Research Article

Pharmacognostic evaluation of *Bergenia ciliata* (Haw.) Sternb

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Abstract

The present study was conducted to investigate the *Bergenia ciliata* (Haw.) Sternb. (Family: Saxifragaceae) for pharmacognostic study including macroscopical and microscopical observations, leaf surface features, Scanning Electron Microscopy and Fluorescence characters. The macroscopy revealed that rhizome was brown in color, hard in fracture, pleasant in odor and astringent in taste. Leaf was cordate, green in color and indistinct in taste and odour, while microscopy of rhizome and leaf showed typical dicot histological differentiation. Leaf was amphistomatic having anomocytic stomata on both the epidermises. The quantitative leaf study showed that the stomatal numbers of upper and lower epidermises were (20 ± 0.7) and (24.2 ± 2.7) (average $\pm$ SEM), while stomatal index was (15.6 ± 2.6) and (27.4 ± 3.9) respectively. Vein islet and vein termination number was (20.6 ± 4.09 and 26.6 ± 5.01), while palisade ratio remained (12.3 ± 3.71). SEM of the powder showed non-glandular trichomes, spiral vessels, parenchymatous tissues and fragments of epidermis with anomocytic stomata. Fluorescence study indicates different shadows of colors like brown, black, greenish, yellowish, red, brown, dark black, pink etc. that revealed the presence of different fluorescent chemical compounds. The current research was done for the first time on *Bergenia* and might be beneficial for its accurate identification, standardization and will give a base for its pharmacognostic applications.

Keywords: *Bergenia ciliata*; Pharmacognostic evaluation; Quantitative leaf microscopy; SEM; Fluorescence study

Introduction

Pharmacognosy is a multi-disciplinary science that covers collection, identification, physical, chemical, pharmacological and other biological evaluation of natural and crude plant and animal sources [1]. It indicates a particular understanding of systems of identification and assessment of natural drugs, which significantly reduces

the unintentional and incorrect endorsements of traditional and natural plant products for pharmaceutical expenditures [2, 3]. Medications derived from plant sources are thought to be much harmless and show an extraordinary effectiveness in the treatment of several disorders and have no side effect [4, 5].

Physical and anatomical assessment is the primary stage to acquire information regarding diagnostic characters of crude drug, which are done by study of the cells, tissue and their structure, organization and cell substances [6, 7]. Along with morphological and histological features, the leaf surface quantitative study is also helpful in the systematic and taxonomic interpretations between various groups of phanerogams. Stomatal variations and diversity and other leaf features have extreme importance in plant taxonomy, systematics and may also help as effective tools in explaining taxonomic problems in the Angiosperm [8]. Fluorescence study is an essential parameter and a significant exhibition possessed by numerous chemical compounds present in plant material. The substances are converted to show fluorescence by dissolving and dipping in various chemical organic solvents like ethanol, methanol, HCl etc [8].

Bergenia ciliata (Haw.) Sternb. belong to Saxifragaceae commonly known as “Elephant ear” in English, “Pakhanbheid” in Urdu and “Makanpath” in Pashto is a herbaceous perennial plant grow up to 24 cm in height [9]. *Bergenia* grows in temperate Himalaya regions of the world up to the altitude of 3000-9000 feet [10]. In Pakistan, it has two species namely *B. stracheyi* and *B. ciliata* which are distributed in Galyat, Shangla, Swat, and Gilgit etc [11]. Therapeutically the rhizome used as astringents, stimulant, anti-acids and purgative. Its powder is given to pregnant women to increase lactation [12]. Cold decoction of leaves is use in kidney stones, cough, cold, and different brain and stomach disease. The leaf is also used in diarrhea, inflammation, GIT Chronic ulcers and various eye disease [13].

Materials and methods

Collection and preservation

The fresh samples of *B. ciliata* was collected

from Kotkay, district Shangla KP and taken to the Department of Botany, University of Peshawar and correctly identified by Mr. Ghulam Jelani. Rhizome and leaf was selected for pharmacognostic study, cleaned, air dried and were grinded.

Macroscopic observations

Morphological and Macroscopic evaluations were carried out organoleptically through five senses that include Size, shape, color, taste, odour, appearance, texture and fracture surface in both fresh and dry states.

Histological evaluation

The histological and microscopic evaluations was carried out by making T.S of rhizome and leaf through hand sectioning mounting in potato slices carefully. The sections were observed under digital microscope fitted with camera and photographs were taken [14]. The micrometry of various types of cells was done using stage and ocular micrometer [15].

Quantitative microscopy

The quantitative leaf surface features including stomatal studies, vein islet number, vein termination number and palisade ratio were also carried out by peeling off both the epidermises from leaf of *B. ciliata* following methods of [15]. The stomatal types and other parameters were studied under microscope and also photographed.

SEM of powder drug

Powder drug study under Scanning Electron Microscope (SEM) was conducted in CRL, Department of Physics, University of Peshawar, Pakistan. Finally, the images were examined carefully and photographs of the identified tissues were printed [16].

Fluorescence analysis

Preliminary fluorescence study determined the presence of various chemical compounds in plant parts. The powder of rhizome and leaf of *B. ciliata* was dissolved in various solvents like Picric acid, Chloroform, Ferric

chloride, Ether, Ethanol, HCL, Acetic acid, Iodine and H₂SO₄ etc. and observed under visible and UV short (254nm) and long (366nm) light and the changing colours were noticed and documented [17, 18].

Results

Macroscopic observations

The macroscopic features were carried out in both fresh and dry rhizome and leaf of *B. ciliata* which showed that the rhizome was average 7-20 cm in length, average 1-2.3 cm in width, cylindrical in shape, brown in

colour, astringent in taste, pleasant in odour, flexible in texture and having fibrous fracture surface when fresh. On drying the rhizome become average 6-19 cm in length, 0.9-2cm in width, irregular and dark brown in shape and color while indistinct in taste and odor (Table 1). The leaf macroscopy showed that it was 5-13 cm in average length, cordate in shape, dark green in colour when fresh, indistinct in taste and odor, soft in fracture, and even in texture surface in both fresh and dry conditions Table 1.

Table 1. Macroscopic features of various parts of *Bergenia ciliata* (Haw.) Sternb

S.No	Parameter studied	Rhizome		Leaf	
		Fresh	Dry	Fresh	Dry
1	Length	7-20cm	6-19cm	5-13cm	4.9-12.8cm
2	Width	1-2.3cm	0.9-2cm	-----	-----
3	Shape	Cylindrical	Irregular	Cordate	Cordate
4	Colour	Brown	Dark brown	Dark Green	Light green
5	Taste	Astringent	Indistinct	Indistinct	Indistinct
6	Odour	Pleasant	Indistinct	Indistinct	Indistinct
7	Fracture	Flexible	Hard	Soft	Soft
8	Fracture surface	Fibrous	Uneven	Even	Even

Histological evaluation

Anatomy of rhizome of *B. ciliata*

The T.S of the rhizome of *B. ciliata* appear circular in shape and surrounded by cork, which is consisted of irregular, in isodiametric thick walled brown color parenchyma cells with average 19μ in length and 10.4 μ width (Table 2, Figure 1). The cork is followed by uniseriate isodiametric small celled epidermis with 19μ and 8μ length and width. Below the epidermis, the rhizome composed of single layered hypodermis whose cells average length and width was 17μ and 9μ respectively. The hypodermis was followed by a multilayer

cortex having large and thin walled isodiametric spherical cells with 27μ and 16μ length and width. The rhizome showed conjoint vascular tissues which was collateral and open type; the phloem was towards the outer side while xylem was towards center. The average length and width of phloem sieve tube cells were 26μ and 15μ. The xylem vessels were also clearly visible with 32μ and 16μ length and width. The center of rhizome was full with thin walled circular parenchymatous pith region with 38μ and 19μ average cell length and width (Table 2, Figure 1).

Table 2. Histological measurements (μ) of various tissues of *Bergenia ciliata* (Haw.) Sternb

S. No	Plant parts	Cells and their size															
		Cork		Epidermis		Hypodermis		Cortex		Pericycle		Phloem		Xylem		Pith	
1	Rhizome	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
		19 μ	10.4 μ	19 μ	8 μ	17 μ	9 μ	17 μ	9 μ	20 μ	15 μ	26 μ	15 μ	32 μ	16 μ	38 μ	19 μ
2	Leaf	Upper Epidermis		Lower Epidermis		Xylem		Phloem		Palisade parenchyma		Spongy parenchyma		Guard cells		Stoma	
		Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
		26 μ	12 μ	30 μ	10 μ	19 μ	12 μ	29 μ	12 μ	19 μ	11 μ	20 μ	9 μ	34 μ	10 μ	26	8 μ

Figure 1. Transvers Section of rhizome of *B. ciliata*

Anatomy of leaf of *B. ciliata*

The TS of leaf of *B. ciliata* showed that it was enclosed by single layered compactly arranged rectangular shaped upper epidermis (26μ length and 12μ width) and lower epidermis with average length and width 26μ and 12μ (Table 2, Figure 2). Upper epidermis was covered by smooth cuticle. Through midrib the area below epidermises was occupied by parenchymatous region and in center composed of crescent shape collateral and closed vascular bundles in dispersed form. Phloem enclosed xylem. The length and width of xylem vessels was

30μ and 10μ and phloem sieve cells was 29μ and 12μ (Table 2, Figure 2). The T.S through lamina occupied by upper and lower epidermis followed by palisade and spongy parenchyma respectively. Palisade was composed of compactly arranged cylindrical cells with average length and width 19μ and 11μ , while spongy parenchyma possess irregular shaped cell with average length and width 20μ and 9μ respectively. Large area between the spongy mesophyll cells was consisted of large intercellular spaces (Table 2, Figure 2).

Figure 2. Transvers Section of leaf of *B. ciliata*

Leaf surface feature

The leaf surface study showed that *B. ciliata* was amphistomatic having anomocytic stomata on both upper and lower surfaces. The guard cells were 34μ and 10μ in average length and width and the stomatal pore was 26μ and 8μ (Figure 3). The mean $\pm$ SEM of stomatal numbers of upper and lower epidermis was (20 ± 0.7) and $(24.2 \pm$

$2.7)$ while stomatal index was (15.6 ± 2.6) and (27.4 ± 3.9) respectively (Figure 3 and Table 3). The vein islet and vein termination number was (20.6 ± 4.09) and (26.6 ± 5.01) . The palisade ratio which is the average number of palisade cells beneath four upper epidermal cells was calculated as (12.3 ± 3.71) (Table 3)

Figure 3. Upper epidermis of leaf showing anomocytic stomata

Table 3. Leaf surface data of the *B. ciliata*

S.No	Leaf surface data	Range	Mean $\pm$ SEM
1	Stomatal number Upper Epidermis	10-28	20 $\pm$ 0.7
2	Stomatal number Lower Epidermis	13-31	24.2 $\pm$ 2.7
3	Stomatal Index of Upper Epidermis	19 – 34	15.6 $\pm$ 2.6
4	Stomatal Index of Lower Epidermis	8.9 – 27.5	27.4 $\pm$ 3.9
5	Vein Islet Number	10 – 25	20.6 $\pm$ 4.09
6	Vein Termination Number	7 – 19	26.6 $\pm$ 5.01
7	Palisade Ratio (Lower Epidermis)	8 – 21	12 $\pm$ 3.71

SEM of the powder drug of *B. ciliata*

The powder drug study of *B. ciliata* through SEM was observed which showed that rhizome powder was dark brown in color with astringent taste and agreeable odor. The leaf powder appears dark green in color with indistinct odor and taste. The Scanning Electron Microscopy of rhizome showed following types of tissues

- i) Fragments of parenchyma region with average cell length and width 33 μ and 20 μ (Table 4, Figure 4),
- ii) Star shaped trichomes with mean length and width 250 μ and 40 μ (Table 4, Figure 5 and 6).
- iii) Cluster of calcium oxalate crystals with each crystal having 26 μ and 11 μ average length and width (Table 4, Figure 7).

The scanning electron microscopy of leaf showed following types of tissues

- i) Various types of non-glandular trichomes i.e tree shaped trichome with average length and width 350 μ and 40 μ , simple uniseriate straight trichomes (390 μ and 74 μ) and star shaped trichome (350 μ and 40 μ) respectively (Table 4, Figure 6, 8a and 8b).
- ii) Fragments of upper epidermis with anomocytic stomata with 39 μ and 20 μ and lower epidermis with 40 μ and 23 μ (Table 4, Figures 9a, 9b and 10a 10b),
- iii) Spirally arranged phloem vessels having 28 μ and 11 μ length and width respectively (Table 4 and Figure 11)

Table 4. SEM measurements (μ) of various tissues of *Bergenia ciliata* (Haw.) Sternb

Plant parts	Anatomical features											
Rhizome	Stellate trichomes		Calcium oxalate Crystals				Parenchyma tissues					
	Length	Width	Length	Width			Length	Width				
	250 μ	40 μ	26 μ	11 μ			33 μ	20 μ				
Leaf	Stellate Trichome		Simple Trichome		Tree shaped trichome		Xylem Vessels		Upper Epidermal cells		Lower Epidermis	
	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width	Length	Width
	450 μ	50 μ	350 μ	74 μ	350 μ	4S0 μ	28 μ	11 μ	39 μ	20 μ	40 μ	23 μ

Figure 4 and 5. Parenchyma cells and trichome in rhizome of *Bergenia ciliate* (Haw) Sternb

Figure 6. Trichome (stellate) in leaf of *B. ciliate*

Figure 7. Calcium oxalate crystals in rhizome *B. ciliate*

Figure 8a and 8b. Trichomes in leaf of *Bergenia ciliate* (Haw.) Sternb

Figure 9a and 9b. Epidermis with stomata in leaf of *Bergenia ciliate* (Haw.) Sternb

Figure 10a and 10b. Epidermis with stomata in leaf of *Bergenia ciliate* (Haw.) Sternb

Figure 11. Vessel cells in leaf of *Bergenia ciliate* (Haw.) Sternb

Fluorescence analysis

The powder of rhizome and leaf of *Bergenia ciliata* showed several shades of colours

from black to brown, yellow, green, dark blue, pink red etc when dipped and dissolved in various chemicals like HCl,

FeCl₃, iodine, Nitric acid, Sulphuric acids and water etc and observed under ordinary and UV short (254nm) and long (366nm)

wavelength, which was clear signal of the existence of various types of chemical substances. Results are shown in Table 5.

Table 5. Fluorescence analysis of different parts of *B. ciliata* (Haw.) Sternb

S.No	Plant parts	Rhizome			Leaf		
		Day light	UV Low (255)	UV High (366)	Day light	UV Low (255)	UV High (366)
1	Iodine	B	Br.B	C.B	G	Br	Br
2	Picric acid	D.Y	Br	B	Y	Gr	B
3	Chrolroform	Br	B	B	G	Gr	D.B
4	Ferric chloride	D.Br	B	C.B	G	D.Gr	D.B
5	Ether	D.B	Br	D.Br	G	D.G	Br
6	Ethanol	B	L.B	D.B	G	Gr	B
7	HCL	Br	D.Br	D.B	G	D.G	B
8	Acetic acid	P	R	Br	P	D.P	D.B
9	H ₂ SO ₄	Re	R	Br	Gr	B	D.B
10	HNO ₃	Br	D.Br	B	L.G	D.G	Br
11	Butanol	D.Br	B	D.B	G	B	B

Keys: B=Black, Br= Brown, Cr= Creamy, D= Dark, G=Green, Gr = Gray, L=Light, P= Pink, R= Red, Y=Yellow, DL= Day light, SWL= Short wavelength, LWL= Long wavelength

Discussion

Macroscopical observation

Macroscopic and organoleptic (sensory) evaluations are the main features in standardization and identification of crude natural drugs and the only parameters that required no involvement of scientific instruments neither any expenses. It gives a valuable, simplest, quickest and easiest information regarding purity and quality for recognition of adulterants in crude drugs [19, 20]. The present work was conducted for the first time on *B. ciliata*, which will be helpful for the correct identification and authentication of crude drug available in herbal market. Several other researchers also carried out similar research work on various other medicinal plant and documented similar observation which are in line with the present work and strongly agree with this work [21- 24].

Microscopical observations

Microscopical observations include anatomical studies, powder drug studies and leaf constants like vein islets Number, vein

termination number, occurrence and types of stomata, stomatal index and palisade ratio etc. Histological and anatomical study which provide help to plant taxonomist and will be used as a valuable source in plant systematics for differentiation between intra-specific and co-generic species of the same family for correct identification [25]. In pharmacognosy microscopic evaluation provide a tool for correct standardization, authentication, and identification of crude form of drugs [22, 26]. The microscopical studies is done by numerous workers on large number of medicinal plants and explore its anatomy, leaf surface features, etc. and support our present work. [27- 31].

Leaf surface feature

The leaf surface quantitative study like stomatal number, stomatal index, vein islet, vein termination number and palisade ratio provide a primary tool for differentiation between various leafy drugs belonging to same and related families [32]. Several other investigators [33-35] have studied leaf surface features of *Mimosa pudica*, *Allium*

sativum, *Verbascum thapsus*, *Heterophragma adenophyllum*, *Holoptelea integrifolia*, and *Zizyphus mauritiana* respectively and recommended the leaf surface and constant features as an important parameter for pharmacognostic and taxonomic fields. Several other workers [30, 36-38] also stress on stomata study to be a used in plant taxonomy to differentiate between closely related specie. The present quantitative work on *B. ciliata* will be provide an important knowledge to pharmacognosists and plant taxonomist on future.

Powder drug study using SEM

Most of the plant obtain drugs are available in market in powder form and sold in the raw form for curing of various disease and also as a main source of synthetic and natural medicines. So most of the powders of several plants are similar in physical appearance and also most of Hakeem and practitioners mix various economic crude drugs with high value drugs. Hence the powder drug study will provide help in identification of pure and specific drugs and also help in detection of adulteration [37]. Similar worked was done on several medicinal plants [29, 30, 38].

Fluorescence analysis

Similarly, [39, 40] reported that fluorescence is important to observe all materials on reaction with different chemical reagents under UV light. According to [41] fluorescence is important to detect the presence of phytoconstituents in powder. [39, 42] studied the powder of leaf and young stem of *Memecylon umbellatum* shows variation colour under day light, short wavelength UV and long wavelength UV light treated with different chemical showed the presence of fluorescence compound. [43] reported that fluorescence can be used as diagnostic tool for testing adulteration if any can be easily identified [44] reported that fluorescence study is an essential parameter

for first line standardization of crude drug. [33, 45] investigated the fluorescence characters of powdered of *Portulaca quadrifida* L. treated with different chemical reagents

Conclusion

From the present study it can be concluded that, *B. ciliata* is a perennial herb distributed in moist temperate regions of Pakistan like Shangla Swat, Gilgit Baltistan. Medicinally it is an Ayurvedic plant and used as astringent, tonic, laxative and in stomach and kidney problem especially in kidney. The present pharmacognostic standards like botanical description, microscopy, anatomy leaf surface features and fluorescence analysis could be useful for its correct identification and standardization and provide a base for its pharmacognostic implementations

Authors' contributions

Conceived and designed the experiments: SA Khan, Performed the Experiments: SA Khan & G Dastagir, Analyzed the Data: Barkatullah & I Ahmad, Contributed reagents/ materials/ analysis tools: U Ali, Wrote the paper: SA Khan & S Ullah.

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