Botanical and phytochemical comparison of three *Bergenia* species

Sharad Srivastava* and Ajay Kumar Singh Rawat

Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow 226 001

Received 20 July 2007; revised 15 October 2007; accepted 18 October 2007

Three species of *Bergenia* [*B. ligulata* (Wall) Eng., *B. ciliata* (Royle) Raizada and *B. stracheyi* Engl.] were evaluated for botanical, physicochemical and chemical studies. Botanical study of rhizomes revealed that *B. ciliata* has large number of starch grains; *B. ligulata* has maximum calcium oxalate crystals while *B. stracheyi* has a lesser amount of starch grains. Physicochemical studies showed that *B. stracheyi* had highest percentage of all physicochemical parameters (total ash 15.8, alcohol and water soluble extractives 13.83 and 16.83, sugar 5.5 and tannins 7.86), except starch and acid insoluble ash, which were highest in *B. ciliata*. A comparative HPTLC study was also carried out.

**Keywords:** *Bergenia*, Bergenin, HPTLC, Pharmacognosy, Pashanbheda

**Introduction**

High incidence of urinary calculi is reported from British Isles, Scandinavian countries, northern Australia, central Europe, India, Pakistan, and Mediterranean countries. Calcium-containing stones are the most common (75% of all urinary calculi), which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%). Different mineral metabolisms are important in the formation of urinary stones or calculi. There are many herbs in the Indian systems of medicine, which play important role in inhibition and removal of calcium oxalate crystals. The effect of aqueous extracts of some common medicinal plants was also studied on the growth and inhibition of calcium oxalate monohydrate (COM) crystals.

Different *Bergenia* species (Family: Saxifragaceae), distributed in South and East Asia and European countries, are important medicinal plants. In India, rhizomes have been used for centuries in the Ayurvedic formulations to dissolve kidney and bladder stones, in leucorrhrea, piles, and pulmonary affections. Alcohol extract of plant has exhibited significant analgesic, anti-inflammatory and diuretic properties. Anti-bacterial, anti-inflammatory and anti-tussive activity of *B. ciliata*, besides anti-viral activity and antilithiatic activity have also been reported. Inhibition of the growth of urinary calcium hydrogen phosphate dehydrate crystals with aqueous extract of *Tribulus terrestris* (Zygophyllaceae) and *B. ligulata* has been reported.

There are reports on ethnobotanical usage of *Bergenia* species as an antilithic, for boils and blisters, in urinary diseases, as anti-diabetic, hemorrhoids, stomach disorders and opthalmia. Leaves are used for dissolving kidney stones. Rhizome is used as tonic, antipyretic, antidiarrhoeal, in opthalmia, kidney stones, and other urinary disorders. Plant contains β-sitosterol-D-glucoside, bergenin and afzelechin. A simultaneous determination of bergenin and gallic acid by HPTLC is also reported in *B. ligulata*.

This study presents comparative botanical and phytochemical evaluation of *B. ligulata* (Wall) Eng., *B. ciliata* (Royal) Raizada and *B. stracheyi* Engl.

**Materials and Methods**

Plant materials were collected in October 2004 from Almora (Uttarakhand, India), authenticated by Dr A K S Rawat, matched with herbarium specimen, and stored in the Institute’s herbarium with following voucher specimen numbers: LWG 222437BC; LWG 222438BL; LWG 222439BS, 2004. Rhizomes preserved in 70% ethanol for histological study. Microtome sections cut and stained with safranin and fast green, and photographed with Nikon F70X camera.
Physico-chemical and HPTLC Studies

Physico-chemical (Fig. 1) and phytochemical studies (Fig. 2) were carried out as per standard procedures\textsuperscript{25-27} using shade dried powdered (100 mesh) plant material. A densitometric HPTLC analysis (Fig. 3) was also performed for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug.

Extraction of Plant Material

Air-dried (45-55°C) powdered rhizomes of three *Bergenia* species (each 1.0 g) were extracted with....
methanol (10 ml). Extracts were concentrated under vacuum, redissolved in methanol, filtered and finally made up to 100 ml with methanol prior to HPTLC analysis.

**Chromatographic Conditions**

Chromatography was performed on Merk HPTLC precoated silica gel 60GF<sub>254</sub> (20 cm x 20 cm) plates. Methanolic solutions of samples and standard compound bergenin of known concentrations were applied to the layers as 6 mm-wide bands positioned 15 mm from the bottom and 15 mm from side of the plate, using Camag Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nl/sec from application syringe. These conditions were kept constant throughout the analysis of samples.

**Detection and Quantification of Bergenin**

Following sample application, layers were developed in a Camag twin through glass chamber that had been pre-saturated with the mobile phase of ethylacetate: formaldehyde: acetic acid: water (10:1:1:2) till proper separation of bands up to 8 cm height. After development,
Plate 1—Macro and microscopic characters of *B. ciliata* rhizome [ICO, inner cortex; CK, cork cells; IVB, inner vascular bundle; OVB, outer vascular bundle; CO, cortex; FR, fibre; ST, starch; VS, vessels; XY, xylem; ED, endodermis; PR, pericycle]
Plate 2—Macro and microscopic characters of *B. ligulata* rhizome [ICO, inner cortex; CK, cork cells; IVB, inner vascular bundle; OVB, outer vascular bundle; CO, cortex; FR, fibre; ST, starch; VS, vessels; XY, xylem; ED, endodermis; PR, pericycle]
Plate 3—Macro and microscopic characters of *B. stracheyi* rhizome [ICO, inner cortex; CK, cork cells; IVB, inner vascular bundle; OVB, outer vascular bundle; CO, cortex; FR, fibre; ST, starch; VS, vessels; XY, xylem; ED, endodermis; PR, pericycle]
layer was dried with a dryer and bergenin was quantified using Camag TLC scanner model 3 equipped with Camag Wincats IV software. Following scan conditions were applied: silt width, 6 mm x 0.45 mm; wavelength, 260 nm; and absorption-reflection mode. To prepare calibration curves, stock solutions of bergenin (1 mg/ml) were prepared and various volumes of solutions were analyzed through HPTLC as mentioned above; calibration curves of peak area vs. concentration were also prepared. Bergenin identified at Rf 0.74 using regression equation (y = 51.790x + 7.075) and r² at 0.996, was found to be: B. ciliata, 5.68; B. ligulata, 5.73; and B. stracheyi, 5.99%.

Results and Discussion
Rhizome can easily be differentiated on the basis of organoleptic characters; odour and taste of rhizome is quite characteristic and is aromatic with astringent taste (Table 1, Plates 1-3). B. ciliata has large number of starch grains; B. ligulata has maximum calcium oxalate crystals while B. stracheyi has lesser amount of starch grains. Physicochemical studies showed that B. stracheyi has almost maximum percentage of all the physicochemical parameters (total ash, 15.8; alcohol soluble extracts, 13.83; water soluble extracts 16.83; sugar, 5.5; and tannins, 7.86%), except starch and acid insoluble ash, which were highest in B. ciliata.

Successive Soxhlet extraction from non-polar to polar solvents (hexane, chloroform, acetone, alcohol, and water) with powdered sample (5 g) of each species (100 mesh) at 70°C showed that B. stracheyi was having almost maximum percentage of all extractives as compared to B. ciliata and B. ligulata, which have almost similar percentages of extractives. Quantitative analysis by HPTLC system showed that bergenin was maximum in B. stracheyi (5.99%) followed by B. ciliata (5.73%) and B. ligulata (5.68%). Thus, B. ciliata and B. stracheyi can be a better source of bergenin and will elicit the desired biological activity better than B. ligulata.

<table>
<thead>
<tr>
<th>Table 1— Macroscopic and microscopic characters of rhizomes of different Bergenia species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characters</td>
</tr>
<tr>
<td>Macroscopic</td>
</tr>
<tr>
<td>Microscopic</td>
</tr>
</tbody>
</table>
Conclusions

Parameters studied are very useful for the identification of *Bergenia* species, which may be useful to pharmaceutical industries for authentication, quality control and standardization of these species in order to identify the correct species used in the formulation.

Acknowledgement

Authors thank Director, NBRI for providing all facilities to conduct this research work.

References

26. *Indian Pharmacopoeia, 2nd edn* (Govt. of India; New Delhi) 1965, 38-40.