Pharmacognostical Studies of *Bryophyllum pinnatum* (Lam.) Kurz.

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**ABSTRACT**

Context: *Bryophyllum pinnatum* (Lam.) Kurz. widely used in traditional as well as folk medicinal systems are locally known as Panphuti. Traditionally, it is used for the treatment of kidney stones, urinary tract infection, burns and diarrhoea. **Aims**: In the present study, pharmacognostic studies of root, stem, and leaf of *B. pinnatum* (Lam.) Kurz. is carried out in order to standardize the plant for its phytochemical, physico-chemical and pharmacognostical. **Materials and Methods**: For standardization of plant material morphological and anatomical characterization was carried out. Physico-chemical parameters viz. ash content, extractive values, heavy metal content was carried out as per Ayurvedic Pharmacopoeia of India. Phytochemical investigations were made to know the presence of various bioactive molecules, amino acid composition. **Results**: Intra-stelar and extra-stelar secondary growth with wood and periderm formation along with deposition of starch grains were observed in the pith region of the root and cortical region of the stem. Calcium oxalate crystals were also present in the cortical region of the stem. Leaf lamina showed spongy parenchyma in mesophyll region and anisocytic type of stomata. Anthocyanin pigment was present below epidermal cells in petiole. Physico-chemical results can be serves as quality control data. Quantitatively carbohydrate, protein, flavonoids, phenolic compounds, saponins and pro-antocyanidins were found to be present in the root, stem and leaf part of *B. pinnatum* (Lam.) Kurz. **Conclusion**: The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant. **Keywords**: *Bryophyllum pinnatum* (Lam.) Kurz., HPTLC, pharmacognosy, physico-chemical, phytochemical

**INTRODUCTION**

Herbal materials are categorized according to sensory, macroscopic and microscopic characteristics. An examination to determine these characteristics is the first step towards establishing the identity and the degree of purity of such materials. Wherever possible, authentic specimens of the material to be identified and samples of pharmacopoeial quality should be available to serve as a reference. Visual inspection provides the simplest and quickest method to establish identity, purity and quality. If a sample is found to be considerably different from the specifications in terms of color, consistency, odor or taste, it is considered as not fulfilling the requirements. Macroscopic identification of herbal materials is based on shape, size, color, surface characteristics, texture, fracture characteristics and appearance of the cut surface.1

*Bryophyllum pinnatum* (Lam.) Kurz. (*Crassulaceae*) is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China, and Australia. The divine herb contains a wide range of active compounds, including alkaloids, triterpenes, glycosides, flavonoid, steroids, bufadienolides, lipids and organic acids, have been isolated from this species. The plant is widely used in traditional medicine for the treatment of a variety of ailments and well known for its haemostatic and wound healing properties. The plant have been found to possess pharmacological activities as immunomodulator, central nervous system depressant, analgesic, antimicrobial, anti-inflammatory, antiallergic, antianaphylactic, antileishmanial, antitumorous, antiulcer, antibacterial, antifungal, antihistamine, antiviral, febrifuge, gastroprotective, immunosuppressive, insecticidal, muscle relaxant, sedative.2

Literature survey revealed that there is no work on pharmacognostic studies of root, stem and leaf of *B. pinnatum* (Lam.) Kurz. Therefore, in the present work, pharmacognostic studies on root, stem and leaf (fresh material and powder) of *B. pinnatum* (Lam.) Kurz. was

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DOI: 10.5530/pj.2014.6.5
carried out, which may be useful for proper identification and authentication of crude drug.

MATERIALS AND METHODS

Plant material

The plants of *B. pinnatum* (Lam.) Kurz. were obtained from Pathare Nursery, Kalyan and grown in the Botanical Garden, Birla College, Kalyan. The plant was identified and authenticated from Blatter Herbarium, St. Xavier’s College, Mumbai. The shade dried material of root, stem, leaf and whole plant were separately cut into small pieces and powdered using mixer grinder. The powdered materials were stored separately in labeled air tight bottles.

Anatomical studies

Morphological features, odor, color and taste of root, stem and leaf were studied. Transverse sections of root, stem, and leaf were taken, stained with safranin and mounted in glycerin. Semi-permanent slides were prepared and observed under compound microscope. Photographs of the sections were taken under ×10 and ×45 magnifications using Nikon camera.

Powder characteristics of root, stem and leaf were studied by staining powder in safranin and observed under compound microscope at ×10 and ×45 magnifications. Histochemical studies, sections of root, stem and leaf were stained with different chemical reagents for the localization of alkaloids, phytosterols, lignins, calcium oxalate crystals and tannins.

Physico-chemical analysis

Root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. were used to study foreign organic matter, loss on drying, total ash content, acid insoluble ash, water soluble ash, alcohol and water soluble extractives and heavy metal content. The above parameters were studied as per standard method of Ayurvedic Pharmacopoeia of India guidelines.

Qualitative phytochemical analysis

Petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of root, stem, leaf and whole plant were tested for the presence of alkaloids by Dragendorffs, Mayers and Wagners reagent; anthraquinones by alcoholic potassium hydroxide and amino acids by ninhydrin reagent; carbohydrates by Molich, Benedict, iodine and phenol-sulfuric acid reagent; proteins by Lowry method; glycosides by sulfuric acid method, saponins by foam test and flavonoids by sodium hydroxide, lead acetate reagent; phenolic compounds and tannins by ferric chloride reagent; cardiac glycosides by Killer–Kilani reagent, terpenoids by Salwoskii and sterols by Libermann–Burchard reagent.

Quantitative estimation of phytochemical

Root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. were quantitatively estimated for total carbohydrate content by anthrone method, total protein content by Lowry method; total phenolic content by Folin–Ciocalteu method; total flavonoid content by aluminium chloride method, proantocyanidin content by vanillin-hydrochloric acid method and total saponin content by vanillin-sulfuric acid method.

Methanolic extracts of the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. were separated on silica gel G F254 pre-coated plates by using following solvent systems for detection of anthraquinone:ethyl acetate:methanol:distilled water (10:1.5:1); terpenoid:toluene:ethyl acetate:methanol (7:2:1), derivatizing reagent used was Lieberman–Burchard; phenolic compounds:toluene:ethyl acetate:formic acid (5:4:1). Derivatizing reagent used was 5% ethanolic ferric chloride; flavonoids:ethyl acetate:formic acid:glacial acetic acid:methanol:distilled water (7:2:0.5:0.5).

RESULTS

Macroscopical studies

The roots were simple, tap root, greenish brown in color when young and light brown when old. Root powder had a pleasant odor and was sweet in taste. Stem of *B. pinnatum* (Lam.) Kurz. was light green in color when young and light brown in color when old (Figure 1). Old stem was rough and had lenticels on surface. Stem powder had a pleasant odor and slightly bitter in taste. The leaves were opposite, decussate, succulent, 10-20 cm in length. The lower leaf were simple, whereas, the upper leaf is 3-4 foliate with long petiole with dark green in color and fleshy, which are distinctively scalloped and trimmed in red. The leaves are furnished with rooted vegetative buds, and leaf apex is obtuse. Petiole was 2-4 cm in length; leaflet blades were oblong to elliptic, margin crenate with each notch bearing a dormant bud competent to develop into a healthy plantlet (Figure 1).
Microscopical characters of young root

Young root was circular in outline and showed outer epiblema, followed by outer and inner cortex. Outer cortex was 3-4 layered thick walled made up of sclerenchymatous cells known as exodermis. Inner cortex was thin walled made up of parenchymatous cells with deposition of starch grains. Stelar region showed presence of vascular tissue (xylem and phloem). Metaxylem was prominent towards center surrounded by protoxylem toward periphery. Parenchymatous pith occupied the central portion of the root section (siphonostele) (Figure 2).

Microscopical characters of old root

Old root was circular in outline with prominent secondary growth in extra and intra stelar region. Extrastelar secondary growth showed the presence of periderm, which get differentiated into phellem, phellogen and phelloderm. Parenchymatous cells of secondary cortex or phelloderm showed the deposition of starch grains. In the intra-stelar growth secondary xylem (wood) occupied the major portion in the form of ring. Primary xylem and phloem get pushed towards the center surrounding the pith. Secondary xylem chiefly made up of tracheids along with fibers, xylem parenchyma and few vessels. Pith showed deposition of starch grains (Figure 2).

Microscopical characters of young stem

Young stem was circular in outline and showed outer layer of thick walled epidermis with cuticle. Beneath the epidermis 3-4 layered hypodermis made up of sclerenchymatous cells was observed. Inner cortex was thin walled parenchymatous, loosely arranged with deposition of starch grains. Endodermis was not prominent. In stelar region, vascular bundles were arranged in a ring. Each vascular bundle was conjoint, collateral and open. Xylem elements were mainly in the form of tracheids, xylem parenchyma fibers with few vessels. Parenchymatous pith in the center showed the deposition of starch grains (Figure 3).

Microscopical characters of old stem

Old stem was wavy in outline and showed both extra-stelar and intra-stelar secondary growth. Extra-stelar secondary growth gave rise to periderm composed of phellem, phellogen and phelloderm forming a bark. While, intra-stelar secondary growth showed development of a broad region of secondary xylem (wood). Secondary xylem was made up of pith, which was broad made up of thin walled parenchyma cells with depositions of starch grain and calcium oxalate crystals (Figure 4).
Microscopical characters of leaf

Leaf of *B. pinnatum* (Lam.) Kurz. showed upper and lower epidermis with cuticle. Midrib region was broad with distinct upper and lower epidermis. The cells between upper and lower epidermis were homogenous and parenchymatous deposited with starch grains and chlorophyll with two vascular bundles found in the center. Each vascular strand was conjoint, collateral with xylem facing toward the upper side. The mesophyll region of the lamina was homogenous and chlorenchymatous and showed spongy parenchyma. Lamina showed distinct upper and lower epidermis with presence of anisocytic type of stomata specifically on the lower epidermis (Figure 5).

Microscopical characters of petiole

Petiole of *B. pinnatum* (Lam.) Kurz. was circular in outline with single layered outer cuticularized epidermis. Beneath the epidermis was a broad region of ground tissue made up, loosely arranged thin walled parenchymatous cells with chlorophyll, starch grains and calcium oxalate crystals. Outermost few cells of ground tissue toward epidermis showed deposition of pink colored anthocyanin pigments, which turned to purple color after staining with safranine. Vascular tissue was grouped together to form crescent-shaped structure with phloem surrounding xylem on one side (Figure 6).
Powder of root, stem and leaf was stained with safranine and microscopic observation was carried out. Powder microscopy of root showed presence of cork cells, fiber, calcium oxalate crystals and xylem vessels with annular thickening. Stem showed trachieds with simple pits on the lateral wall, vessels with spiral wall thickening, xylem parenchyma with profused deposition of starch grains, and fibers. Leaf showed presence of prismatic oxalate crystals, starch grains, stomata with epidermal cell and pitted vessels annular wall thickening.

**Physico-chemical analysis**

Plant materials free from visible signs of contamination by molds or insects and other animal contaminants were collected. It was without abnormal odor, discoloration, etc. Thus, the plant material was free from any foreign organic matter.

Root, stem, leaf and whole plant powder of *B. pinnatum* (Lam.) Kurz. were studied to find out total ash, acid insoluble ash and water soluble ash content. Total ash content in the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. was 10.30 ± 0.30%, 13.50 ± 0.43%, 13.26 ± 1.01% and 11.03 ± 0.32% respectively. Root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. showed 0.86 ± 0.05%, 0.93 ± 0.11%, 1.73 ± 0.05% and 1.03 ± 0.11% of acid insoluble ash content respectively. Water soluble ash content in the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. was 1.30 ± 0.26%, 2.53 ± 0.25%, 2.13 ± 0.15% and 0.96 ± 0.11% respectively (Table 1).

Extractive value for the root, stem, leaf and whole plant powder of *B. pinnatum* (Lam.) Kurz. was estimated by using ethanol and distilled water as solvents. Ethanol soluble extractive value for the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. was 6.48 ± 0.08%, 5.49 ± 0.44%, 10.18 ± 0.12% and 23.58 ± 0.41% respectively (Table 1), whereas water soluble extractive value for root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. was 18.50 ± 0.44%, 11.70 ± 0.16%, 22.53 ± 0.12% and 29.30 ± 0.20% respectively (Table 1).

Loss on drying was found to be 80.06 ± 0.75%, 77.66 ± 0.83%, 91.46 ± 0.80% and 79.00 ± 0.60% in root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. respectively (Table 1).

Arsenic content in root, stem and leaf of *B. pinnatum* (Lam.) Kurz. was 0.712, 1.876 and 0.516 ppm, respectively (Table 2). However metals like cadmium and mercury were absent in root, stem and leaf of *B. pinnatum* (Lam.) Kurz. Lead content in root, stem and leaf of *B. pinnatum* (Lam.) Kurz. was 0.045, 0.089 and 0.093 ppm, respectively (Table 1).

**Phytochemical analysis**

Preliminary phytochemical analysis showed the presence of terpenoids, glycosides, anthraquinones, flavonoids, sterols, proteins, amino acids and carbohydrates in different extracts of the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. Preliminary phytochemical evaluation may help further in the estimation of the phytoconstituents quantitatively from different parts of *B. pinnatum* (Lam.) Kurz.

Quantitative phytochemical estimation by standard methods showed a higher content of carbohydrates and phenolic compounds in root. Proteins, flavonoids, saponins, pro-anthocyanidins were found to be higher in leaf of *B. pinnatum* (Lam.) Kurz. (Table 2).

Phytochemicals present in the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. were extracted and separated using thin layer chromatography (TLC). The different phytoconstituents were identified by phytochemical analysis. The R<sub>f</sub> values obtained can be

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign organic matter</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Percentage loss on drying</td>
<td>80.06±0.75</td>
<td>77.66±0.83</td>
<td>91.46±0.80</td>
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<td>Percentage total ash</td>
<td>10.03±0.30</td>
<td>13.50±0.43</td>
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<tr>
<td>Percentage acid insoluble ash</td>
<td>0.86±0.05</td>
<td>0.93±0.11</td>
<td>1.73±0.05</td>
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<td>Percentage water soluble ash extractive</td>
<td>13.00±0.26</td>
<td>2.53±0.25</td>
<td>2.13±0.15</td>
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<tr>
<td>Percentage alcohol soluble extractive</td>
<td>6.48±0.08</td>
<td>5.49±0.44</td>
<td>10.18±0.12</td>
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<tr>
<td>Percentage water soluble extractive</td>
<td>18.50±0.44</td>
<td>11.70±0.16</td>
<td>22.53±0.12</td>
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<tr>
<td>Lead</td>
<td>0.045</td>
<td>0.089</td>
<td>0.093</td>
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<tr>
<td>Arsenic</td>
<td>0.712</td>
<td>1.876</td>
<td>0.516</td>
</tr>
<tr>
<td>Cadmium</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mercury</td>
<td>ND</td>
<td>ND</td>
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</table>

<Not present, ND: Not detected, B. pinnatum: Bryophyllum pinnatum>

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
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</thead>
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<tr>
<td>Carbohydrate</td>
<td>87.44±1.90</td>
<td>78.55±1.92</td>
<td>54.10±1.90</td>
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<td>Proteins</td>
<td>17.16±0.28</td>
<td>47.16±0.57</td>
<td>73.88±0.28</td>
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<td>Total phenolics</td>
<td>106.22±0.38</td>
<td>-</td>
<td>12.88±0.38</td>
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<tr>
<td>Total flavonoids</td>
<td>59.13±1.15</td>
<td>33.13±0.23</td>
<td>89.13±1.15</td>
</tr>
<tr>
<td>Total saponins</td>
<td>22.26±1.15</td>
<td>18.26±1.15</td>
<td>40.93±1.15</td>
</tr>
<tr>
<td>Total proanthocyanidin</td>
<td>41.83±1.44</td>
<td>21.83±1.44</td>
<td>46.83±1.44</td>
</tr>
</tbody>
</table>

*Values are mean of three determinants, B. pinnatum: Bryophyllum pinnatum*
used as a reference to check the quality of the plant during bulk collection. TLC method developed in the present work is reliable, fast and economic. The fingerprint pattern obtained by TLC was distinctive for different phytoconstituents present in *B. pinnatum* (Lam.) Kurz. (Figure 7).

**DISCUSSION**

If the monograph of plants is given or mentioned in pharmacopoeias, it provides the simplest and quickest means of establishing the identity and purity of herbal raw material. Microscopic analysis is based on observation of specific microscopic characteristics. The anatomical characters can also be used to identify the spurious drugs from the original one.

In the present work, macroscopic characters of root, stem and leaf were studied, which may help to identify the plant by its external morphology. Microscopic section of root showed broad region of secondary xylem, stem showed development of periderm after secondary growth. Leaf showed anisocytic type of stomata, presence of starch grains. Presence of anthocyanin pigment and prismatic crystals was seen in petiole. These anatomical characters can be used for proper identification of *B. pinnatum* (Lam.) Kurz.

The major problem faced in the herbal industry is the identification of authenticated raw material and in the absence of data one can use adulterant in the drug formulation. The detailed systematic pharmacognostical evaluation of plant and plant material provides means of standardization of a herb that can be used as a drug or as raw material.

The anatomical characters studied can be used for proper identification and will avoid the use of the adulterant of plant raw material to be used in herbal pharmaceutical industries. Data obtained from macroscopic and microscopic studies may be considered as a distinguishing parameter to identify and decide the authenticity of the plant material, and this can be included as pharmacognostic standards in pharmacopoeia.

As per the WHO guidelines, the quality control of medicinal or herbal plant is mandatory before using for consumption. The physico-chemical parameters will help in judging the purity and quality of the herbal plants.

From the present study it was observed that foreign organic matters were absent in the root, stem and leaf of *B. pinnatum* (Lam.) Kurz, this may be due to collection of plant material from non-polluted area. Ash content was less in root, stem, leaf and whole plant powder of *B. pinnatum* (Lam.) Kurz. due to low content of carbonates, phosphates, silicates and silica. The results showed that the root, stem, leaf and whole plant of *B. pinnatum* (Lam.) Kurz. showed higher water soluble extractive value in comparison with the alcohol extractive values. It indicates the presence of a considerable amount of polar compounds and large quantity of water soluble constituents such as sugar, glycosides, phenolic and tannins.

Amongst the four heavy metals analyzed, cadmium and mercury were absent in the root, stem and leaf of *B. pinnatum* (Lam.) Kurz. Arsenic and lead were in permissible limits in root, stem and leaf of *B. pinnatum* (Lam.) Kurz. Data obtained from physico-chemical parameters viz. ash value, extractive value, loss on drying and heavy metal content will be useful to check the quality and to confirm the authenticity of *B. pinnatum* (Lam.) Kurz.

Phytochemicals, generally have a wide range of pharmacological activities or actions. Most of these

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**Figure 7:** Thin layer chromatography fingerprints of phytochemicals from *Bryophyllum pinnatum* (Lam.) Kurz. RM: Methanolic extract of root, SM: Methanolic extract of stem, LM: Methanolic extract of leaf and WM: Methanolic extract of whole plant.
Phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs. Natural products are of great significance to man, fulfilling the roles of medicines, stimulants, perfumes, spices, antimicrobial agents, hallucinogens and as components of industrial products. Phytoconstituents have been known to possess various health benefits viz. antimicrobial, anti-inflammatory, cancer preventive, anti-diabetic and antihypertensive effects. Phytochemical analysis can be an important path of information for selection of active constituents in pharmacological studies as secondary metabolites are responsible for various biological activities.

CONCLUSION

The present study confirms the presence of bioactive constituents such as flavonoid, terpenoid and mucilage in root, stem leaf and whole plant of *B. pinnatum* (Lam.) Kurz. Further studies are required to be carried out for *in vivo* pharmacokinetic evaluation and assess the bioavailability of *B. pinnatum* (Lam.) Kurz.; efficacy study, which may provide the necessary evidence for rational use of the plant as potent herbal medicine.

REFERENCES


Source of Support: None. Conflict of Interest: None declared.