Research Article

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

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Citation

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 ± 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 pharmacognistic 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 organoliptically 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 \( \text{H}_2\text{SO}_4 \) 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>
<thead>
<tr>
<th>S&gt;No</th>
<th>Parameter studied</th>
<th>Rhizome</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Length</td>
<td>7-20cm</td>
<td>6-19cm</td>
</tr>
<tr>
<td>2</td>
<td>Width</td>
<td>1-2.3cm</td>
<td>0.9-2cm</td>
</tr>
<tr>
<td>3</td>
<td>Shape</td>
<td>Cylindrical</td>
<td>Irregular</td>
</tr>
<tr>
<td>4</td>
<td>Colour</td>
<td>Brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>5</td>
<td>Taste</td>
<td>Astringent</td>
<td>Indistinct</td>
</tr>
<tr>
<td>6</td>
<td>Odour</td>
<td>Pleasant</td>
<td>Indistinct</td>
</tr>
<tr>
<td>7</td>
<td>Fracture</td>
<td>Flexible</td>
<td>Hard</td>
</tr>
<tr>
<td>8</td>
<td>Fracture surface</td>
<td>Fibrous</td>
<td>Uneven</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th>S. No</th>
<th>Plant parts</th>
<th>Cells and their size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cork</td>
</tr>
<tr>
<td>1</td>
<td>Rhizome</td>
<td>Length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19µ</td>
</tr>
<tr>
<td>2</td>
<td>Leaf</td>
<td>Length</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26 µ</td>
</tr>
</tbody>
</table>

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*](image)

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 ± SEM of stomatal numbers of upper and lower epidermis was (20± 0.7) and (24.2 ± 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)
Table 3. Leaf surface data of the *B. ciliate*

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

**SEM of the powder drug of *B. ciliate***

The powder drug study of *B. ciliate* 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 33μ and 20μ (Table 4, Figure 4),

ii) Star shaped trichomes with mean length and width 250μ and 40μ (Table 4, Figure 5 and 6).

iii) Cluster of calcium oxalate crystals with each crystal having 26μ and 11μ 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 350μ and 40μ, simple uniseriate straight trichomes (390μ and 74μ) and star shaped trichome (350μ and 40μ) respectively (Table 4, Figure 6, 8a and 8b).

ii) Fragments of upper epidermis with anomocytic stomata with 39μ and 20μ and lower epidermis with 40μ and 23μ (Table 4, Figures 9a, 9b and 10a 10b),

iii) Spirally arranged phloem vessels having 28μ and 11μ length and width respectively (Table 4 and Figure 11)
Table 4. SEM measurements (µ) of various tissues of *Bergenia ciliata* (Haw.) Sternb

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Anatomical features</th>
<th>Rhizome</th>
<th></th>
<th></th>
<th>Leaf</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stellate trichomes</td>
<td>Length</td>
<td>Width</td>
<td>Length</td>
<td>Width</td>
<td>Length</td>
<td>Width</td>
</tr>
<tr>
<td>Rhizome</td>
<td>Calcium oxalate Crystals</td>
<td>250µ</td>
<td>40µ</td>
<td>26µ</td>
<td>11µ</td>
<td>33µ</td>
<td>20µ</td>
</tr>
<tr>
<td>Leaf</td>
<td>Stellate Trichome</td>
<td>50µ</td>
<td>350µ</td>
<td>74µ</td>
<td>350µ</td>
<td>28µ</td>
<td>11µ</td>
</tr>
</tbody>
</table>
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 ciliata* (Haw.) Sternb

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

Figure 11. Vessel cells in leaf of *Bergenia ciliata* (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

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant parts</th>
<th>Rhizome</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reagent Used</td>
<td>Day light</td>
<td>UV Low (255)</td>
</tr>
<tr>
<td>1</td>
<td>Iodine</td>
<td>B</td>
<td>Br.B</td>
</tr>
<tr>
<td>2</td>
<td>Picric acid</td>
<td>B.Y</td>
<td>Br</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Br</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>Ferric chloride</td>
<td>D.Br</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>Ether</td>
<td>D.B</td>
<td>Br</td>
</tr>
<tr>
<td>6</td>
<td>Ethanol</td>
<td>B</td>
<td>L.B</td>
</tr>
<tr>
<td>7</td>
<td>HCL</td>
<td>B</td>
<td>D.Br</td>
</tr>
<tr>
<td>8</td>
<td>Acetic acid</td>
<td>P</td>
<td>R</td>
</tr>
<tr>
<td>9</td>
<td>H₂SO₄</td>
<td>Re</td>
<td>R</td>
</tr>
<tr>
<td>10</td>
<td>HNO₃</td>
<td>Br</td>
<td>D.Br</td>
</tr>
<tr>
<td>11</td>
<td>Butanol</td>
<td>D.Br</td>
<td>B</td>
</tr>
</tbody>
</table>

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 adenophylum, 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 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 Ulllah.

**References**


