Indian Journal of Natural Products and Resources
Vol. 13(3), September 2022, pp. 362-373
DOI: 10.56042/ijnpr.v13i3.40840

Pharmacognostic study of roots and aerial parts of less explored *Heracleum candicans* Wall. ex DC.from Betaab Valley, Pahalgam, Kashmir, India

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Received 18 September 2020; revised received 07 June 2022; accepted 17 June 2022

Plant derived products have sparked considerable attention due to their versatile applications. The plants are among the richest bio-resource of drugs for traditional and modern medicines and leads for synthetic drug development and various pharmaceutical intermediates. *Heracleum candicans* Wall. ex DC. is reported as a medicinal herb of Apiaceae family. It is also known as White-leaf Hogweed and traditionally reported to be effective in various disease conditions like skin diseases, sunburn, and external tumours. The present work deals with the pharmacognostic evaluations of root and aerial parts of *H. candicans*. Macroscopic and microscopic analysis was carried out along with various physicochemical analysis, phytochemical screening as well as thin layer chromatography studies. Moreover, the presence of heavy toxic metals by ICP-OES method was also evaluated. The pharmacognostic studies of *H. candicans* showed the presence of prismatic calcium oxalate crystals, anomocytic stomata, reticulate and spiral xylem vessels along with lignified fibres with two types of covering trichomes. Preliminary phytochemical studies revealed the occurrence of carbohydrates, flavonoids, phenols, tannins, saponins, phytosterols, diterpenes, coumarins, cardiac glycosides, fats and oils. The estimation of various physicochemical constants could be beneficial in determining various quality control standards for crude drug. The findings from this study would be beneficial for the identification of *H. candicans*.

Keywords: Flavonoids, *Heracleum candicans*, Microscopy, Phenols, Quality control.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 125/00, A61K 127/00

Introduction

The search for medicines for various disease conditions from natural resources has been performed since ancient times. Various knowledge exchange, memorials, and written documentation are evidence for this. Around two-thirds of novel, medications were directly or indirectly developed from plants. Hence, comprehensive studies on phytoconstituents from traditional medicines using phytochemical, pharmacological, and analytical methods are the need of the hour[1]. The standardization of crude drugs is of paramount importance that begins with the collection of botanical materials and ends with their packaging and usage as medicine. Microscopy, macroscopy, physicochemical characteristics, extractive values, fluorescence analysis, and heavy metal analysis are utilized to set pharmacognostical standards. These characteristics, in turn, can serve to ensure the drug's quality[2]. Amongst the family of Angiosperms, Apiaceae or Umbelliferae is the largest family and recognized as diverse group of species of medicinal value[3]. The family comprises of about 450 genera and 3,700 species worldwide[4]. In India, of the total 186 species (representing 55 genera) of this family, 150 (80.6%) species representing 45 genera (81.8%) are found in the Himalayan region[5].

Heracleum is a derivative of Latin word, *Herâclêus* or belonging to *Hercules* (itself derived from the Greek) means 'glory of Hera'. Around 125 *Heracleum* species are present globally with the Sino-Himalayan and Caucasus region as the major centres of diversity[6-8]. Genus *Heracleum* (family Umbelliferae) or 'Hogweed' is a perennial plant spread in Asia, Europe, North America and Abyssinia[9]. This genus is widely distributed in Asia[10]

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with around 15 species in India, out of which 5 are prevalent to Peninsular, India[11-12]. The genus is made up of tall or dwarf perennial or monocarpic aromatic plants with simple leaves that are lobed (1 to 2), trisect to biternate, hairy or glabrous. The sepals are tiny, the petals are white or greenish, and the fruits are spiky or glabrous[11]. However, because the taxonomy of this genus is challenging, botanists require more and better samples for authentication[11].

The species *H. candicans* is of substantial commercial importance being a major source of xanthotoxin. The xanthotoxins are widely used in the treatment of leucoderma as a component of sun-tan lotion. It has been isolated from many Himalayan and non-Himalayan plant species of *Heracleum* for instance, *H. mantegazzianum* Somm. and Lev., *H. spondylium* L., *H. yunngningense, H. rapula* Franchet, *H. lanatum* (Michx.) Dorn, *H. persicum* L., *H. sibiricum* L. and some other *Apiaceae* species like *Ammi majus* L. and *Angelica japonica* A. Gray. Among the Himalayan species, *H. candicans* Wallich ex de Candolle has a maximum percentage of xanthotoxin (1.5%) followed by *H. cachemiricum* Cl. (0.05%), *H. canescens* Lindl. (0.005%) and *H. pinnatum* Cl. (0.005%)[10-13].

Most of the *Heracleum* species are utilized as medicinal plants, herbs, or spices. Several traditional uses exist for *H. persicum, H. sphondylium*, and *H. candicans* in particular. They are used to treat epilepsy, flatulence, stomachache, used as analgesic, carminatives, digestives, antiseptics, and anticonvulsants[14]. Many specialized metabolites such as coumarins, flavonoids and lignans have been isolated and identified from the genus *Heracleum*[15]. This genus has the potential to develop new coumarin derivatives. These compounds exhibit anti-Alzheimer's, anti-neurodegenerative, antioxidant, anticancer, antidiabetic, antibacterial, antiviral, and anti-inflammatory effects. Researchers have studied a few of the biological effects *Heracleum* including antibacterial, antiproliferative, and anti-inflammatory[16].

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Materials and Methods

Procurement and authentication of plant material

Aerial and ground plant parts of *H. candicans* were collected in the month of July 2014, from an altitude of 2850-2900 m above sea level, from the foothills of Betaab Valley, Pahalgam, District Anantnag, Jammu and Kashmir, India. Herbarium specimen was prepared and the plant was authenticated and identified by the Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir with voucher specimen number 2118-KASH.

Macroscopic study

Macroscopic evaluation is an important parameter to establish the identity of crude drugs. Macroscopic characters of a drug include its visual appearance to the naked eye. Thus, detailed morphological study of the characters could be beneficial in distinguishing them. The macroscopic evaluation of *H. candicans* was done as per the method described earlier[17].

Microscopic study

Various cellular structures like epidermal trichomes, calcium oxalate crystals, starch granules, lignified tissues were carefully examined with their size, shape, and histochemistry was also performed by using various staining reagents[18]. The dried root, leaf, and stem were powdered and the microscopic characters were examined by using various staining reagents[19]. The characteristic structures of the cells and its components were observed followed by capturing photomicrographs. Other staining reagents like ruthenium red, toluidine blue, were also used for microscopy[20].

Physicochemical evaluation

The physicochemical parameters like extractive values, ash values and foaming index was evaluated as per the standard method[21-22].

Determination of pH

The pH of aqueous solution (1 and 10%) of the powdered drug was carried out using a calibrated glass electrode.

Extract preparation

After collection and authentication, aerial and root plant material were dried in shade and powdered. The powdered material was sieved by 40 mesh sieves, accurately weighed and used for extraction. The successive extraction was done by different solvents like petroleum ether, chloroform and methanol in Soxhlet apparatus on the other hand, decoction method was used for aqueous extraction. The extracts were concentrated under reduced pressure through rotary evaporator and dried. The resulting extract was weighed and stored in airtight containers (amber coloured) at temperature between 5-7°C till further use[23].

Fluorescence and behavioural analysis

Several plant materials exhibit fluorescence on exposure to UV light and this property is utilized in plant identification. The fluorescence study of the samples was carried out in daylight and UV light (254 and 366 nm) both. Likewise, after treatments with different chemical reagents for instance nitric acid, sodium hydroxide, iodine, acetic acid, picric acid, hydrochloric acid, ferric chloride, etc. were also analyzed[24-25]. Behavioural analysis of powdered plant material with various chemical reagents was also conducted.

Preliminary phytochemical investigation

Plant extracts obtained were then exposed to qualitative phytochemical screening to identify the presence of various primary and secondary metabolites like glycoside, alkaloids, flavonoids, tannins, sterols and steroids, terpenes and terpenoids, phenolic compounds, coumarins, mucilage, resins, carbohydrates, protein, amino acids, saponins, and fats and oils, etc[26].

Thin layer chromatography

The samples of plant extracts were applied on silica gel G TLC plates with the help of capillary tubes. TLC chamber was then utilized for the development of these plates using appropriate mobile phase. The mobile phase was optimized based on the hit and trial method. Chromatographic rectangular glass chamber (16.5x29.5) and pre-saturated TLC chamber were used for this purpose. The plates were exposed to iodine vapours after completion of airdrying. Retention factor (R*f*) was estimated individually for all the samples[27].

Heavy metal analysis by ICP-OES instrument

The heavy metals analysis of powdered plant material is an important tool to evaluate the quality of crude drugs. Heavy metal evaluation for root and aerial parts of *H. candicans* was done by ICP-OES method at Avon Food Lab (Pvt) Ltd, Delhi (NABL accredited). Four main heavy toxic metals Cd, As, Pb, and Hg were evaluated for their presence in the root and aerial parts of *H. candicans*.

Results

Pharmacognostic evaluation

Macroscopic evaluation

The leaves of the plant are broad ovate, around 3-9 cm in length and 3-6 cm wide, with lower surface of leaves having papery texture. The stem is elongated, yellowish green in colour, around 1-2 cm in diameter and 10-18 cm long. The powder of the aerial parts was yellowish green in colour with pleasant odour and astringent taste. The roots are elongated, 3-8 cm long and 1-3cm wide with rough surface. The powder of the root part was brownish in colour with pleasant odour and astringent taste. The pleasant odour was more intense in the root part than the aerial part. The macroscopic study of the aerial and root parts of *H. candicans* revealed the presence of white-felted underside of the large pinnately lobed leaves with characteristic shape and faint odour. Lower and basal leaves were pinnate; 2-3 pinnate pairs, ovate-oblong, 4-13 cm long, 3-6 cm wide, densely white tomentose, abaxially silvery, margins serrate with obtuse apex. Flowers, white; the outer petals of the flowers are larger, bilobed. Stem is solitary, branched. White petals, minute calyx teeth, radiant outer flowers of umbels, fruit, obovoid, around 6-12 mm long, 4-7 mm wide, glabrous when mature. Roots are stout, cylindrical 2-3 cm wide. Numerous unicellular and non-lignified covering trichomes with elongated cells were present (Fig. 1).

Microscopic evaluation

The diagnostic features of powder microscopy of aerial part of *H. candicans* in surface view revealed lamina fragments. Upper epidermis was formed by wavy walled polygonal cells, irregularly thickened and beaded. There was more elongation in the cells over the vein's region, underlying small palisade cells

Fig. 1 — Macroscopic study of aerial and root parts *H. candicans*. a-b) Dried andelongated root; c) *H. candicans* in its habitat; d) Stem of *H. candicans*; e) Surface view of Leaf.

were closely packed. The detailed microscopic studies are depicted in Fig. 2. The cytological structures of lower epidermis were smaller than upper epidermis; numerous small anomocytic stomata were also found; occasionally lignified cicatrices and abundance of covering trichomes were also found.

The abundant crystals of prismatic calcium oxalate were found in powder of aerial and root parts of *H. candicans*. They were variable in size and some of them were moderately large. From the veins and rachis, fibres which were lignified and groups of vascular tissue were also seen. Thickened walls of fibres; few pits and the vessels were also lignified which were thickened reticulately or annularly. Lignified xylem vessels were found to be of two types i.e., reticulated and spiral-shaped in the powder of *H. candicans*. Simple starch grains were also seen in the powder microscopy particularly in the aerial part. Stomata that were seen in the parenchymatous tissue of aerial powder were of anomocytic type. The detailed microscopic characters for the roots and aerial parts are presented in Fig. 2-4.

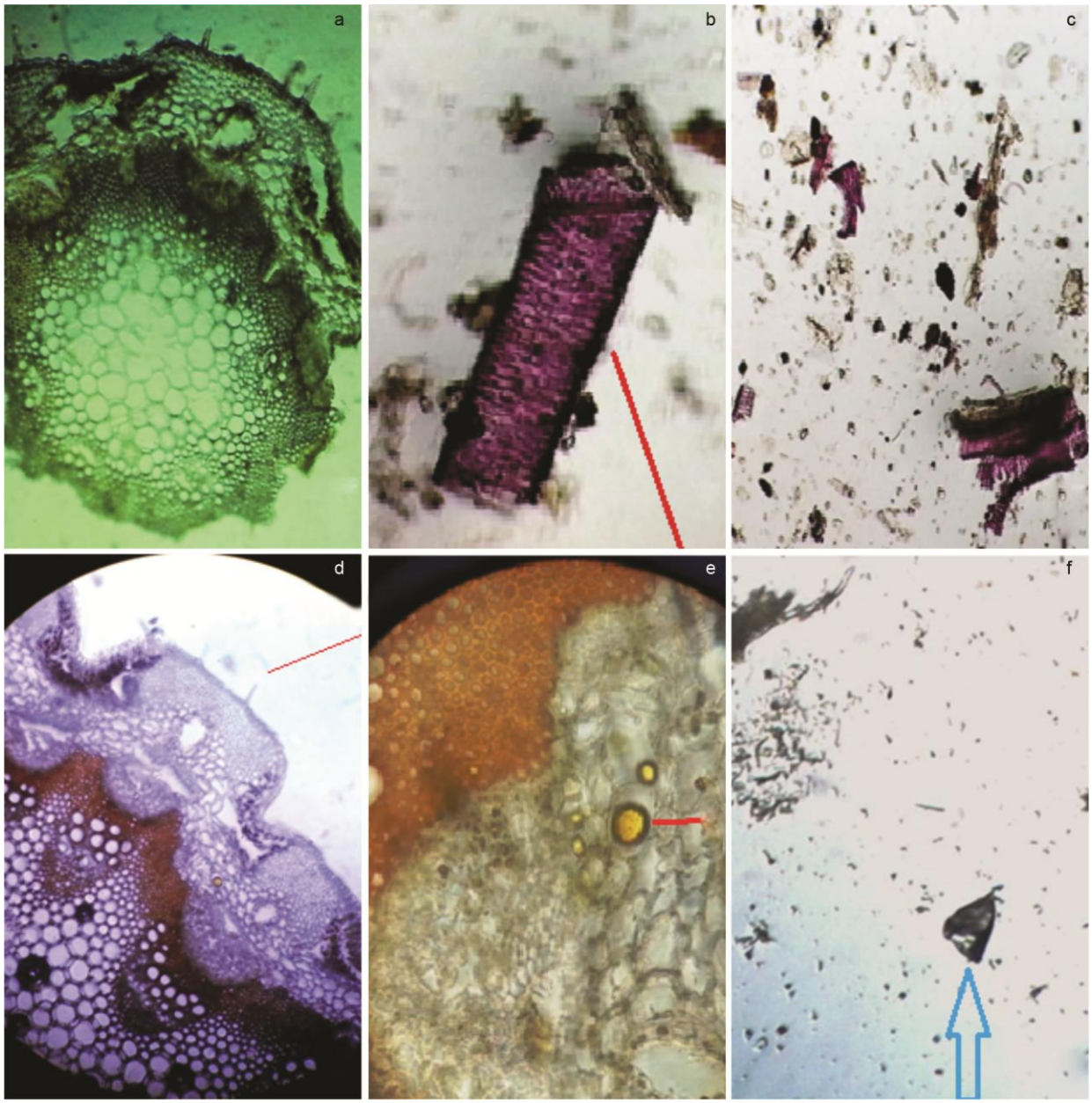

Fig. 2 — Microscopic study of *H. candicans* root. a) TS of roots; b) Lignified xylem vessels in the root powder; c) Root powder showing Ca oxalate crystals and xylem vessels along with starch grains; d) TS of roots showing trichomes; e) TS of roots showing oil cells (glands); f) Prismatic calcium oxalate crystals in the roots powder.

Extraction values of H. candicans (aerial parts and roots)

While carrying out the cold extraction of root part, the maximum extractive value of 17.9% w/w was found in aqueous extract followed by chloroform (1.90% w/w), methanol (1.40% w/w), and petroleum ether (1.03% w/w). Similarly, in the case of aerial part also, the maximum cold extractive value was found in aqueous extract (16.30% w/w) followed by methanol (13.99% w/w), chloroform (2.71% w/w) and petroleum ether (1.10% w/w). Hot extractive values of aqueous, methanol, chloroform and petroleum ether extracts of aerial part were 24.10, 18.90, 4.90, and 2.20% w/w respectively and for root part, the hot extractive values of aqueous, methanol, chloroform and petroleum ether were 25.80, 19.60, 4.60, and 2% w/w respectively. Successive extractive value in aerial part was maximum in methanol (17.38% w/w) followed by aqueous (14.80% w/w), chloroform (5.60% w/w) and petroleum ether (2.90% w/w). Similarly, the successive extractive value in roots was

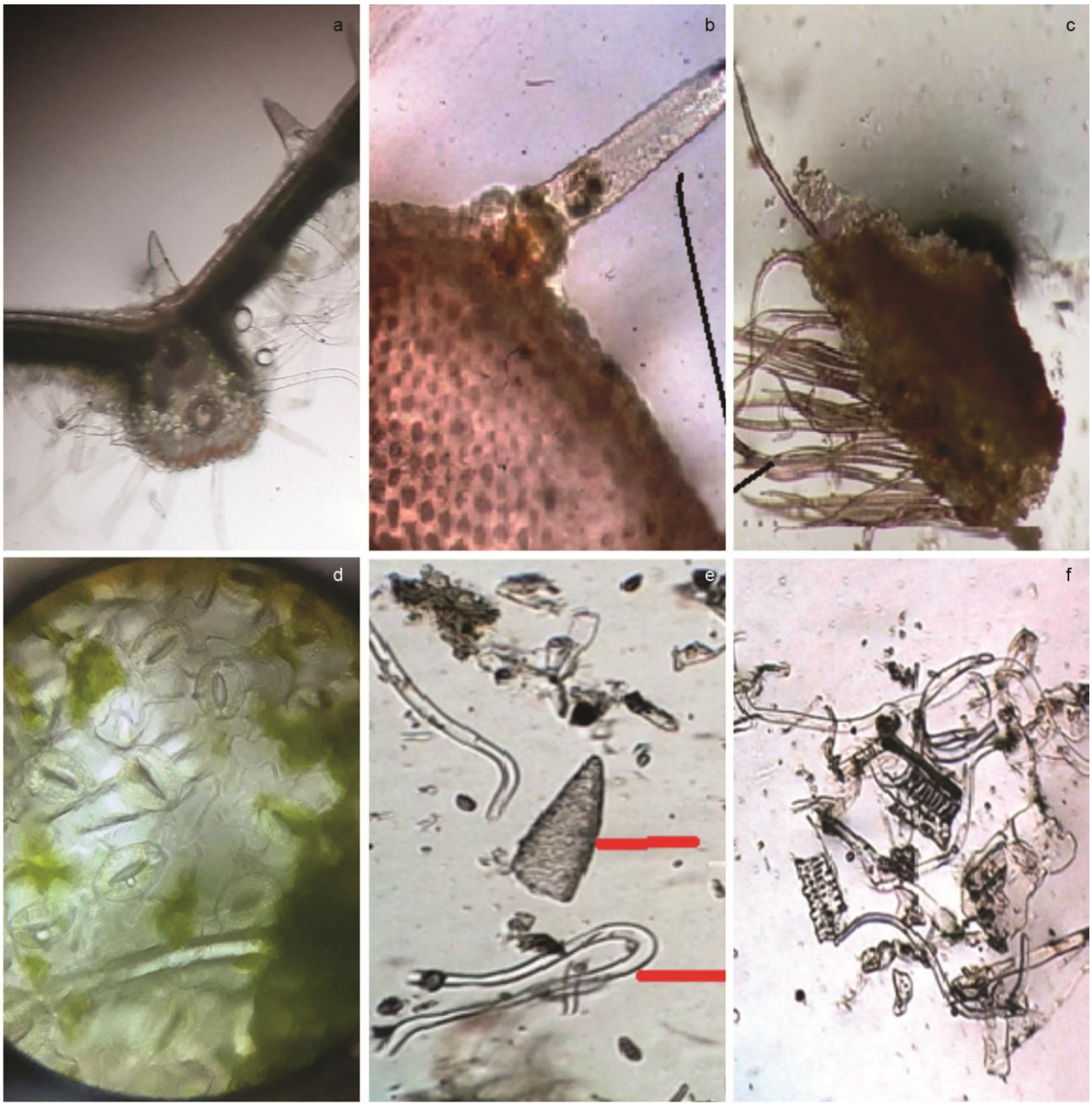

Fig. 3 — Microscopic study of *H. candicans* leaf and aerial part, a) T.S. of leaf; b) Unicellular covering trichome; c) Presence of abundant covering trichomes in the lower leaf surface; d) Anomocytic stomata in aerial part; e) Covering Trichomes at upper and lower surface of leaf; f) Prismatic Ca oxalate crystals and spiral xylem vessels in aerial part.

maximum in methanol (18.20% w/w) followed by aqueous (16.34% w/w), chloroform (4.80% w/w) and petroleum ether (2.70% w/w) respectively. The details are presented in Fig. 5.

The pH value of 1 and 10% solution of leaf in distilled water were found to be 6.24 and 6 and the pH value of 1 and 10% solution of root in distilled water were found to be 6.61 and 6.33 respectively representing presence of different constituents of acidic nature in this plant. The active chemical constituents' percentage in crude drugs is calculated on air dried basis. The loss on drying (LOD) of dry powder of leaves was 11.73% and that of roots was 13.93%. The foaming index was found to be less than 100 for both aerial and root parts of *H. candicans*. The total ash value which represents the inorganic and non-physiological matter such as silica occurring naturally in the drug or adhering to it. The ash value

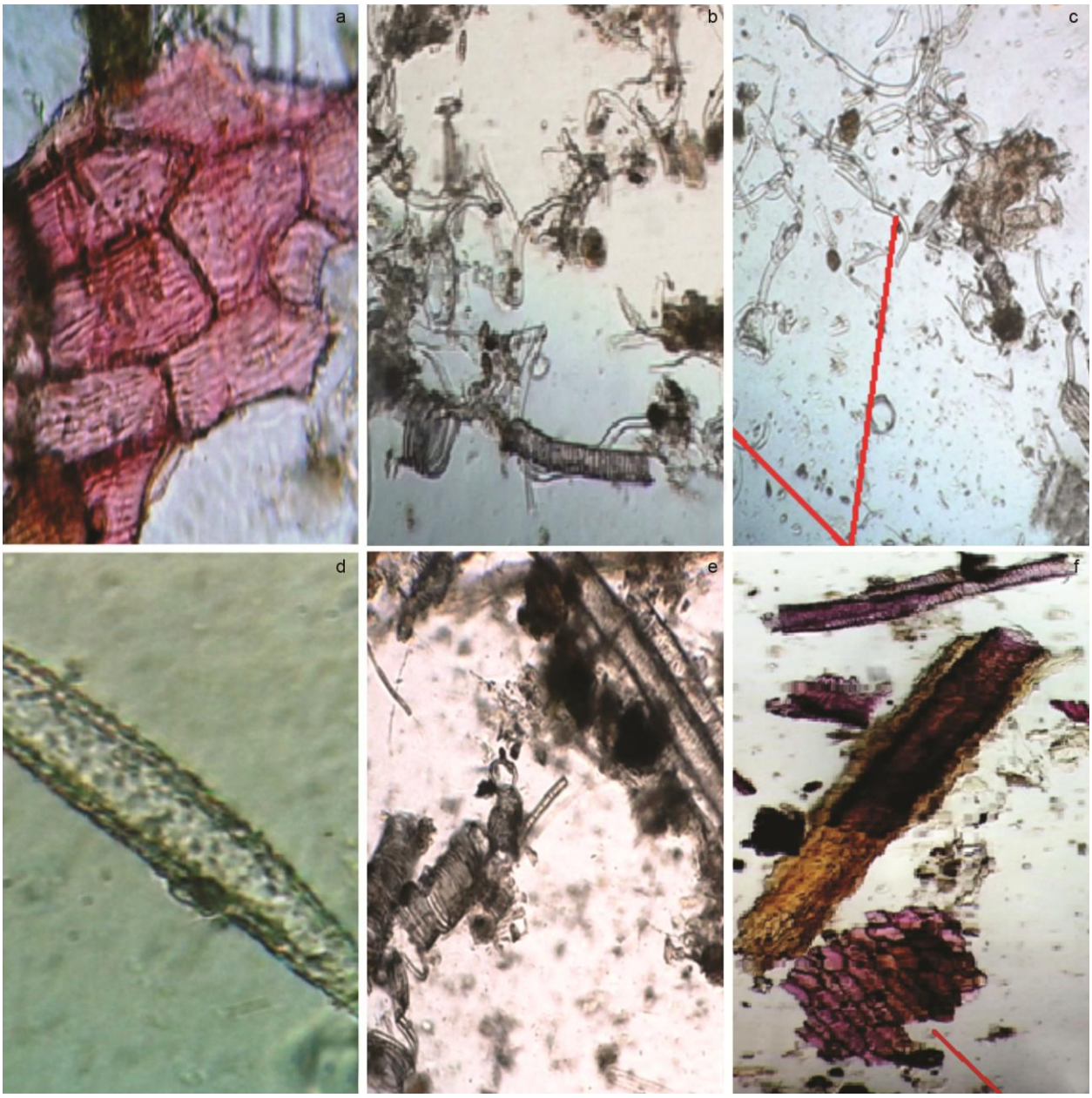

Fig. 4 — Powder microscopic study of *H. candicans* aerial part, a) Lignified pitted parenchyma in aerial part; b) Aerial part showing spiral & reticulated xylem vessels; c) Abundant covering trichomes in aerial part; d) Aerial powder showing a covering trichome under 40x; e) Reticulated xylem vessels in aerial part; f) Lignified pitted parenchyma in aerial part.

was found to be 12.16 and 12.15% in leaves and roots respectively. The details pertaining to physicochemical analysis are presented in Table 1.

Fluorescence analysis

Fluorescence analysis was performed with various reagents to observe the difference in the physical characteristics of the crude drugs. The fluorescence analysis results of this plant are presented in Table 2.

Preliminary phytochemical screening

The preliminary phytochemical screening was done to identify the constituents of the plant and the results have been tabulated in Table 3 which indicated the presence of various phytoconstituents like carbohydrates, alkaloids, flavonoids, polyphenolic compounds, tannins, and terpenoids. Similarly, behavioural studies of the powdered drug was conducted to observe the fundamental behavioural

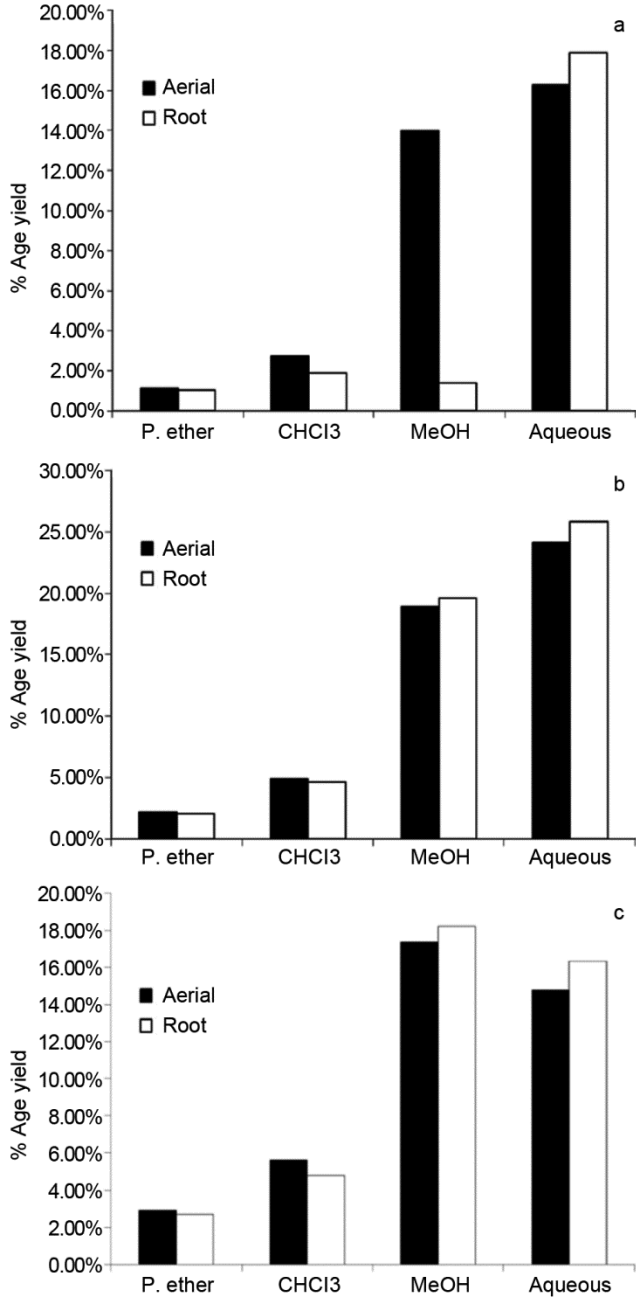

Fig. 5 — Cold, hot and successive extractive values of *H. candicans* aerial parts and roots.

studies of the crude drug. The results of behavioural analysis are presented in Table 4.

Thin layer chromatography

After preliminary screening, an attempt was made to separate the individual chemical constituents from various extracts by TLC method. The details of optimized mobile phases are given in Table 5. TLC analysis clearly revealed separation of substantial phytoconstituents in almost every extract as presented in Fig. 6. The results of TLC studies showed that highest separation was occurred in methanolic root extract (6 spots with Rf values 0.82, 0.78, 0.49, 0.41, 0.32, and 0.15) and petroleum ether extract of leaf (6 spots at Rf values 0.75, 0.56, 0.48, 0.39, 0.32, and 0.21). Followed by this, the aqueous extract of roots and leaves also exhibited the presence of 5 spots at Rf values 0.67, 0.53, 0.41, 0.39, 0.34 and 0.82, 0.71, 0.41, 0.30, 0.13 respectively. Methanolic leaf extract showed the presence of 4 spots and petroleum ether extract of roots showed 3 spots. The details of Rf values are presented in Fig. 7.

Table 1 — Physicochemical analysis of roots and aerial parts of *H. candicans*

Parameter	Roots	Aerial part
Loss of drying	13.93	11.73
Total ash value (%w/w)	12.15	12.16
Water soluble ash value	7.20	5.66
Acid insoluble ash value	3.6	1.2
Foaming index	<100	<100
pH (1%)	6.61	6.24
pH (10%)	6.33	6.0

Heavy metal analysis

Heavy metal analysis is an important tool to check the quality of crude drugs. The heavy metal evaluation was done by ICP-OES method at Avon Food Lab (Pvt) Ltd, Delhi. Four main heavy toxic metals Cd, As, Pb and Hg were evaluated for their presence in the root and aerial parts of *H. candicans* and it was found that all these toxic metals were absent in the plant. This may be due to the geographical condition of the Himalayan region. The details of heavy metal analysis are present in Table 6.

Discussion

Physico-chemical parameters are also vital for the standardization and quality control of herbal drugs which included foreign matter analysis, loss on drying, ash content, pH, swelling index, foaming index etc. Herbal materials should be devoid of any kind of contamination, so foreign matter analysis of powdered drugs can be considered as an important parameter in order to check the purity of herbal drugs[28]. The present work demonstrates the pharmacognostic, physicochemical, and preliminary phytochemical evaluation of aerial and root parts of *H. candicans*, which will help in appropriate identification of this plant for future investigation.

Table 2 — Fluorescence analysis of powdered drug of *H. candicans*

	Root			Aerial part		
Drug treatment	Visible light	UV 254 nm	UV 360 nm	Visible light	UV 254 nm	UV 360 nm
Powder drug as such	Water grey	Espresso	Black	Water grey	Espresso	Black
Powder drug + Dist. Water	Brown	Cherry	Black	Brown	Cherry	Brown
Powder drug + conc. HCl	Brown	Blackish brown	Black	Brown	Blackish brown	Black
Powder drug + Dil. HCl (10%)	Light brown	Espresso	Black	Light brown	Espresso	Black
Powder drug + H_2SO_4	Blackish brown	Brown	Black	Blackish brown	Brown	Black
Powder drug + dil. H_2SO_4(10%)	Steel grey	Cherry	Black	Steel grey	Cherry	Light brown
Powder drug + Nitric acid	Buff	Brown	Black	Buff	Brown	Black
Powder drug + dil. $HN0_3$ (10%)	Brown	Espresso	Black	Brown	Espresso	Black
Powder drug + 10% NaOH	Brown	Brown	Black	Brown	Brown	Brown
Powder drug + picric acid	Dark green	Brown	Blackish brown	Dark green	Brown	Black
Powder drug + Iodine solution	Oxford blue	Brown	Black	Oxford blue	Brown	Brownish black
Powder drug + Methanol	Brownish black	Espresso	Black	Brownish black	Espresso	Black
Powder drug + Ethanol	Blackish brown	Brown	Black	Blackish brown	Brown	Black
Powder drug + acetic acid	Espresso	Brown	Blackish brown	Espresso	Brown	Brownish black
Powder drug + Chloroform	Sugar creek	Espresso	Black	Sugar creek	Espresso	Black
Powder drug + Pet. ether	Sugar creek	Brown	Black	Sugar creek	Brown	Black
Powder drug + Ferric chloride	A.D grey	Espresso	Brown	A.D grey	Espresso	Black
Powder drug + Ammonia solution	Sugar creek	Brown	Black	Sugar creek	Brown	Brown

Table 3 — Phytochemical screening of powdered drug of *H. candicans*

Test	Inference	MeOH aerial extract	Aqueous aerial extract	MeOH root extract	Aqueous root extract
	Carbohydrates				
Molisch's test	Violet ring	+	+	+	+
Fehling's test	Brick red precipitate	+	+	+	+
Benedict's test	Orange red precipitate	+	+	+	+
	Tannins				
5% $FeCl_3$ test	Yellow colour	+	+	+	+
Lead acetate test	White precipitate	+	+	+	+
Gelatin test	White precipitate	+	+	+	+
	Flavonoids				
Shinoda test	Pink colour	+	+	+	+
Alkali reagent test	Intense yellow colour which becomes colourless on addition of dil. acid	+	+	+	-
Lead acetate test	yellow colour precipitate	+	+	+	+
	Phenols				
1% FeCl3	Bluish colour	+	+	+	+
	Phytosterols				
Salkowski test	Golden yellow ring at junction	+	-	+	-
Liebermann's test	Brown ring at junction	+	-	-	-
	Proteins				
Xanthoproteic test	Yellow colour	+	+	+	+
Biuret test	Blue colour	-	-	-	-
	Saponins				
Foam test	Foaming	+	+	+	+
Froth test	Frothing	+	+	+	+
	Diterpenes				

(*Contd*.)

Table 3 — Phytochemical screening of powdered drug of *H. candicans* (*Contd.*)

Test	Inference	MeOH aerial extract	Aqueous aerial extract	MeOH root extract	Aqueous root extract
Copper acetate test	Emerald green colour	+	+	+	+
	Fats and Oils				
Filter paper test or Stain test	Permanent stain on filter paper	+	+	+	+
	Coumarins				
Filter Paper test under UV light	Yellowish green Fluorescence	+	-	+	-
	Cardiac Glycosides				
Keller Kiliani test	Brown ring at junction	+	+	+	+
Legal test	Pink colour	+	+	+	+
	Alkaloids				
Mayer's test	Cream precipitate	-	-	-	-
Hager's test	Yellow precipitate	-	-	-	-
Dragendorff's test	Orange precipitate	-	-	-	-
Wagner's test	Reddish brown precipitate	-	-	-	-
	Anthraquinone Glycosides				
Bontrager's test	Pink colour	-	-	-	-

Table 4 — Behavioural analysis of powdered drug of *H. candicans* (aerial and root part) with various chemical reagents

Treatment with chemicals	Aerial part	Root part
Powder drug + Dist. Water	Buff colour	Buff colour
Powder drug + conc. HCl	Golden Fleece	Golden Fleece
Powder drug + Sulphuric acid	Burnt brick	Burnt brick
Powder drug + Nitric acid	Marengo	Marengo
Powder drug + 10% NaOH	El. Greco Bronze (brownish)	El. Greco Bronze (brownish)
Powder drug + picric acid	Ra. Gold	Ra. Gold
Powder drug + Iodine solution	Espresso	Espresso
Powder drug + Methanol	Tata mimosa	Tata mimosa
Powder drug + Ethanol	Dark lime bright	Dark lime bright
Powder drug + acetic acid	Water grey	Water grey
Powder drug + Chloroform	Golden Fleece	Golden Fleece
Powder drug + Pet. Ether	Light Golden Fleece	Light Golden Fleece
Powder drug + Ferric chloride	Brownish gold	Brownish gold
Powder drug + Ammonia solution	Green	Green

The pharmacognostical study is a primary and reliable criterion in the identification and assessment of quality and purity of crude drugs. As per World Health Organization (WHO), macroscopic and microscopic description of a medicinal plant is the initial move towards establishing its identity and purity and should be completed before any tests are undertaken[1].

Table 5 — Thin layer chromatography of various extracts of *H. candicans* in different mobile phase ratio

Extract	Optimized mobile phase
Petroleum ether extract of root	Toluene: Ethyl acetate (8:2)
Methanol extract of root	Chloroform: Methanol (8:2)
Petroleum ether extract of aerial part	Toluene: Ethyl acetate (9.5: 0.5)
Methanol extract of leaves	Chloroform: Methanol (8: 2)
Aqueous extract of root and aerial part	Ethyl acetate: Formic Acid: Glacial Acetic Acid: Water (100:11:11:26)

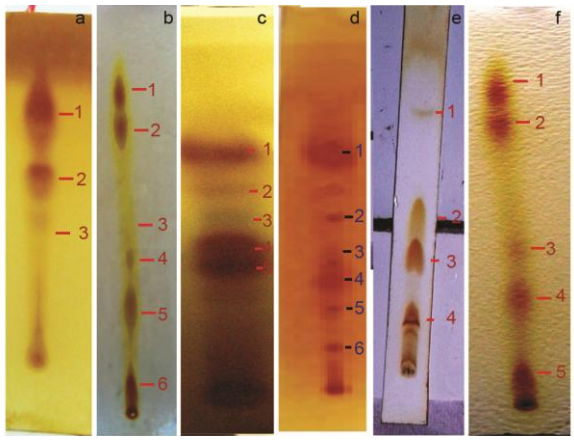

Fig. 6 — TLC chromatography of different extracts of *H.candicans*, a) Petroleum ether extract of root (Toluene: Ethyl acetate 8:2); b) Methanolic extract of root (Toluene: Ethyl acetate 8:2); c) Aqueous extract of root (Ethyl acetate: Formic Acid: Glacial acetic acid: H2O 100:11:11:26); d) Petroleum ether extract of leaf (Toluene: Ethyl acetate 9.5: 0.5); e) Methanolic extract of leaf ($CHCl_3$: CH_3OH 8: 2); f)Aqueous extract of leaf (Ethyl acetate: Formic acid: Glacial acetic acid: Water (100 : 11: 11: 26).

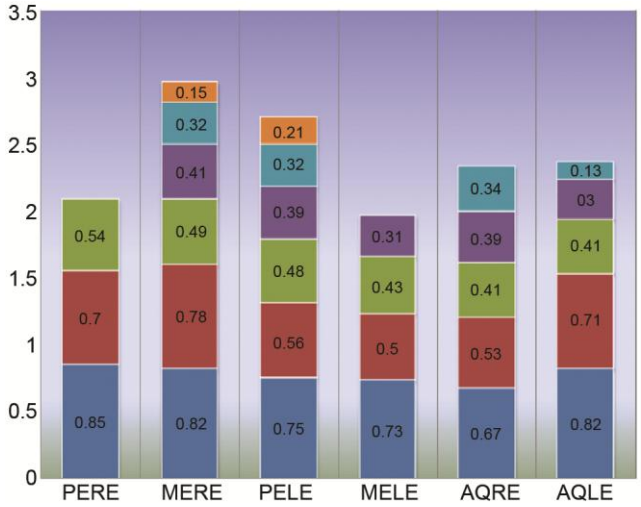

Fig. 7 — Graph showing number of phytoconstituents separated from various extracts through TLC with their Rf values (PERE: Petroleum ether extract of roots; MERE: Methanol extract of roots; PELE: Petroleum ether extract of leaf; MELE: Methanolic extract of leaf; AQRE: Aqueous extract of root; AQLE: Aqueous extract of leaf).

Table 6 — Heavy metal residue of powdered drug of *H. candicans* (aerial part & root parts)

Test parameters	Instrument used	Result	MDL
Cadmium (Cd)	ICP-OES	Not detected	0.10 mg/kg
Lead (Pb)	ICP-OES	Not detected	0.10 mg/kg
Arsenic (As)	ICP-OES	Not detected	0.10 mg/kg
Mercury (Hg)	ICP-OES	Not detected	0.10 mg/kg

MDL = Method detection limit

The abundant prismatic calcium oxalate crystals of variable size were found in powder of aerial and root parts of *H. candicans*. Some other diagnostic cellular structures include lignified fibres; few pits and the reticulate and spiral vessels. Stomata that were seen in the parenchymatous tissue of aerial powder were of anomocytic type. Similar observations have been reported for *Heracleum persicum* aerial parts from Iran[29].

Extractive value plays a vital role in the evaluation of crude drugs and give an idea about the active constituents present in the drug. It is also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. Extractive values are primarily useful for the determination of exhausted or adulterated drugs[30]. High alcohol soluble and water-soluble extractive values (cold, hot and successive) in both aerial and root parts revealed that the plant mainly contains polar substances. Further, the higher methanolic and aqueous extractive values (cold, hot, and successive) of roots in comparison to the aerial part indicated that the percentage of polar substances in roots is greater than aerial part. The pH value of 1 and 10% solution of leaf and root in distilled water respectively represented the presence of acidic constituents in the plant.

Preliminary phytochemical screening revealed the presence of various phytoconstituents like carbohydrates, alkaloids, flavonoids, tannins, phenolic compounds and terpenoids. The phytochemical screening of *H. afghanicum* Kitamurahas been found to show phenolic substances (flavonoids and total phenols), sugars, resins, and sterols, but no alkaloids or saponins[31]. In a similar manner, the qualitative analysis of stem, leaves, flowers, and fruits of *H. persicum* showed the presence of various phytochemicals like flavonoids, tannins, fixed oils and steroids while the carbohydrates, alkaloids, anthraquinones, cyanogenetics and saponins were not found in samples[29]. The alkaloid content of *H. candicans* extracts might have anticonvulsant and cytotoxic effects as reported for alkaloids of *H. persicum*[32]. Moreover, the method for TLC of the plant was also developed and it was found that almost all the extracts utilized in the TLC studies showed substantial amount of phytoconstituents.

Conclusion

The results of preliminary phytochemical analysis of various *H. candicans* extracts revealed the presence of carbohydrates, tannins, phenols, flavonoids, phytosterols, coumarins, proteins, saponins, diterpenes, cardiac glycosides, fats and oils. However, alkaloids and anthraquinone glycosides were found to be absent in all the extracts. The pharmacognostic studies of *H. candicans* revealed the presence of prismatic calcium oxalate crystals, anomocytic stomata, and simple starch grains along with two types of covering trichomes which were more numerous in the lower epidermis of the leaves. The presence of spiral and reticulate xylem vessels (lignified) along with lignified fibres which can assist in proper identification of this less explored drug was also found. The estimation of various physicochemical constants such as extractive values, ash values, loss on drying, pH value, swelling index, foaming index, heavy metal analysis, fluorescence analysis and TLC values can be helpful in determining various quality control standards for the crude drug. Additionally, the absence of heavy metals

in the plant also depict the better environment conditions of the study area for the cultivation of the medicinal plants. Pharmacognostic study of this less explored plant will be useful in the correct identification of this plants for future references and preparation of monographs.

Conflict of interest

The authors declare that there are no conflicts of interest.

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