



BOTANICAL STANDARDIZATION OF RAW HERBAL DRUG *PASHANABHEDA* [*BERGENIA CILIATA* (HAW.) STERNB.] USED IN INDIAN SYSTEMS OF MEDICINE

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Abstract

Bergenia ciliata (Haw.) Sternb., belonging to the family Saxifragaceae, commonly known as *Pashanbheda*, is an important medicinal plant distributed in temperate regions of the Himalaya. It is used in Indian Systems of Medicine (ISMs) and is also known to have high ethnomedicinal value. Its root, rhizome and whole plant parts are used and have a very high demand in the Indian herbal market. Due to confusion in common names and lack of unique identification characters in dried and fragmented traded herbal samples, the identification of this plant's raw herbal material is problematic. Correct identification of herbal samples is required to ensure the safety and efficacy of herbal medicines. The present study aimed to study the detailed qualitative and quantitative botanical characters of different plant parts of *B. ciliata*, to ensure quick, easy and cost-effective identification of its dry herbal samples. Macroscopic characters were studied under the stereomicroscope and quantitative and qualitative microscopic features, including powder characters, were observed under the light microscope. Botanical characters summarised in the present study can be used as a reference for the identification of herbal samples of *B. ciliata* in fresh and dried forms.

Key words: Raw drug samples, Zakhmehayat, Pashanbheda, botanical characterization, reference standards.

Introduction

The genus *Bergenia*, a native to Central Asia from Afghanistan to China and the Himalaya, is reported to have ten species (Khan and Kumar, 2016). Three species of the genus *Bergenia* occur in India, with rhizomes of each species used in the Indian Systems of Medicine (ISM) (Kirtikar and Basu, 1935; Chopra *et al.*, 1956; API, 2001). In UTs of Jammu and Kashmir and Ladakh, different plant parts of the three species of *Bergenia* viz., *Bergenia ciliata* (Haw.) Sternb., *Bergenia pacumbis* (Buch.-Ham. ex D. Don) C.Y. Wu & J.T. Pan and *B. stracheyi* (Hook. f. & Thomson) Engl. are reported to be used by indigenous communities to treat various health ailments (Gairola *et al.*, 2014). *Bergenia ciliata* belonging to the family Saxifragaceae in the order Saxifragales, commonly known as Zakhmehayat,

Pashanbheda and Rock foil, is found distributed in the cold temperate Himalayan region from Kashmir to Bhutan between an altitudinal range of 2000 and 3000 m asl (API, 2001; TPL, 2013). It is a small perennial herb up to 40 cm tall, grows in moist, shady places between rocks, with thick rhizome and rosette succulent, broad, oval to obovate shaped leaves. Its flowering and fruiting occurs from March to mid-June.

Bergenia ciliata is known to have high medicinal importance, mentioned in the ancient Ayurvedic texts such as Vedas, Charaka Samhita and Sushruta Samhita (Sanjay, 2015). Its different plant parts are reported to have different medicinal properties. In Ayurveda, its rhizomes are used to treat bladder stone and urinary tract related diseases and are part of many Ayurvedic formulations, such as Asmarihara Kasaya Churna and Mutravirecaniya Kasaya Churna (API, 2001). In the Himalayan region, local inhabitants use dried rhizomes of *B. ciliata* for making tea and as a tonic to cure fever, diarrhea and muscular pain (Khan *et al.*, 2012). The herbal healers in Nanda Devi Biosphere Reserve,

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Uttarakhand, use the roots of *B. ciliata* as a hair tonic and to treat liver problems (Rana *et al.*, 2013). Leaf, root and rhizome samples of *B. ciliata* are ethnomedicinally used in different regions of UTs of Jammu & Kashmir and Ladakh as a tonic, in fever, headache, skin diseases, abrasions, wound healing, digestive ailments, diarrhea, kidney stones, bladder stone, menstrual irregularities, pulmonary infections and asthma (Gairola *et al.*, 2014).

Roots and rhizomes of *B. ciliata* contain important secondary metabolites like bergenin, catechin, phenolic compounds, arbutin, gallicin, gallic acid, tannic acid, glucose, albumens, mucilage, wax, albumen, mineral salts, flavonoids, glycosides, sterols, saponins and terpenoids (Khan and Kumar 2016, Chauhan *et al.*, 2013; Khan and Kumar, 2016). 'Bergenin' is the major compound present in rhizomes of *B. ciliata* and is considered to be responsible for its anti-ulcer activity (Ali *et al.*, 2018). The plant is reported to have antiurolithiatic (Anonymous, 1988), astringent and diuretic (Anonymous, 1986), anti-tussive (Sinha *et al.*, 2001a), antimicrobial (Pokhrel *et al.*, 2014), antibacterial (Sinha *et al.*, 2001b), antiviral (Kakub and Gulfraz, 2007), antidiabetic (Bhandari *et al.*, 2008) and anti-neoplastic (Venkatadri *et al.*, 2011) activities.

The whole plant, including root and rhizome of *B. ciliata*, have a very high annual trade value of 1000-2000 metric tonnes (NMPB, 2020). In the herbal drug industry, the plant material used in medicinal preparations is generally procured in a dried, fragmented, or broken forms. The problem of misidentification and adulteration of herbal samples due to several reasons including careless collection, confusing vernacular names, lack of identification features in dried or fragmented herbal samples, cost, scarcity of herbal drugs, etc. are common (Kokate, 2007; Mukherjee, 2002; Parkash *et al.*, 2013; Nithaniyal *et al.*, 2016; Poonam, 2016). The correct identification and authentication of dried herbal samples are important to ensure herbal medicines' quality and efficacy. In various pharmacopeias, botanical identification of the pharmaceutically important species is made by macroscopic and microscopic studies (Ginko *et al.*, 2016). Keeping the points mentioned above in view, the present study was conducted for botanical standardization of a high value raw herbal drug Pashanabheda (*B. ciliata*) used in ISM. The detailed information on macroscopic, anatomical and powder characters of Pashanabheda were generated, which can be used as a reference standard for quick and cost-effective identification of herbal samples of *B. ciliata* in fresh and the dried state.

Material and Methods

The authentic plant specimens of *Bergenia ciliata* (Haw.) Sternb. were collected from Dhera top, Udhampur, UT of J&K, located at an altitude of 3015 m asl. Herbarium sheets were prepared from collected samples following standard herbarium procedures (Rao and Sharma, 1990). Identified herbarium samples were submitted to Janaki Ammal Herbarium (RRLH) at CSIR-IIIM, Jammu, India (accession no. RRLH-23736). Dried crude herbal samples were submitted to the Crude Drug Repository at CSIR-IIIM, Jammu (accession no. CDR-4056).

The macroscopic characterization involved studying surface features, color, texture and appearance of the cut surface of samples under a stereomicroscope (LEICA S9i). Microscopic characterization involved the anatomical study of transverse sections and powder samples. For the anatomical study, freehand transverse sections (T.S.) were obtained by using a razor blade. The fine T.S. of adventitious root and rhizome samples were observed in water mounted slides (without staining) to observe the cell contents in transverse sections. The staining of T.S. was done as per Kumar *et al.*, (2018) with minor modifications. Thin T.S. were dehydrated in series of alcohol gradients (30%, 50% and 70% alcohol, each for 10-15 min), stained in safranin stain for 5-7 minutes and in fast green for 2-3 minutes, each followed by decolorization in 70% alcohol for 3-5 min. The sections were dehydrated in 90% alcohol and then in absolute alcohol for 5-7 minutes each and finally mounted in Canada balsam. The final slides were observed under a compound microscope (LEICA DM 750) with an associated camera (LEICA ICC50E). The fine powder samples were observed in water mounted slides to study the various cell types and cell contents. Iodine test was performed on rhizome powder to study the shape and size of starch grains.

Results

The observations made in the current study are presented in Figs. 1, 2 & 3 and table 1.

Botanical description

The plant is a perennial rhizomatous spreading herb, with a spirally arranged bunch of rosette leaves, about 45-60 cm in height, flowers pink colored, bisexual or occasionally unisexual, present in cyme inflorescence (flowers rarely solitary) and subtended by an ovate leafy bract. The morphological features of the plant are presented in Fig. 1A & 1B and table 1.

Leaves are simple glabrous, with broad obovate

Table 1: Quantitative anatomical characters of different plant parts of *B. ciliata*.

Plant part	Layer	Min (µm)	Max (µm)	Mean ±SD (µm)
Leaf	Upper epidermis (L×B)	14.12×13.04	33.12×19.07	25.05±1.80×17.84±1.04
	Lower epidermis (L×B)	16.4×15.44	30.15×23.04	23.82±1.34×20.04±0.97
	Cortex cells size (L×B)	46.57×40.82	89.95×79.24	64.55±4.17×55.84±3.59
	V.B. size (L×B)	96.15×74.41	264.10×239.46	179.37±18.18×150.11±17.42
	Lumen diameter of xylem vessel	8.78	17.93	14.20±0.87
Petiole	Epidermis (L×B)	20.45×16.9	43.79×30.51	30.78±2.26×20.05±1.27
	Hypodermis (L×B)	28.23×27.84	47.84×36.84	41.20±2.05×33.46±1.60
	Cortex (L×B)	35.09×29.31	84.9×66.37	64.17±4.68×53.41±3.64
	V.B. size (L×B)	159.57×115.31	335.47×304.05	253.69±17.88×207.65±17.15
	Lumen diameter of xylem vessel	10.10	17.10	13.27±0.81
Rhizome	Cortical cell size (L×B)	49.06×46.34	85.93×38.48	68.45±3.65×42.70±1.09
	Pith cell size (L×B)	37.24×24.26	62.02×50.7	49.84±2.52×42.62±2.80
	Starch grain (L×B)	12.88×7.34	35.91×19.01	23.24±2.47×13.51±1.40
	Rosette crystals	29.54×27.36	48.66×42.64	37.39±2.08×35.05±1.63
Root	Cortical cell (L×B)	17.95×15.85	38.62×24.67	28.52±2.33×24.07±1.89
	Lumen diameter of xylem vessel	16.62	34.26	26.46±1.56

lamina, about 5-30 cm long and 2.5-15 cm wide, base cordate or rotunded, usually exstipulate, apex abruptly rotunded, margin entire to slightly denticulate Fig. 1A. Fresh petiole appeared fleshy and cylindrical with length ranging from 5-10 cm or more Fig. 1B. Rhizomes are cylindrical, solid, 1-2 cm in thickness, branched at some points, compact, dark brown colored and dried samples brittle. Surface rough, scaly with remnants of leaf bases, longitudinally wrinkled and covered with numerous root scars and leaf scars. Adventitious roots 0.1-0.5 cm in thickness, dark brown colored and brittle on drying Fig. 1C.

The rhizome's cut surface was circular to oval or irregular in outline with an outer thin dark-colored cork layer followed by a small dark reddish colored cortex zone and then by creamish brown colored, patchy ring of the vascular region. Next to the vascular ring, a broad dark reddish colored central pith region was present Fig. 2B. The root's cut surface was also circular shaped, with an outer scaly cork zone, inner cortex zone and dark red colored central vascular zone, with spoke like xylem rays in the central region Fig. 2A.

Microscopic characters

Anatomy of leaf

The T.S. of the leaf (lower and upper part) consists of the midrib and lamina regions Fig. 1D, 1E and 1F. The outermost zone was the single-layered upper epidermis followed by a few-layered (20-25 layered) cortical zone with parenchymatous cells having intercellular spaces. The mesophyll region in the lamina region was less distinguishable into palisade and spongy parenchyma. Cortex (in the midrib region) was observed with several

randomly present vascular bundles of variable sizes in the lower region of the leaf Fig. 1D, while only a few vascular bundles in the cortex near the upper region of T.S. of the leaf Fig. 1E & 1F. Xylem faces the upper epidermis while the phloem faces the lower epidermis. The lower epidermis was single-layered with smaller sized cells compared to cortical cells. Trichomes were not observed. Quantitative anatomical characters, including the size of epidermal cells, cortical cells and lumen diameter of xylem vessels, are shown in table 1.

Anatomy of petiole

The T.S. of the petiole near the middle region appeared circular in outline and consisted of the outermost single-layered epidermis followed by single-layered hypodermis and then by a broad cortical region with parenchymatous cells having intercellular spaces. Cortical cells were comparatively large-sized than hypodermal and epidermal cells Fig. 1G & H. The cortex region was observed with secretory canals and numerous scattered vascular bundles of variable size. The vascular bundles were oval to circular shaped; conjoint, collateral and closed; formed of phloem present towards the epidermis and xylem towards the center Fig. 1H. Quantitative anatomical characters are shown in Table 1.

Anatomy of a rhizome

The rhizome's T.S. was oval to circular in outline with outermost multilayered, compactly packed, brownish, lignified transversally elongated cork cells, followed by multilayered (20-25 layered) broad cortex. The cortex zone was observed with secretory canals and with parenchymatous, oval-shaped cells having intercellular spaces Fig. 2F & 2H. Cortex cells are filled with rosette

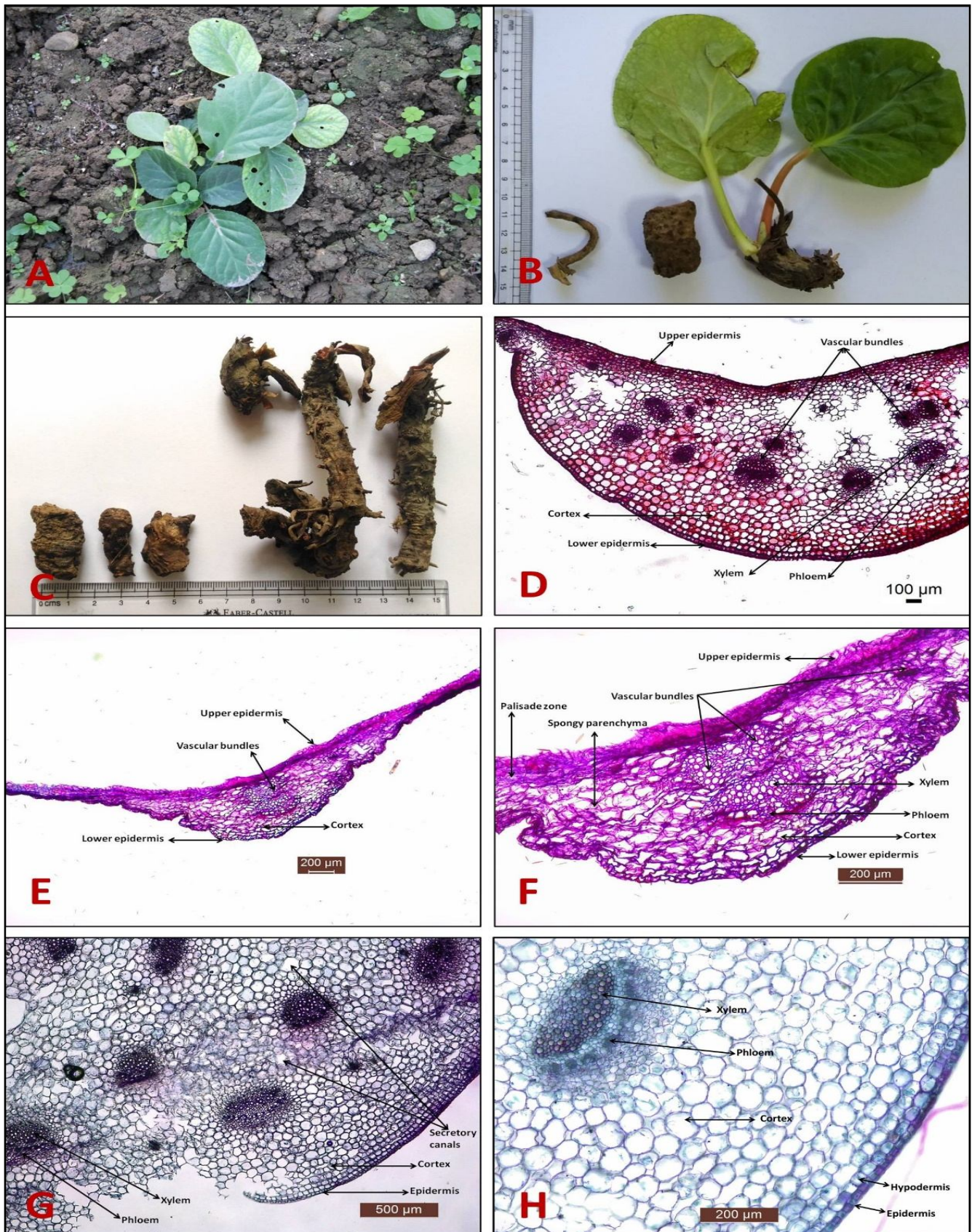


Fig. 1: *Bergenia ciliata*, A). Habit, B). The fresh plant, C). Dry rhizomes and whole plant, D). T.S. of the lower portion of the leaf, E). T.S. of the upper portion of the leaf, F). T.S. of the upper portion of the leaf enlarged view, G). T.S. of the petiole, H). T.S. of the petiole showing vascular bundles.

Botanical standardization of raw herbal drug *Pashanabheda* [*Bergenia ciliata* (Haw.) Sternb.] used in Indian Systems of Medicine

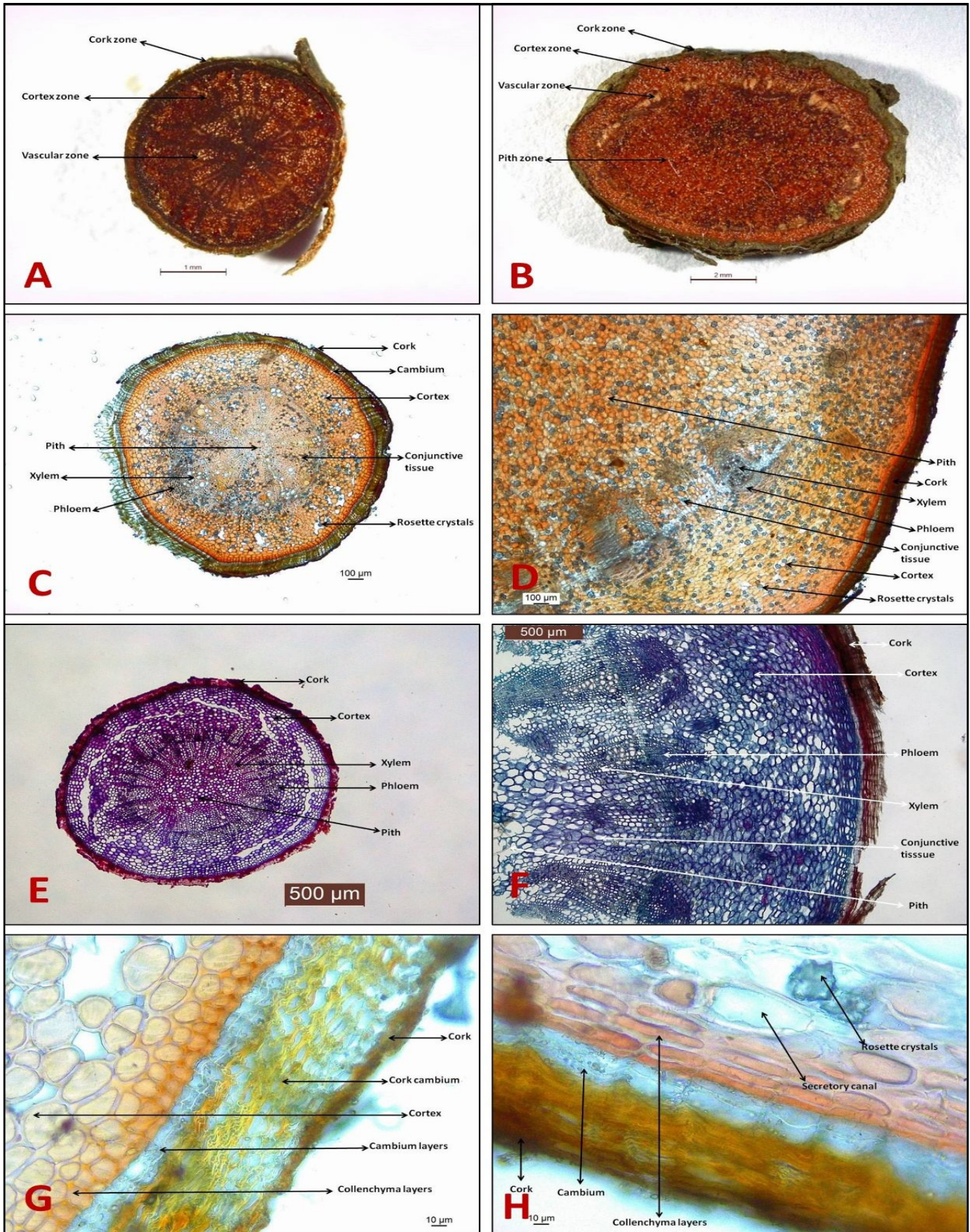


Fig. 2: *Bergenia ciliata*, A). Cut surface view of the adventitious root, B). Cut surface view of the rhizome, C). Unstained T.S. of the root, D). Unstained T.S. of the rhizome, E). Stained T.S. of the root, F). Stained T.S. of the rhizome, G). Unstained T.S. of the outer region of root, H). Unstained T.S. of the outer region of the rhizome.

calcium oxalate crystals, while some with reddish-brown contents Fig. 2D. Endodermis and pericycle were not observed. Vascular bundles were arranged in a ring-like pattern and formed of phloem tissue towards the outer region and elongated xylem towards the pith region and separated from each other by conjunctive tissue. The central part comprises a large pith with oval or rounded, brownish parenchymatous cells Fig. 2D, 2F & 2H.

Anatomy of root

The T.S. of the root was circular in outline with outermost thick-walled compact cell layers of a cork followed by multilayered (10-12 layered) parenchymatous, oval-shaped, isodiametric cortical cells with intercellular spaces Fig. 2E & 2G. Cortex cells were observed with abundant rosette calcium oxalate crystals Fig. 2C. Next to the cortex, vascular bundles present in a circular pattern, comprised of phloem tissue towards the epidermal region and elongated, ray-like xylem tissue towards the central pith region. Vascular bundles were separated by parenchymatous conjunctive tissue. The central region was occupied by small, nearly circular pith with

parenchymatous cells. The study of unstained T.S. of root and rhizome revealed outer deep reddish cork zone, uniformly orange-reddish parenchymatous cells in the center. Parenchymatous cells of the cortex of root and rhizome were observed with an abundant presence of rosette crystals Fig. 2C & 2D.

Powder characteristics

Organoleptic characters of the rhizome powder sample were observed. The powder was dark brown colored with a sandy and gritty texture, strong aromatic odor and pungent taste. Microscopic examination revealed few xylem vessel fragments with spiral wall thickening, few brown colored fragments of irregular shapes and variable size Fig. 3A & 3B; few parenchyma and cork cells Fig. 3B; abundant starch grains (most elongated, few oval and triangular), rosette calcium oxalate crystals and few prismatic crystals Fig. 3C & 3D.

Discussion

For botanical identification of raw herbal drugs, the study of detailed macroscopic and microscopic characters

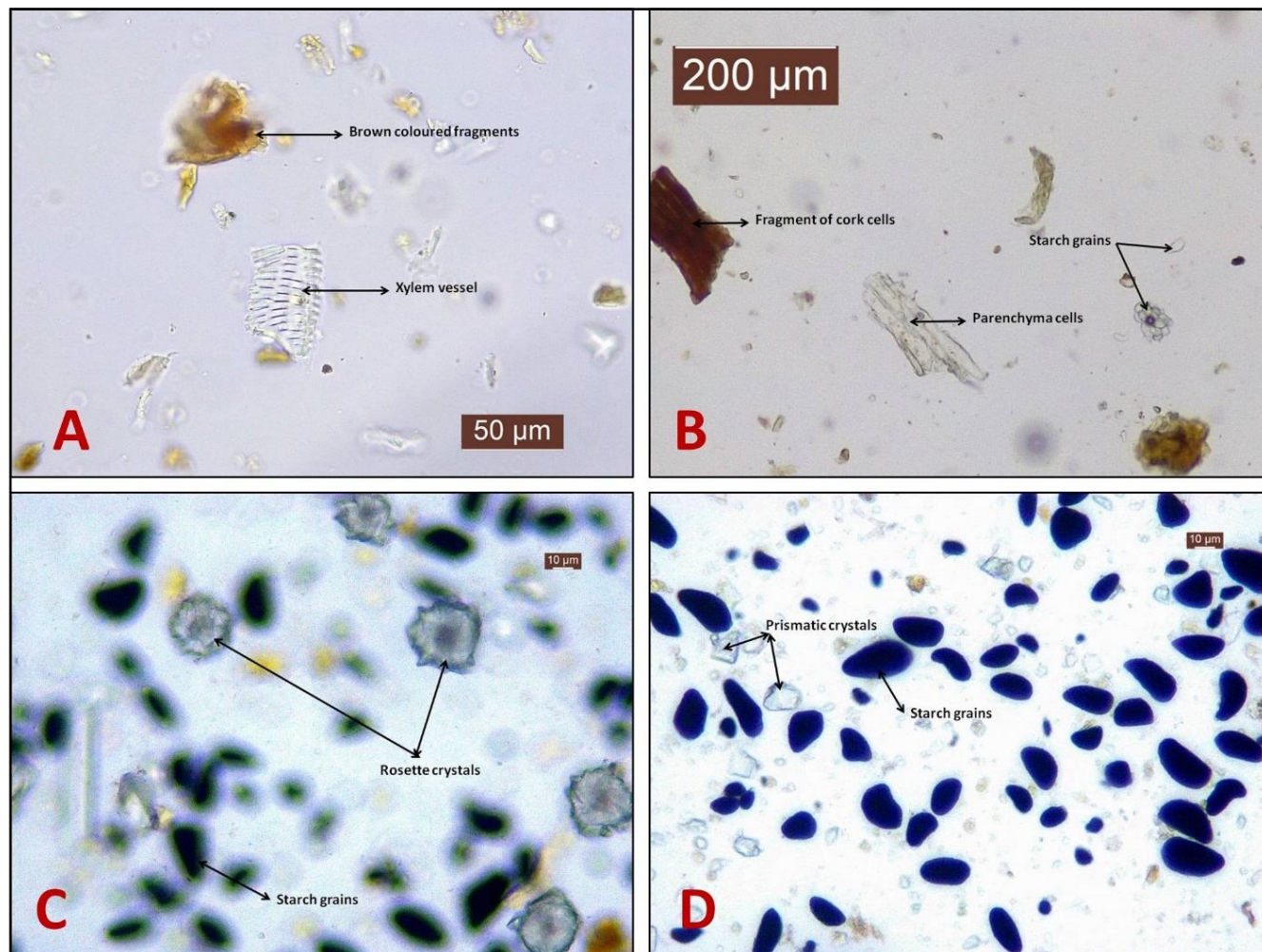


Fig. 3: Powder characteristics of the root and rhizome of *B. ciliata*.

of herbal samples is considered a suitable method (Manohan *et al.*, 2013). Earlier, Ginko *et al.*, (2016) used the diameter of the largest vessel in the anatomical characterization of species of Asteraceae. Qualitative and quantitative morpho-anatomical characters of aerial and underground parts of raw herbal samples can be used to standardize raw herbal samples. Singh *et al.*, (2020) described quantitative anatomical characters (of epidermal, hypodermal, cortex cells, starch grains, etc.) for botanical standardization of raw herbal drug *Fritillaria cirrhosa*. The microscopic study of herbal drugs' powder samples is useful in species identification (Sereena and Sreeja, 2014). Cortella and Pochettino (1994) described starch grains as useful in the identification of herbal drug material.

Bergenia ciliata is an important medicinal plant well known since ancient Vedic times. Due to immense medicinal importance, aerial and underground parts of *B. ciliata* are in high demand in the Indian herbal market (NMPB, 2020). The correct identification of raw herbal samples is essential to avoid adulteration and ensure herbal medicines' purity, quality and efficacy (Sahoo *et al.*, 2010). In the present study, detailed botanical information on macroscopic and microscopic characters of aerial and underground raw herbal drug samples of *B. ciliata* is provided. Anatomical characters of leaf and rhizome samples of *B. ciliata* were described in some previous studies (API, 2001; Srivastava and Rawat, 2008; Chowdhary and Verma, 2010; Ghimire *et al.*, 2012; Pokhrel *et al.*, 2014; Khan *et al.*, 2017). As compared to the microscopic structures of leaf observed in previous studies, in the current study, less prominent stomata and trichomes in T.S. of leaf and petiole, scarcity of starch grains and rosette calcium oxalate crystals in the parenchyma cells of the cortex in T.S. of leaf and petiole were observed. In the present study, some secretory canals in the cortical region in T.S. of petiole were observed. Anatomical rhizome characters observed in the present study were similar to the characters reported in the previous studies. However, some macroscopic and microscopic characters were described for the first time. The macroscopic study included a description of cut surface features of root and rhizome samples, description of T.S. in water mounts (without any staining) for visualization of cell and tissue color and cell contents and study of powder microscopic features, including a description of cell types, cell contents and shape and size of starch grains.

Conclusion

In the herbal drug industry, the herbal samples

procured are generally in broken or disintegrated forms. The identification of such samples in dried form is considered as problematic. For such samples, an authentic botanical monograph helps in the correct identification of the material to be used in medicine. A detailed microscopic study, including qualitative and quantitative characterization of rhizome, root, leaf and petiole samples of *B. ciliata*, will help in easy and cost-effective identification of intact, fragmented, or powdered samples. Botanical characters studied in the present study can be used as a reference for identification and to ensure quality, purity and efficacy of *B. ciliata* herbal material in the fresh and dried state.

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