Original Research

PHARMACOGNOSTICAL EVALUATION OF AERIAL PARTS OF *IPOMOEA AQUATICA* FORSK.

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ABSTRACT

Ipomoea aquatic (Forsk), also known as water spinach, of Convolvulaceae family has been originated in China and distributed throughout India, Sri Lanka, Tropical Asia, Africa, and Australia. *Ipomoea aquatic* have been used in traditional medicine as a laxative, recommended for piles, and certain conditions with sleeplessness and head-ache while the plant has a calming effect. It is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka. No detailed work on pharmacognostic and development of quality parameters has been done on aerial parts of this plant specifically to determine the anatomical and other physicochemical standards required for its quality control. The present study deals with Pharmacognostic evaluation of leaves and aerial parts in terms of morphological, anatomical, quantitative microscopical examinations, including powder microscopy and physiochemical parameters including determination of different physical constants like ash values, extractive values etc. and also fluorescence analysis. The findings of the current study can be useful to progress and surge further scientific investigation on the leaves and aerial arts of this species and would be of immense use to identify and establish the authenticity of the plant *Ipomoea aquatic* Forsk.

KEY WORDS: *Ipomoea aquatic* Forsk., Convolvulaceae, Pharmacognostic study, Quality control

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INTRODUCTION

Pharmacognostic study is the preliminary step in the standardization of crude drugs. The detailed pharmacognostic 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].

Ipomoea aquatic Forsk., the aquatic plant grows wild and is cultivated throughout Southeast Asia and is usually consumed as a green leafy vegetable in the region [3]. It is edible, semiaquatic, herbaceous, vegetable plant and used in traditional medicine as a laxative, recommended for piles, sleeplessness and head-ache.[4],[5]. Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka [6].

Ipomoea aquatic Forsk is also considered a tonic as the species contains several vitamins, including A, B, C, E, and "U" (S-methyl-methionine) [7]. The species also contains aliphatic pyrrolidine amides, hentriacontane, β-sitosterol and its glycosides, prostaglandin, leukotrine, N-trans- and N-cisferuloyltyramines [8][9].

Detailed 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 *Ipomoea aquatic* Forsk. 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 Cooch Behar, 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 with accession no. 09696, dated the 20th May2013 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 [10, 11].

Microscopic 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 [12].

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 character s of the plant material in different solvents observed using visible, short UV and long UV light [13]. Alcohols, mineral acids in different concentrations, halogens and other various chemical and organic reagents used to perform fluorescence analysis [14].

Physicochemical analysis:

The physicochemical characteristics of powdered leaf were determined as per WHO guidelines [15].

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 [16, 17].

RESULTS:

Morphological characters:

Ipomoea aquatica (Forsk) is a vascular, glabrous, semi-aquatic trailing vine with milky sap, grows in water or floating on water, native to tropics and subtropics that grow wild and sometime cultivated. Water spinach is an herbaceous perennial plant belonging to the family *Convolvulaceae*. It has 2–3 meters (7–10 ft.) or more long hollow and viny stem, grow prostrate or floating in aquatic situations and the roots are produced from the nodes and penetrate into wet soil or mud. Leaves are alternate, simple, with glabrous petioles 3–14 cm (1–6 in) long. Leaf shape varies from typically sagittate (arrow head-shaped) to lanceolate, 5–15 centimeters (2–6 in) long and 2–8 centimeters (0.8–3 in) broad. The margins entire or angular, and sub lobed, surface glabrous or rarely pilose. Peduncles are erect, 2.5 to 5 centimeters long, with 1 or 2 flowers, borne in the axils of the leaves. Sepals are green, oblong, about 8 millimeters long. Corolla is narrowly bell-shaped, about 5 centimeters long, and purplish; limb nearly white or pale pink purple, about 5 centimeters in diameter, the tube deeper purple inside (Fig.1).Detail of morphological characters of leaves and stems has been mentioned (Table.1 and table.2).

Microscopical characters:

Under microscope, the stomata were found distributed on both abaxial surface and adaxial surface. Both the surface characteristically contains Paracytic stomata (Fig.2).Stomatal density is more in upper epidermis than lower epidermis. Frequent vein islet and vein terminals are observed in upper surface (Table.3).

The unicellular covering trichomes are 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 double 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. Starch granules found to be present in midrib portion.(Fig.3).

Transverse section of the stem is round in shape, glabrous and contains a large hollow pith in the center. Cortex portion is thick and consists of Exodermis, Hypodermis(collenchyma) and Endodermis (parenchyma) layers. Large numbers of vascular bundles are arranged in a concentric ring with pericycle. Xylem vessels appear prominent and large followed by phloem in each vascular bundle which is capped by sclerenchyma fiber. Few starch grains are also foundin both below and above the vascularbundle ring(Fig.4, 5).

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, paracytic stomata, prism crystals of calcium oxalate,oil cells, phloem fibers etc. (Fig.6-Fig.13).

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).

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 (Tab.5).

Table.1: Morphological characters of leaf

Sl no.	Particulars	Observation
1	Colour	Green
2	Odour	No
3	Taste	No
4	Length	5.0-15.0cm.
5	Margin	Entire
6	Apex	Acute
7	Base	Symmetric,petiolate.
8	Surface	Glabrous-pilose.
9	Shape	Sagittate.
10	Vein	Reticulate.
11	Stipules	Absent.
12	Type	Simple
13	Main nerves	One
14	Petioles	Present

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

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

Sl no.	Parameters	Values obtained
1	Stomatal no. in upper epidermis	305/ square mm
2	Stomatal no. in lower epidermis	375/ square mm

3	Stomatal index in upper epidermis	14.78
4	Stomatal index in lower epidermis	17.16
5	Palisade ratio in upper epidermis	7.24
6	Vein-islet no. in upper epidermis	40.66/ square mm
7	Vein termination no. in upper epidermis	25.33/ square mm

Table.4: Fluorescence Study

Powdered drug	Visible light	UV light(short)	UV light(long)
Powder	Green	Greenish brown	Greenish brown
Powder + 5%$FeCl_3$	Deep green	Green	Black brown
Powder + 1 N HCl	Pale green	Green	Brown
Powder + 1 N HNO_3	Reddish brown	Green	Brown
Powder + 10% $K_2Cr_2O_7$	Reddish	Green	Brown black
Powder + 1M NaOH	Green	Reddish green	Red
Powder + $AgNO_3$	Green	Greenish brown	Light brown
Powder + Ammonia	Citrine green	Greenish black	Brown
Powder + 1 N H_2SO_4	Green	Green	Brown black
Powder + Br_2 water	Reddish green	Brown	Light brown
Powder + 5% H_2O_2	Pale green	Green	Brown
Powder + CCl_4	Green	Green	Greenish brown
Powder + Methanol	Green	Brown	Dark brown
Powder + CH_3COOH	Green	Reddish brown	Dark brown
Powder + Xylene	Greyish green	Grey	Orange
Powder + 5% KOH	Cascade green	Reddish brown	Dark brown
Powder + I_2	Reddish green	Brown	Dark brown

Table.5: Physiochemical analysis

Sl. No.	Particulars	Result(%w/w)
1	Loss on drying	4.30
2	Total ash	9.90
3	Water soluble ash	1.75
4	Acid insoluble ash	6.50
5	Water soluble extractive value	42.31
6	Chloroform soluble extractive value	5.98
7	Ethanolic extractive value	30.07
8	Methanolic extractive value	35.15
9	Swelling index	25.63
10	Foaming index	16.42

Fig.1: Leaves and aerial parts of *Ipomoea aquatica*Forsk.

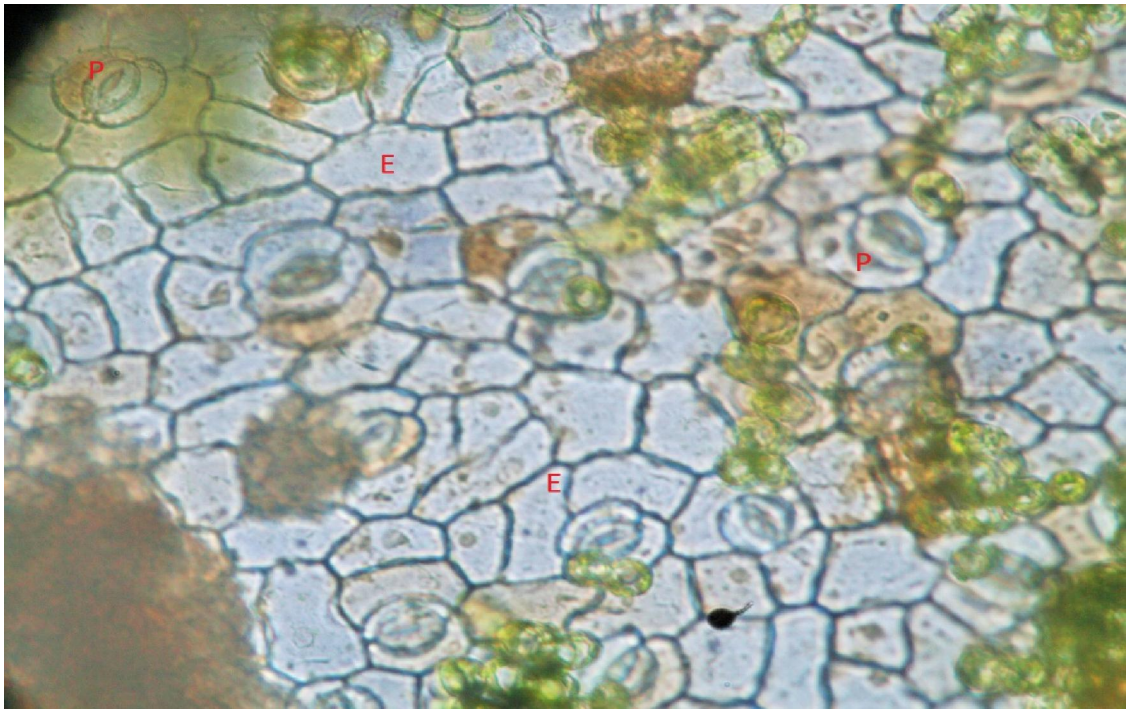

Fig.2: Paracytic stomata in upper surface of *Ipomoea aquatica*; P: Paracytic stomata, E: Epidermal cells

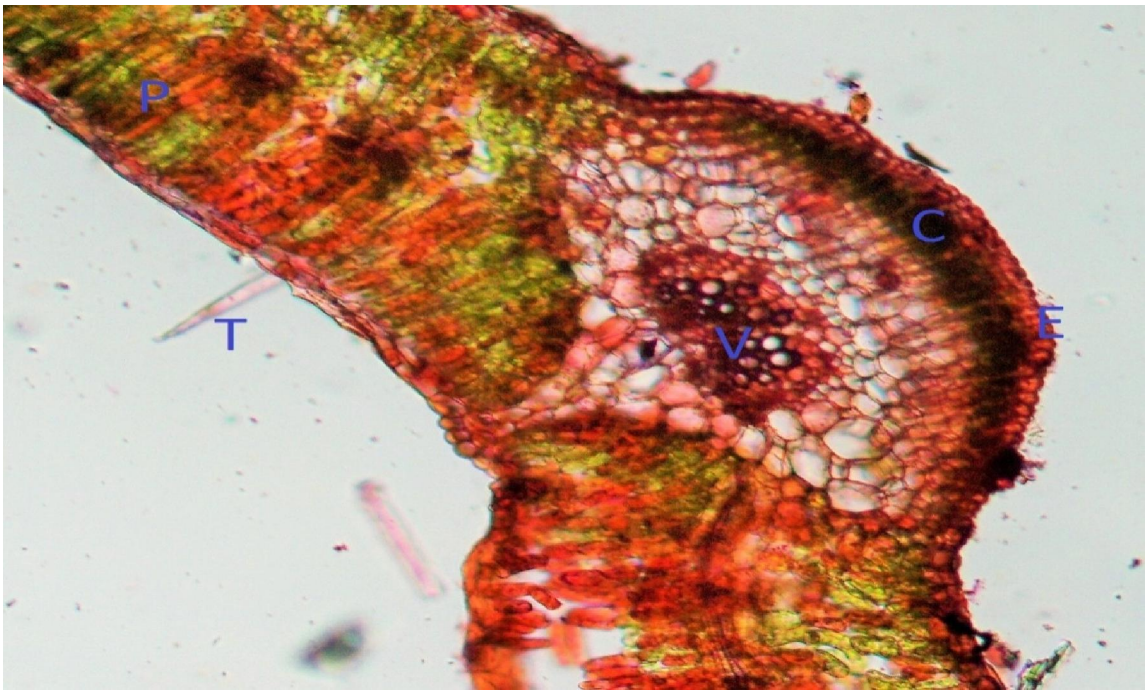

Fig.3: T.S. of *Ipomoea aquatica* leaf; T: Trichome, P: Palisade cells, V: Vascular bundle, C: Collenchyma, E: Epidermis

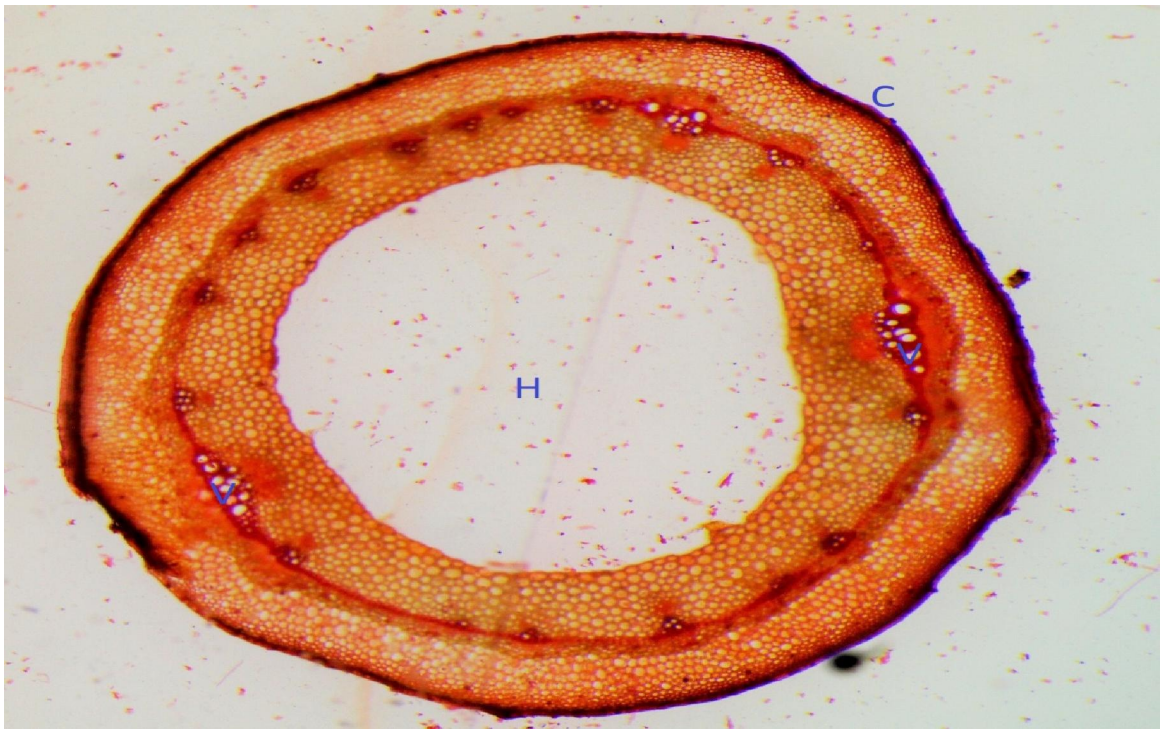

Fig.4: T.S. of *Ipomoea aquatica* stem; H: Hollow pith, C: Cortex, V: Vascular bundle

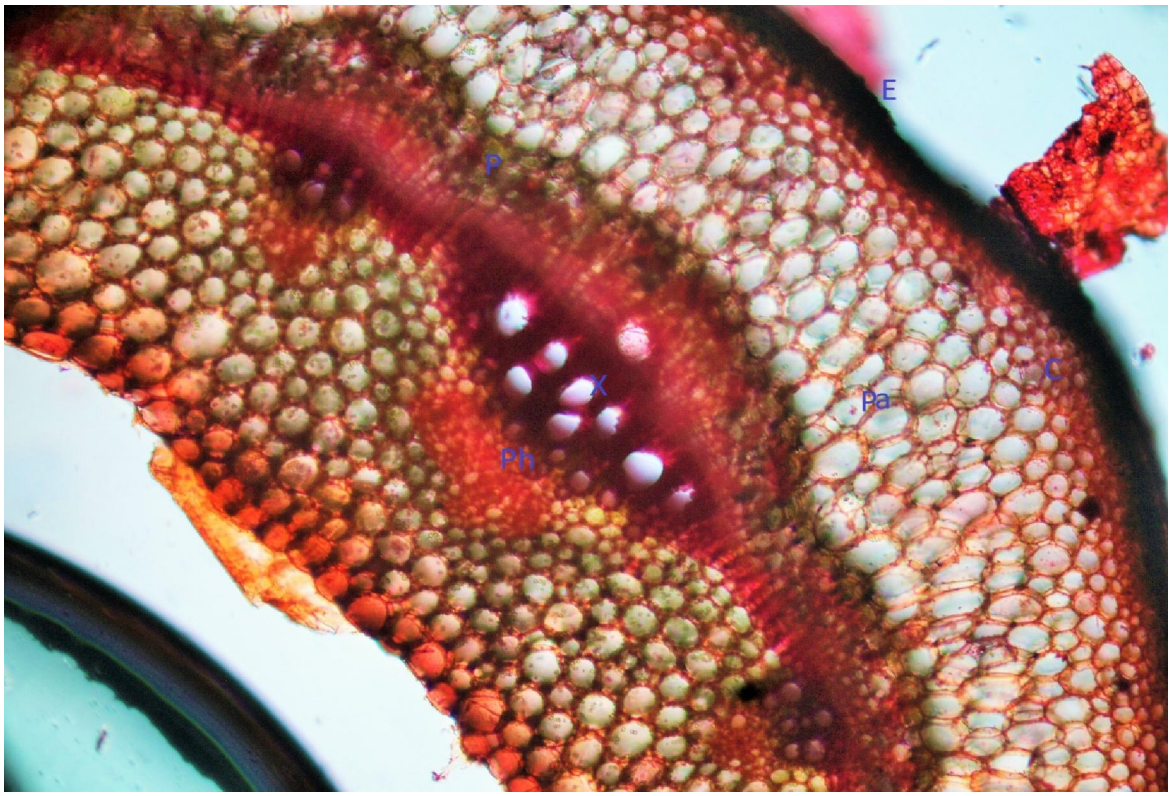

Fig.5: T.S. of stem of *Ipomoea aquatica*; E: Exodermis, Pa: Parenchyma, C: Collenchyma, X: Xylem vessele, P: Pericycle, Ph: Phloem

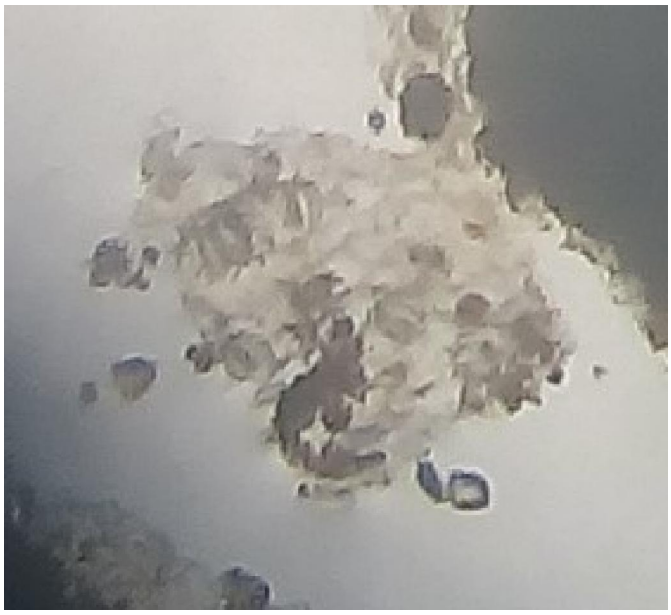

Fig.6: Powder microscopy: Epidermal cells

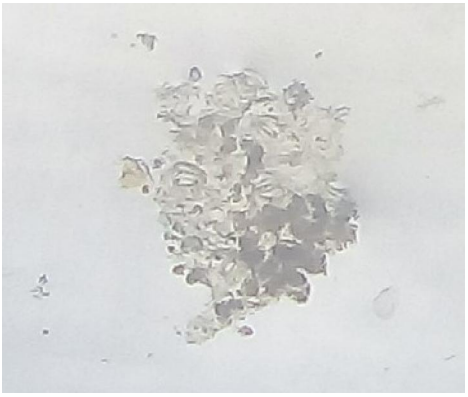

Fig 7: Powder microscopy: stomata

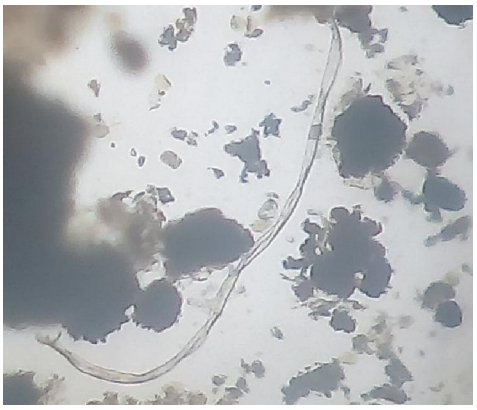

Fig.10: Powder microscopy: Phloem fibre

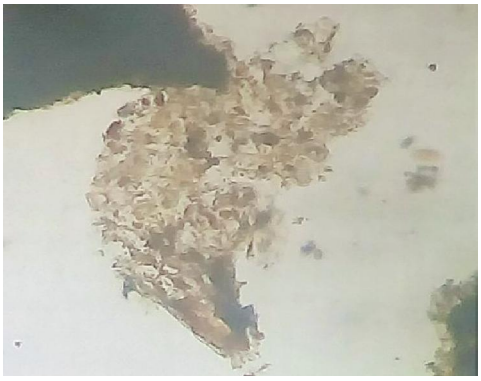

Fig.8: Powder microscopy: Laticiferous cells

Fig.11: Powder microscopy: Annular vessels

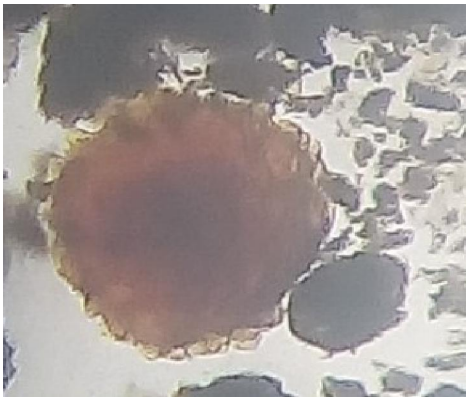

Fig.9: Powder microscopy: volatile oil cell

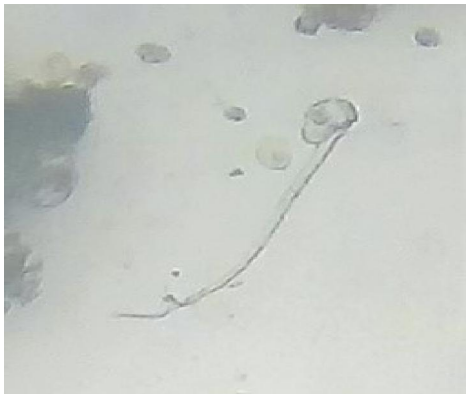

Fig.12: Powder microscopy: Unicellulartrichome

Fig.13: Powder microscopy: calcium oxalate

DISCUSSIONS

In the present investigation, the detailed pharmacognostic account of *Ipomoea aquatic* Forsk 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 *Ipomoea aquatic* Forsk possess unicellular covering and glandular trichomes on both epidermises, contains paracyticstomata in same as well as in both surfaces which are comparatively more on lower epidermis.Prisms of calcium oxalate, oil cells and phloem fibers 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 pharmacognostic evalution can provide useful information for identification and authentication of plant. The pharmacognostic standard for the aerial parts of *Ipomoea aquatic* Forsk.is laid down 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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AUTHORS CONTRIBUTION

S. Deb carried out the pharmacognostic studies, participated in the sequence alignment and drafted the manuscript. A. Chowdhury carried out the photographic investigations. S. Das participated in the design of the study. U.Sharma conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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