer outer epidermis composed of thick walled rectangular shaped epidermis (7–12µm in length and 5–8µm in width) which is followed by single layer of thin walled collenchymatous cell (10–15µm in length and 8–12µm in width). Below the collenchymatous layer appears the cortex region showing 10–25 layers of irregular shaped parenchymatous cells with sizes ranging from 10–25µm in length and 10–15µm in width. The parenchymatous cells of the cortical region showed the presence of numerous simple and compound starch grains along with raphides of calcium oxalate crystals. The cortex region is followed by a single continuous layer of lignified pericyclic fibers which are made up of sclerenchymatous cells (6–10µm in length and 2–4µm in width). Below the pericyclic layer is the vascular bundle layer (18–20 in number) which are conjoint in nature and are collateral. Phloem is represented by sieve elements while xylem is represented by vessels, tracheids, fibres and xylem parenchyma all showing strong lignifications on their walls. The innermost layer of the rhizome is made up of wide central pith which is composed of irregular shape parenchymatous cells (15–30µm in length and 12–25µm in width) filled with numerous starch grains (Fig. 4).

Powder microscopy of whole plant shows the presence of slender shaped fibers with size ranging from 250–350µm in length and 5–8µm in width and in many places it also shows raphides of calcium oxalate crystals. The trichomes (150–250µm in length and 10–15µm in width, and xylem vessels with spiral thickening (203–381µm length and 15–20µm in width) were also found (Fig. 5).

Physicochemical evaluation and Quantitative estimations

Evaluation of physicochemical parameter is important in the determination of adulterants and improper handling of drugs. The ethanol soluble and water soluble extractive values were found to be 12.8% w/w and 14.9% w/w

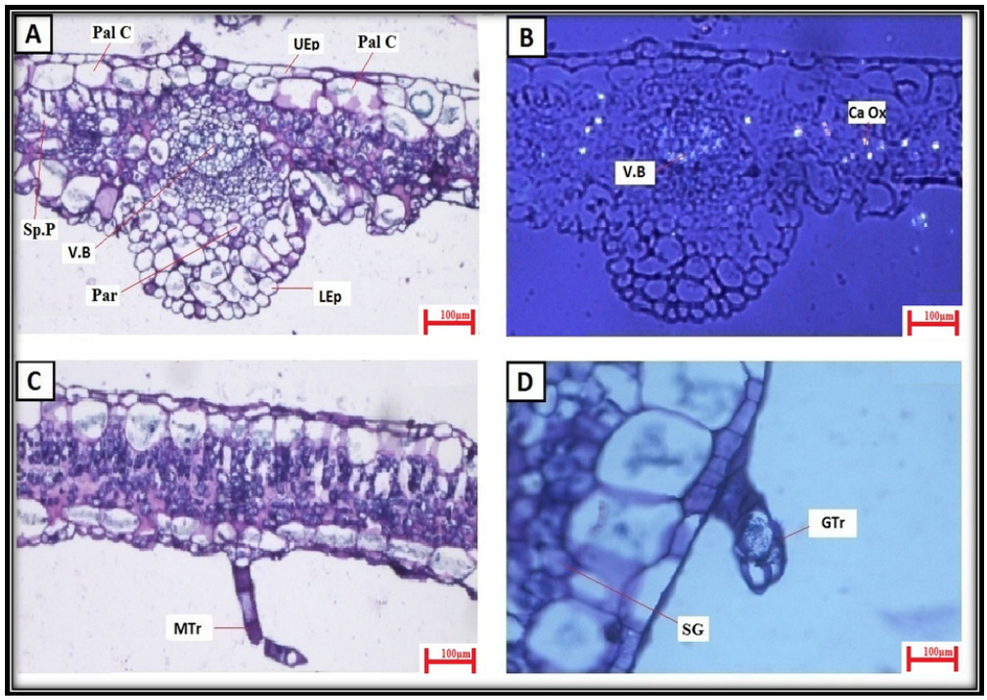
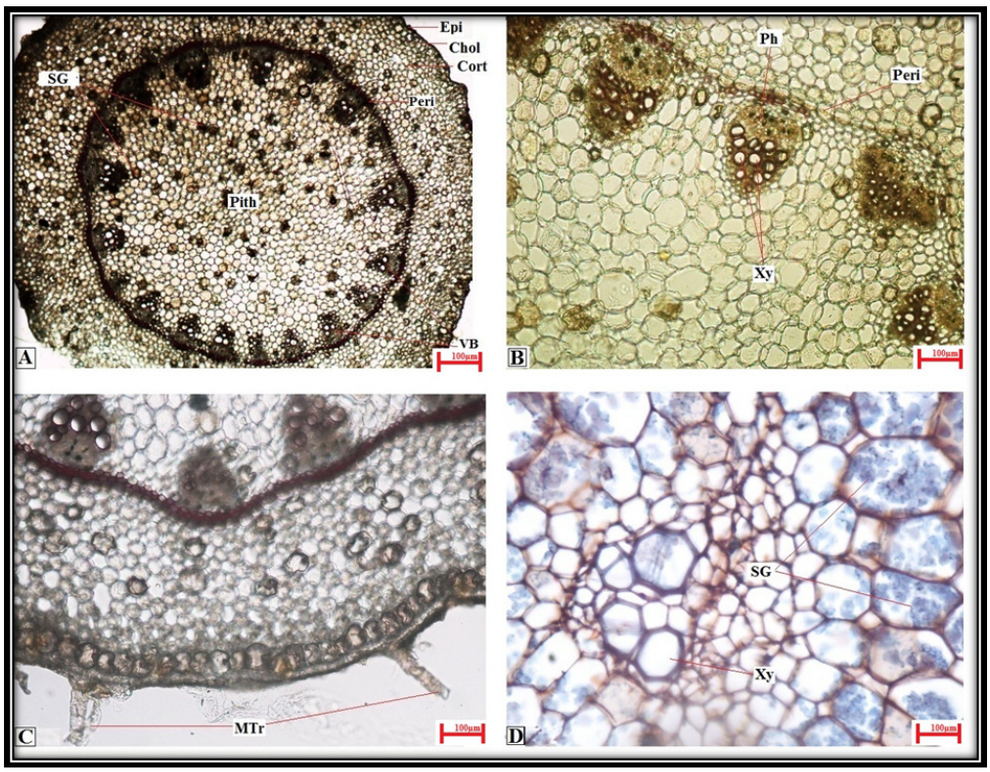


Figure 3. Histological study of *H. cordata* leaf. [A]: transverse section (TS) of midrib portion of leaf (10 X), [B]: TS showing vascular bundles (V.B) and calcium oxalate crystals (Ca.Ox) under polarized light, [C–D]: TS of lamina of leaf showing multicellular (MTr) and glandular (GTr) trichomes, [UEp – upper epidermis; LEp – lower epidermis; PalC – palisade cell; Sp.P – spongy parenchyma; Par – parenchymatous cells; V.B – vascular bundels; SG – starch grain].



Fgure 4. Histological study of *H. cordata* rhizome. [A]: TS of rhizome (10X), [B]: TS of rhizome (100X) showing arrangement of vascular bundles (VB), [C]: TS showing multicellular trichomes (MTr), [D]: TS showing iodine stained starch grains (SG). [Epi – epidermis; Chol – chollenchyma; Cort – cortex; Peri – pericycle; Pith – pith; Xy – xylem; Ph – phloem].

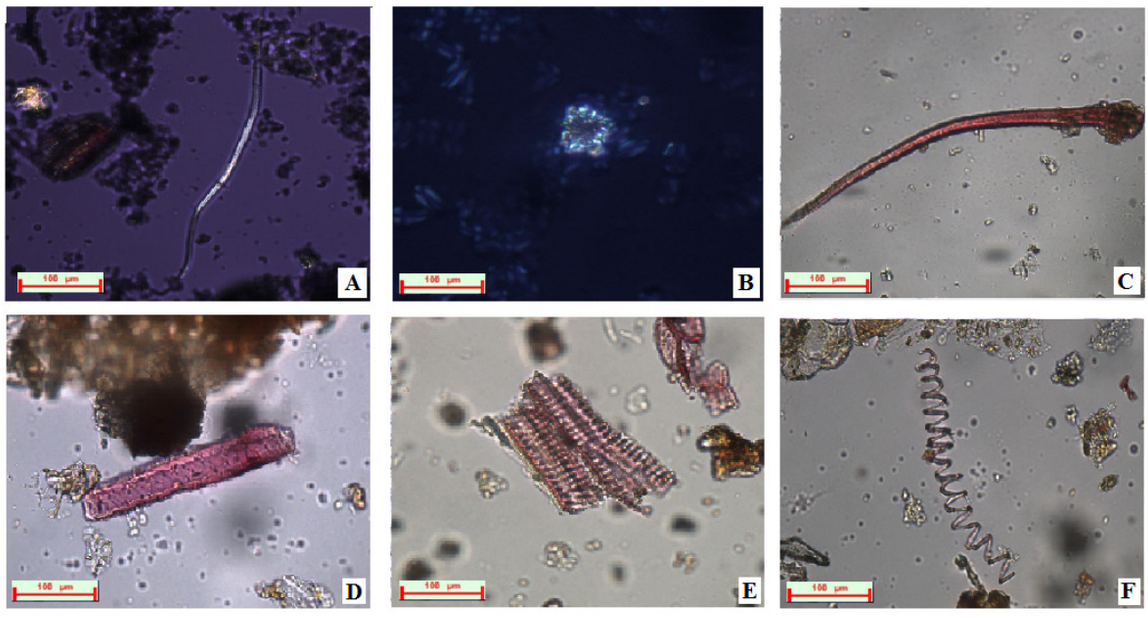


Figure 5. Powder characteristics study of *H. cordata.* [A]: Slender shaped fiber, [B] Raphides of calcium oxalate crystals, [C]: Lignified trichome, [D–F]: Vascular bundles with spiral thickening.

respectively. The total ash value of the crude drug was found to be 14.13% w/w, while water soluble ash, acid insoluble ash and sulphated ash values were determined as 1.12%, 4.02% and 3.15% w/w, respectively. Loss on drying at 105°C is determined since the presence of excess moisture is conclusive to the promotion of mould and bacterial growth, and subsequently to deterioration and spoilage of the drug. Loss on drying content was

determined to be 3.42% w/w. Crude fibre content was found to be 13.10% w/w. Microbial contamination of powder was confirmed to be within limits as shown in Table 1. Heavy metal analysis revealed that each element was present within specified limits as per WHO guideline 2002 as results are shown in Table 2. Color reaction of powdered drug and their fluorescence analysis with different reagents were studied (Table 3).

Phytochemical evaluation

Yield of sub fractions and preliminary phytochemical screening

The percentage yield of the EHC obtained by soxhlation was found to be 13.2% w/w, whereas, the percentage yield of various fractions *viz.* successive fractionation of EHC gave hexane (5.3% w/w), chloroform (3.9% w/w), ethyl acetate (2.2% w/w) and aqueous fraction (6.1% w/w). The results for the preliminary phytochemical screening of EHC and its successive fraction are represented in table 4. The phytochemicals present were found to be polyphenolics (tannins, phenolic and flavonoids), steroids, proteins, amino acids, carbohydrates and alkaloids. Whereas, glycoside, sapponin and mucilage components seem to be absent. Ethyl acetate fraction was found to be rich in phenolics and flavonoids (Table 4). Preleminary phytochemical screening gives a brief idea about the qualitative nature of active phytoconstituents present in the *H. cordata* extract/fractions.

Quantitative estimation

Preliminary phytochemical analysis of the extract revealed the presence of phenols, flavonoids, tannins and alkaloids as a major component. Total phenolic content of *H. cordata* was reported to be 45.74mg/g tannic acid equivalent while total tannin content was estimated to be 33.29mg/g tannic acid equivalent. Total flavonoid and flavonol content were found to be 104.55 and 17.16mg/g rutin equivalent. Total alkaloid content estimated in the plant material were reported to be 0.13% w/w. HPTLC studies revealed well resolved peaks of EHC containing quercetin. The spots of the entire chromatogram were visualized under UV 254nm and the percentage of

Table 1. Microbial contamination test.

Parameter	Specified limit	Value
Total plate count	<1000 cfu/g	102 cfu/g
Yeast & Mould	<100 cfu/g	13 cfu/g
E. Coli	Negative	Negative
Salmonella	Negative	Negative
Staphylococcin	Negative	Negative

CFU: colony forming unit per gram

Table 2. Heavy metal content.

Heavy metal	Specified limit (ppm)	Result (ppm)
Arsenic (As)	< 2	0.091
Lead (Pb)	< 2	0.0189
Cadmium	< 2	0.0003
Mercury	< 2	0.078
Zinc	< 2	0.019

ppm: parts per million

Table 3. Fluorescence powder drug analysis of *Houttuynia cordata* Thunb.

S. No.	Powder + Chemical	Fluorescence in day light	Fluorescence at λ_{max}254nm	Fluorescence at λ_{max}365nm
1	Powder as such	Brown	NF	NF
2	Powder + 1N NaOH in methanol	Dark olive green	NF	Corn silk
3	Powder + 1N NaOH in water	Maroon	NF	Light green
4	Powder + 1N HCL in methanol	Dark red	NF	Corn silk
5	Powder + 1N HCL in water	Khaki	NF	Pale green
6	Powder + 1N HNO_3 in methanol	Brown	NF	Blanched almond
7	Powder + 1N HNO_3 in water	Orange	Golden red	Yellow green
8	Powder + Iodine (5%)	Orange red	NF	NF
9	Powder + $FeCl_3$ (5%)	Dark olive green	NF	NF
10	Powder + KOH (50%)	Maroon	Lawn green	Spring green
11	Powder + NH_3 (25%)	Dark red	NF	Green yellow
12	Powder + Picric Acid	Yellow	NF	NF
13	Powder + Acetic Acid	Dark orange	NF	Ivory

NF: No Fluorescence

Table 4. Preliminary Phytochemical screening of *Houttuynia cordata* Thunb.

Phytochemical class	Ethanolic extract (EHC)	Successive fractions from EHC			
		Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Aqueous fraction
Alkaloids	+	–	+	+	–
Phytosterol	–	+	+	+	–
Phenolic	+	–	+	+	+
Tannins	+	–	–	–	+
Flavonoids	+	–	–	+	+
carbohydrates	+	–	–	–	+
Reducing sugars	+	–	–	+	+
Hexose sugars	+	–	–	+	+
Saponins	–	–	–	–	–
Proteins	+	–	–	+	–
Amino acids	+	–	–	–	+
Cardiac Glycosides	–	–	–	–	–
Anthraquinon glycosides	–	–	–	–	–

(+) indicates present, (-) indicates absence

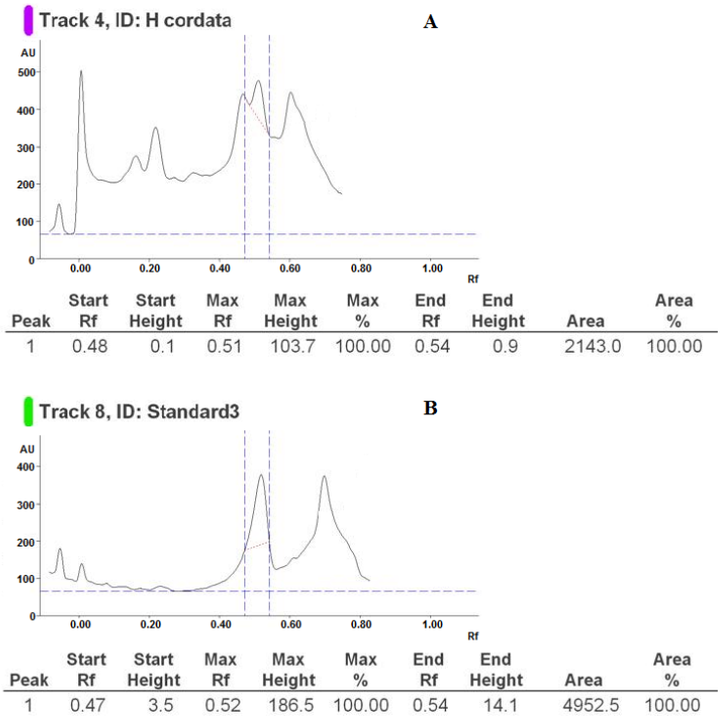


Figure 6. HPTLC densitogram of quercetin in ethanol extract of *H. Cordata* (HC). In figure A: Peak of quercetin present in HC and B: Standard peak of quercetin.

quercetin (R_f 0.51) in *H. cordata* extract (EHC) was found to be 4.39% (w/w) (Fig. 6).

DISCUSSION

Currently, there is a prominence on the standardization of medicinal plant materials for their therapeutic potentials. The modern pharmacognostic techniques available make the identification and evaluation of crude drugs more reliable, accurate and inexpensive. According to the World Health Organisation (WHO), pharmacognostical standards are considered to be the primary step for diagnosis of the herbal drug, which includes macroscopic and microscopic evaluation of the particular plant/plant parts.[19] Further, the evaluation of crude drugs on the

basis of histological studies helps to broaden the views about the type of characters and their occurrence in plant tissues, which in turn is necessary in proper identification of plant/plant parts.[20] Thus, the present work was carried out on *H. cordata* to lay down the standards which could be useful for establishing its authenticity and maintaining its quality, safety and reproducibility. On the basis of the pharmacognostical studies it was observed that there are various diagnostic features present in the *H. cordata* plant which can serve as useful information in maintaining the genuine nature of the drug. The specific diagnostic character of leaf showed the presence of arm palisade cells in both below the upper and lower epidermis which confirms its isobilateral nature. Moreover, another important features are the presence of trichomes which are multicellular and glandular types, raphides of calcium oxalate crystals and stomata of ranunculaceous type. However, the rhizome showed the presence of lignified pericyclic fibers, ring of collateral vascular bundles, compound starch grains and raphides of calcium oxalate crystals.

The physicochemical evaluation is an important parameter which helps in detecting adulteration or improper handling of the drug. The ash values are quantitative standards that represent the presence of various impurities like carbonate, oxalate and silicate which may be naturally occurring or deliberately added to crude drug as a form of adulterant. Total ash includes both physiological as well as non–physiological ash, while acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs.[21,22] Extractive values are useful to assess the amount of active chemical constituents present in the plant/plant parts using different solvents.[12] Loss on drying indicates that the drug is safe regarding any growth of bacteria, fungi and yeast**.** Quantitative determination of pharmacognostical parameters is efficient to set up standards for crude drugs. Heavy metal (As, Pb, Ca, Hg and Zn) and microbial load for powder drug were found to be present within the limit of WHO guidelines, indicating that the plant is safe to be used free from any unwanted contaminations. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins and steroids in different fractions of *H.cordata*. Such phytochemical screening is helpful in the prediction of nature of phytoconstituents present in the tested drugs since phytochemicals are proven to be responsible for the activity of the drugs. Moreover, the chemical standardization of EHC was also performed with the help of HPTLC and the amount of quercetin was quantified as a chemical marker.

Recently, there has been great demand for plant derived products in developed countries. These products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics.[23] In order to avoid the misuse of harmful plant material pharmacognostic studies and phytochemical screening can serve as a basis for proper identification, collection and investigation of the plant. These parameters are to be useful in the preparation of the herbal monograph for its proper identification. Any crude drug which is claimed to be *H. cordata*, but whose characters significantly deviate from the above accepted standards would then be rejected as contaminated, adulterated or downright fake. Hence the present study will serve as useful information with respect to the identification, authentication and standardization of *H. cordata* herb.

CONFLICT OF INTEREST

No conflict of interest has been reported by all the authors.

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