



Original Research Article

Pharmacognostic Study and Development of Quality Parameters of Aerial Part of Plant *Enhydra fluctuans* Dc.

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ABSTRACT

Enhydra fluctuans DC. (Family: Compositae), commonly known as Helencha, is an edible semi aquatic herbaceous vegetable plant. As traditional medicines it is commonly used in India for cooling, carminative, tonic, liver tonic, leprosy, coughs and especially in Homeopathy. There were no pharmacognostical reports of this plant to determine the anatomical and other physicochemical standards required for its quality control. Pharmacognostic evaluation in terms of morphological, anatomical, quantitative microscopical examination including powder microscopy and physicochemical parameters including determination of different physical constants like ash values, extractive values etc and also fluorescence analysis were carried out. The findings of the current study can be useful to progress and surge further scientific investigation on the leaves and aerial parts of this species. The present study aims at developing a standardized profile of leaves and aerial parts of *Enhydra fluctuans* Lour which would be of immense use to identify and establish the authenticity of the plant *Enhydra fluctuans* DC.

Keyword: *Enhydra fluctuans* DC.; compositae; pharmacognostic study; physicochemical standard

INTRODUCTION

Pharmacognostical study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostical evaluation gives valuable information regarding the

morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting

observations have been incorporated in various pharmacopoeias [1]. There are a number of crude drugs where the plant source has not yet been scientifically identified. Hence pharmacognostic study gives the scientific information regarding the purity and quality of the plant drugs [2].

Enhydra fluctuans DC (Compositae) is available in tropical parts of India and it is commonly seen in ponds, Jheels etc. It is edible, semiaquatic, herbaceous, vegetable plant with strong pungent odour and as traditional medicine commonly used in India for cooling, carminative, tonic, liver tonic, leprosy, coughs, oedema, skin diseases, as antidyspeptic & laxative, as well as in neurological ailments[3][4]. Sesquiterpene lactones, gibberelins, cholesterol derivatives and flavonoids have been reported to be present in this plant [5][6].

Pharmacognostic and preliminary phytochemical studies have not been reported for the leaves and aerial part of this plant. Therefore the main aim of the present study was to Pharmacognostical investigation such as organoleptic, morphologic, microscopic and other applicable physico - chemical parameters of leaves and aerial parts of *Enhydra fluctuans* DC. which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Collection and authentication of the plant

Whole plants were collected from the pond side of Jalpaiguri, North Bengal and authenticated by Dr. A. P. Das, Professor and Head of Taxonomy and environmental botany and Herbarium- in-charge, The North Bengal University, Darjeeling. A herbarium was deposited in the Department of botany of accession no. 09695, dated the 20th May 2013 in The North Bengal University, Darjeeling.

Morphological studies

Morphological characters of fresh leaves and stems were examined properly. The following macroscopic characters for the fresh leaves were noted: size and shape, color, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odor and taste. Also different characters like nodes, internodes, different buds, surface including colour and odour of stem was studied [7, 8].

Microscopical studies

The outer epidermal membranous layer (in fragments) of both surface of fresh leaf were cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence, types and distribution of stomata and epidermal cell was observed. Stomatal number, stomatal index, vein- islet number and veinlet termination number of fresh leaves were determined by using camera Lucida and stage micrometer. Transverse section of the leaf and stem were also cleared, mounted, stained and observed [9].

Powder microscopy

The leaves were shade dried and powdered using mechanical grinder for powder analysis. Small amount of powdered drug mounted on slide and treated it with Phloroglucinol in HCl and iodine solution to clear the view and watch under the microscope to know about its powdered characters.

Fluorescence analysis

The fluorescence characters of the plant material in different solvents observed using visible, short UV and long UV light [10]. Alcohols, mineral acids in different concentrations, halogens and other various chemical and organic reagents used to perform fluorescence analysis [11].

Physicochemical analysis

The physicochemical characteristics of powdered leaf were determined as per WHO guidelines [12]. Various physicochemical parameters like LOD, ash values (total ash, acid insoluble ash, water soluble ash, sulphated ash), extractive values (aqueous, chloroform, ethanol, methanol), swelling index and foaming index of the powdered materials were established [13, 14].

RESULTS

Morphological characters

Enhydra fluctuans DC. of family Compositae is a prostrate, spreading, semi-aquatic herbaceous plant. The herb is quite glabrous sometimes pubescent glandular. The stems are somewhat fleshy, 30 centimeters or more in length,

elongated simple, branched, divaricating rooting at the lower nodes, and somewhat hairy. The leaves are dorsiventral, simple, opposite, sessile, linear-oblong, 3 to 5 centimeters in length, pointed or blunt at the tip, usually truncate at the base, and somewhat toothed at the margins. The flowers are white or greenish-white. Fruits are achenes enclosed by rigid receptacle-scales. The flowering heads are without stalks, are borne singly in the axils of the leaves, and excluding the bracts, are less than 1 centimeter in diameter. The outer pair of the involucrel bracts is ovate and 1 to 1.2 centimeters long; the inner pair is somewhat smaller (Fig.1). Detail of morphological characters of leaves and stems has been mentioned (table 1 and table 2).



Fig.1: Aerial part of *Enhydra fluctuans* DC.

Table.1: Morphological characters of leaf

Sl no.	Particulars	Observation
1	Colour	Green
2	Odour	No
3	Taste	No
4	Length	3.0-5.0cm.
5	Margin	Serrate/Crenate
6	Apex	Acute
7	Base	Truncate, sessile.
8	Surface	Glabrous-pubescent.
9	Shape	Linear-oblong.
10	Vein	Reticulate.
11	Stipules	Absent.
12	Phyllotaxy	Opposite
13	Main nerves	One
14	Petioles	Absent

Table.2: Morphological characters of stem

Sl no.	Particulars	Observation
1	Colour	Green
2	Odour	No
3	Taste	No
4	Surface	Pubescent.
5	Nodes	Possess appendages, i. e. leaves, branches and flowers.
6	Internodes	2-2.5 cm long, hollow.
7	Axillary buds	Present in the axis of leaves on the stem.
8	Lenticels	Absent
9	Leaf scar	Absent
10	Terminal buds	Present
11	Flower buds	Present

Microscopical characters

Under microscope, the stomata were found distributed on both abaxial surface and adaxial surface. Both the surface characteristically contains Paracytic and Actinocytic stomata

together (Fig.2 and Fig.3). Stomatal density is more in upper epidermis than lower epidermis. Frequent vein islet and vein terminals are observed in upper surface (Table 3).

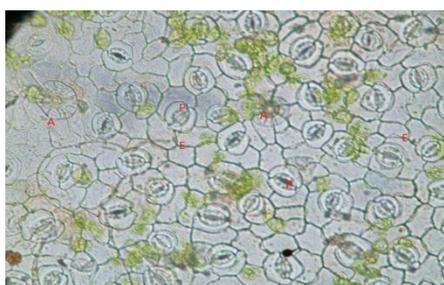


Fig. 2: Paracytic stomata in upper leaf surface of *Enhydra fluctuans* A: Actinocytic stomata P: Paracytic stomata; E: Epidermal cell

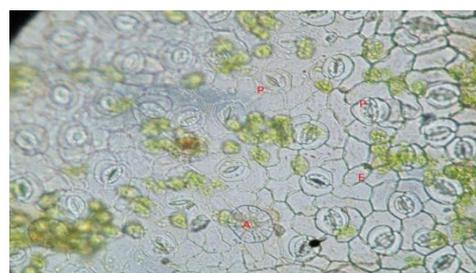


Fig. 3: Paracytic stomata in lower leaf surface of *Enhydra fluctuans* A: Actinocytic stomata P: Paracytic stomata; E: Epidermal cell

Table.3: Quantitative analytical microscopical parameter (leaf constants) of leaves

Sl no.	Parameters	Values obtained
1	Stomatal no. in upper epidermis	410/ square mm
2	Stomatal no. in lower epidermis	365/ square mm
3	Stomatal index in upper epidermis	19.43
4	Stomatal index in lower epidermis	17.67
5	Palisade ratio in upper epidermis	8.54
6	Vein-islet no. in upper epidermis	34.33/ square mm
7	Vein termination no. in upper epidermis	22.83/ square mm

The multicellular covering as well as glandular trichomes were observed on both surfaces, more frequent on upper surface of midrib portion. Transverse section through midrib with lamina in both side revealed the dorsiventral character of the leaf. The transverse section of leaf exposed a layer of epidermis composed of compact rectangular cells as outermost covering on both upper and lower layer. The upper epidermis was enveloped with deposition of cuticle. In lamina portion a single layer of large, elongated, compact, chlorophyll containing palisade parenchyma underneath the upper epidermis occupying more than one

third portion of the mesophyll tissue was found. Remaining portion of mesophyll was occupied by few layers of spongy parenchyma with large intercellular spaces. In midrib portion, epidermis was followed by few layers of collenchymatous hypodermis in continuation with few layers of parenchyma cells. Xylem and phloem portion of vascular bundle consist of their basic elements (Fig.4).

The stem contains vascular bundle arranged in a concentric ring. Epidermis appears thicker and below the epidermis lie parenchyma cells of cortex. Each vascular bundle is capped by sclerenchyma fiber (Fig.5).

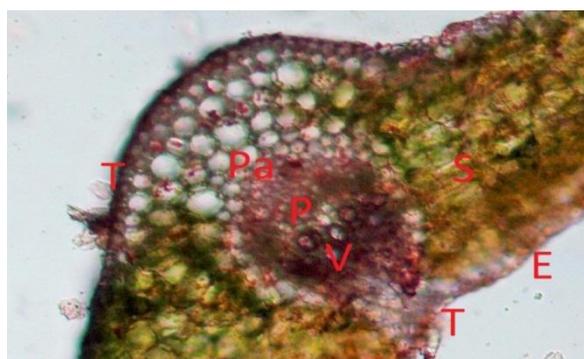


Fig.4: T.S. of leaf through midrib with lamina in both sides.

T: Trichome, E: Epidermis, V: Vascular bundle, C: Collenchyma
P: Pericyclic fibres, S: Spongy parenchyma, Pa: Parenchyma

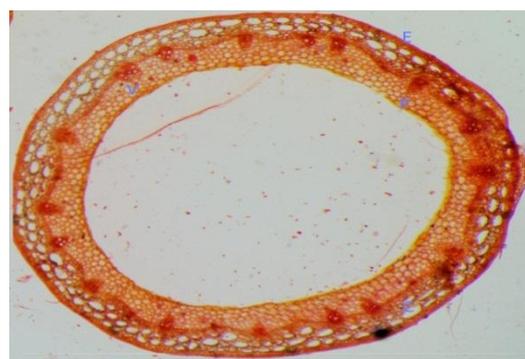


Fig. 5: T.S. of stem of *Enhydra fluctuans* V:
Vascular Bundle; C: Cortex; E: Epidermis; P: Parenchyma

Powder microscopy

Powder of the herb is fine, greyish green, fibrous, tasteless and odourless. When stained with phloroglucinol in sulphuric acid and iodine solution separately and observed under microscopic observation, powder of the herb

shows presence of epidermis (cells with thin, slightly sinuous walls), long unicellular uniseriate covering trichomes, prism crystals of calcium oxalate, annular vessels of vascular bundles, oil cells, starch granules, phloem fibers, oil globules etc (Fig.6-Fig.14).

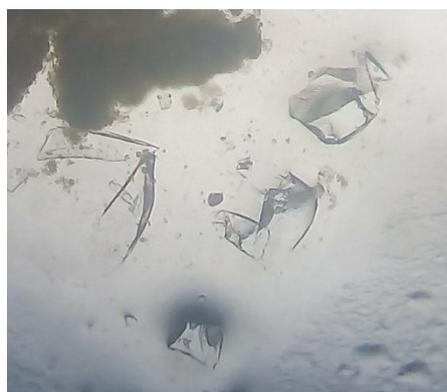


Fig. 6: Powder microscopy- Cluster crystals of Calcium oxalate.



Fig. 7: Powder microscopy- Unicellular covering trichome



Fig. 8: Powder microscopy- Phloem fibre.



Fig. 12: Powder microscopy- Annular vessels from vascular bundle



Fig 9: Powder microscopy- Oil globules



Fig.13: Powder microscopy- Oil cells



Fig. 10: Powder microscopy- Parenchyma cells.

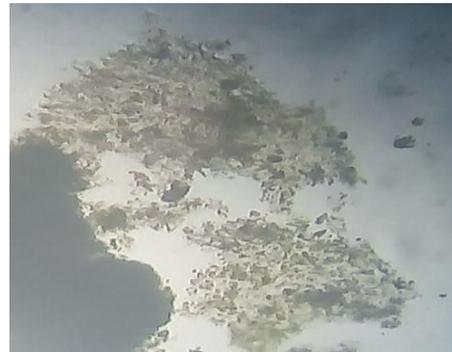


Fig.14: Powder microscopy- Laticiferous cells.



Fig. 11: Powder microscopy- Epidermal cells

Fluorescence analysis

The fluorescence characters of the powdered plant material in different solvents like Alcohols, mineral acids in different

concentrations, halogens and other various chemical and organic reagents observed using visible, short UV and long UV light and mentioned (table 4).

Table.4: Fluorescence Study

Powdered drug	Visible light	UV light(short)	UV light(long)
Powder	Green	Greenish brown	Greenish brown
Powder + 5%FeCl ₃	Greenish blue	Greenish black	Black
Powder + 1 N HCl	Pale green	Green	Brown
Powder + 1 N HNO ₃	Reddish brown	Greenish brown	Brown
Powder + 10% K ₂ Cr ₂ O ₇	Pale brown	Deep green	Black
Powder + 1M NaOH	Citrine green	red	Brick red
Powder + AgNO ₃	Green	Greenish brown	Light brown
Powder + Ammonia	Citrine green	Greenish black	Brown
Powder + 1 N H ₂ SO ₄	Green	Greenish brown	Brown black
Powder + Br ₂ water	Reddish green	Brown	Light brown
Powder + 5% H ₂ O ₂	Pale green	Green	Black brown
Powder + CCl ₄	Green	Green	Greenish brown
Powder + Methanol	Green	Brown	Dark brown
Powder + CH ₃ COOH	Green	Reddish brown	Dark brown
Powder + Xylene	Greyish green	Grey	Orange green
Powder + 5% KOH	Cascade green	Reddish brown	Dark brown
Powder + I ₂	Reddish green	Brown	Dark brown

Physicochemical analysis

Various physicochemical parameters of powdered leaves like ash values viz., total ash, acid insoluble ash, water soluble ash and sulphated ash; extractive values viz., alcohol soluble extractive value, water soluble

extractive value, methanol soluble extractive value and chloroform soluble extractive values; loss on drying, swelling index and foaming index were calculated and recorded as per WHO guidelines (Table 5).

Table.5: Physiochemical analysis

Sl. No.	Particulars	Result (%w/w)
1	Loss on drying	6.5
2	Total ash	15.34
3	Water soluble ash	8.30
4	Acid insoluble ash	4.15
5	Water soluble extractive value	16.46
6	Chloroform soluble extractive value	11.57
7	Ethanollic extractive value	13.14
8	Methanollic extractive value	15.10
9	Swelling index	36.21
10	Foaming index	13.46

DISCUSSION

In the present investigation, the detailed pharmacognostic account of *Enhydra fluctuans* DC is given which includes macroscopic and microscopic characters with leaf constants, which will be helpful for the correct botanical identification of the drug. Leaves of *Enhydra fluctuans* DC possess unicellular covering and glandular trichomes on both epidermises, exceptionally contains paracytic and actinocytic stomata in same as well as in both surfaces which are comparatively more on upper epidermis, prisms of calcium oxalate found in powder microscopy. Ash values, extractive values and fluorescence analysis can be used as reliable aid for detecting adulteration. The extractive values confirmed the presence of more amount of polar or water soluble phyto constituents, ash values representing the presence of more water soluble inorganic salts, swelling index and foaming index result reflects the presence of considerable amount of mucilaginous substances and saponins.

CONCLUSION

The evaluation of a crude drug is an integral part of establishing the correct identification of a plant material. For this the pharmacognostical evaluation can provide useful information for identification and authentication of plant. The pharmacognostic standard for the aerial parts of *Enhydra fluctuans* DC is laid down for the first time in this study. It can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. To conclude, this study could be used as a diagnostic tool for the standardization of this medicinal plant and will helpful in characterization of crude drug.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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