PHARMACOGNOSTICAL EVALUATION OF LEAF OF BLEPHARISPERMUM SUBSESSILE DC. (ASTERACEAE)

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Received: 29/01/2013; Revised: 20/02/2013; Accepted: 27/02/2013

ABSTRACT

Blepharispermum subsessile DC (Asteraceae), a less explored folklore medicinal plant, is a glabrous shrub, found in the forest of Odisha, Karnataka, Madhya pradesh and Maharashtra. It is used as one of the source plants of Rasna. Its root, stem and leaves are claimed to be beneficial in the treatment of Rheumatism. In this study, a detailed pharmacognostical investigation on its leaves, which includes macroscopic and microscopic characters, histochemical tests, quantitative microscopy and preliminary physicochemical analysis, following the prescribed guidelines of Ayurvedic Pharmacopoeia of India. The microscopical study result showed the presence of undifferentiated palisade and spongy tissue, anomocytic stomata, bicollateral vascular bundles, unicellular trichomes, calcium oxalate crystals, oil globules, tannins etc. in the leaves. The quantitative surface microscopy study showed 174 numbers of epidermal cells, each epidermal cell measured about 22.48µm², through veins 29.36µm² and stomatal index 25. The purity test showed loss on drying (9.14%w/w), total ash (9.68%w/w), water soluble extractive (30.91%w/w), alcohol soluble extractive (5.73%w/w) and pH (6.5).

KEY WORDS:
Asteraceae, Ayurveda, Blepharispermum subsessile DC., Ethnomedicine, Pharmacognosy, Rasna.
INTRODUCTION

*Blepharispermum subsessile* DC. (Asteraceae), known as *Rasnajhadi* in Odisha, *Naama banta* in Kannada and *Adavi banti* in Telugu, is a glabrous shrub with small close globose cluster head, leaves alternate, entire or toothed with short petiole. It is distributed in Odisha, Karnataka, Madhya pradesh and Maharashtra. Ethno-botanical studies reports its use in common cold and rhinitis, as wormicidal, as tonic (A.K.Gupta et al., 2004), diarrhoea (Dash et al., 2006), eye troubles, backache and rheumatism (A.B.Prusti et al., 2007), irregular menstruation (Rajesh et al., 2008) and rheumatism (C.P.Khare, 2008). The pharmacognostical characters of its leaf, one of the parts used, is not reported yet. So, the present study deals with the macroscopic and microscopic characters of its leaf including preliminary physicochemical analysis.

MATERIALS AND METHODS

Collection and authentification:

*Rasnajhadi*, identified by local traditional practitioners, growing in Gurudongar medicinal plants conservation area of Nuapada district of Odisha, India, was authenticated by expert taxonomist as *Blepharispermum subsessile* DC., (Asteraceae), on the basis of characters given in Flora of Orissa (Saxena et al., 1990). The fresh plant samples were collected from its natural habitat, Odisha, in the month of October 2011 and voucher specimen has been preserved in the pharmacognosy laboratory of IPGT and RA, vide no 36561. The collected plant samples were shaken to remove adherent soil and dirt. The leaves were separated from the stem, washed with running fresh water and few pieces stored in solution of AAF (Alcohol: Acetic acid: Formalin) in the ratio of (90:5:5) (Johnsen, 1940) to utilize them for microscopic studies. The remaining leaves were shade dried and then powdered with mechanical grinder and passed through mesh no.80# and preserved in an air-tight glass container.

Pharmacognostic studies:

Morphological characters were studied by observing the leaves as such and also with the help of the dissecting microscope. For detailed microscopical observation, free hand thin transverse section passing through the midrib were taken, and cleared with chloral hydrate and observed as such for the presence of any crystals, then were stained with Phloroglucinol and Hydrochloric acid to notice the lignified elements like fibers, vessels etc. of the meristele and other parts (Khandelwal K.R., 2008). Photographs of the section were taken with the help of Canon digital camera attached to Zeiss microscope. Powder characters were observed and histochemical tests carried out, as per the guidelines of Ayurvedic Pharmacopoeia of India, (Anonymous, 1999).

Quantitative microscopy:

Determination of certain leaf constants or diagnostic characters of leaves like micrometric evaluation of stomatal number, stomatal index, stomatal size and epidermal size were taken by mean value (3-5 successive results) carried out as per the method described in Ayurvedic Pharmacopoeia of India (Evans, 2009). [fig 2.g-i] [table-2, 3].

\[
I = \frac{S * 100}{E + S}
\]

(I= stomatal index, \(S=\) no. of stomata per unit area, \(E = \) no. of epidermal cells in the same unit area).

Phytochemical evaluation:

Preliminary physic-chemical investigations were carried out following standard procedures recommended by API (Anonymous, 1999).

RESULTS AND DISCUSSION

Morphology:

Leaves simple, alternate, stipulate, sessile, measures about 10–12 × 4–6 cm, ovate to elliptic, margin, entire with acute tip and symmetrical base, upper surface dark green, glabrous while lower surface light green in colour, Mid rib strong, 5-7 pairs of veins, venation reticulate. (fig 1-a,c)
Photo plate: 1 Microscopic characters of *Blepharispermum subsessile DC.* leaf

Fig-1.a leaf arrangement

Fig-1.b T.S. of leaf through mid-rib

Fig-1.c individual sub sessile leaf

Fig-1.d Upper epidermis through veins

Fig-1.e Upper epidermis with thick cuticle

Fig-1.f Bicollateral vascular bundle

Fig-1.g Chlorophyll pigments

Fig-1.h undifferentiated spongy & palisade tissue

Fig-1.i. oil globules

Fig-1.j lower epidermis with stomata

Fig-1.k lower epidermis with unicellular trichomes
Microscopic description:

**T.S. of the leaf passing through the mid rib**

1. Midrib convex dorsiventrally with lateral extensions of the lamina on its either side.
2. Epidermis single layered, covered with thick cuticle. Upper epidermal cells wavy walled, large in size than the lower epidermis. Lower epidermis, interrupted with number of stomatas along with simple unicellular trichomes aroused from it.
3. The mesophyll consisted of undifferentiated palisade and spongy tissues. Through mid rib both the upper and lower epidermis consisted of thick compactly arranged collenchymatous cells. Vascular bundles located at the centre of the ground tissue. Ground tissue was made up of simple parenchyma cells with many calcium oxalate crystals at the lower side. The meristele, as seen in midrib, in the form of an arc having the xylem towards the upper surface arranged in radiating bands spreading towards the lower surface. Protoxylem being pointed towards the upper side and the phloem bands towards
the lower side. Medullary rays and cambium tissue occasionally was visible in well developed big vascular bundles. Phloem patches could be seen on both sides of the xylem and hence vascular bundle is bicollateral. The vascular bundle was surrounded by parenchymatous bundle sheath.

4. Stomata- Epidermal cells of the leaf in surface view were wavy in outline and stomata were found mostly on the lower surface of the lamina. The stomata were mostly anomocytic in which guard cells were surrounded by 4 subsidiary cells. (fig 1.b, d–k).

**Histochemical test:**

The results of various histochemical tests carried out to detect lignin, crystals of Calcium oxalate and tannin are depicted in table no.1

**Quantitative microscopy**

No. of epidermal cells 174 and 5 veins were passing through in 1 mm (32, 38, 33, 33, 38). In surface study, each epidermal cell measured about 22.48 µm², whereas through veins 29.36 µm². In transverse section lower epidermal cells measured about 134.16 µm², whereas, ground tissue 299.33 µm² measurement of stomata and stomatal index is shown in table 2-3. (fig.2 g–i).

**Table no.1: Histochemical test**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Observation</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phloroglucinol</td>
<td>+</td>
<td>Pink colour</td>
</tr>
<tr>
<td>Concentrated HCL(1:1)</td>
<td></td>
<td>Effervescence</td>
</tr>
<tr>
<td>FeCl₃ solution</td>
<td>Black colour</td>
<td>Tannin present</td>
</tr>
</tbody>
</table>

**Table no.2: Measurement of stomata**

<table>
<thead>
<tr>
<th>Length (micrometre)</th>
<th>Breadth (micrometre)</th>
<th>Circumference (Square micrometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.03</td>
<td>14.11</td>
<td>207.1</td>
</tr>
<tr>
<td>17.27</td>
<td>12.63</td>
<td>183.79</td>
</tr>
<tr>
<td>17.37</td>
<td>12.5</td>
<td>186.4</td>
</tr>
</tbody>
</table>

**Table no.3: Stomatal index**

<table>
<thead>
<tr>
<th>Stomata through veinlets</th>
<th>Epidermal cells</th>
<th>Stomatal index (S*100/E+S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>222</td>
<td>25</td>
</tr>
<tr>
<td>69</td>
<td>207</td>
<td>25</td>
</tr>
<tr>
<td>82</td>
<td>246</td>
<td>25</td>
</tr>
</tbody>
</table>
Table no-4: Preliminary Physicochemical analysis of *B. subsessile* leaves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreign matter</td>
<td>Nil</td>
</tr>
<tr>
<td>Loss on drying (% w/w)</td>
<td>9.14%</td>
</tr>
<tr>
<td>Total ash content (% w/w)</td>
<td>9.68%</td>
</tr>
<tr>
<td>Water soluble extractive value (% w/w)</td>
<td>30.91%</td>
</tr>
<tr>
<td>Alcohol soluble extractive value (% w/w)</td>
<td>5.73%</td>
</tr>
<tr>
<td>pH</td>
<td>6.5</td>
</tr>
</tbody>
</table>

**Powder microscopy:**

**Organoleptic characters:**

Organoleptic evaluation of leaves of *B. subsessile* revealed its yellowish green colour, aromatic odour and slightly bitter taste. Diagnostic characters of the leaf powder were presence of prismatic crystals of Calcium oxalate, lignified fibers, anomocytic stomata from lower epidermis, spiral and annular vessels from stellar portion, dark yellowish brown content (Tannin), oil globules and many unicellular trichomes. (fig.2 a–f).

**Preliminary Physicochemical analysis:**

While observing the physicochemical characters for purity test, the loss on drying was not more than 9.14% w/w, ash value was not more than 9.68% w/w, water soluble extractive was not more than 30.91% w/w and the alcohol soluble extractive was not more than 5.73% w/w and pH is 6.5. (Table no.4) Total ash content signifies the level of inorganic matter and silica content. The high solubility of the sample in Water denotes that the drug is best suited for extraction with water or water based preparations.

**CONCLUSION**

Leaf of *Blepharispermum subsessile* DC. (Asteraceae) can be identified on the basis of key microscopical characters like undifferentiated palisade and spongy tissue, anomocytic stomata, bicollateral vascular bundles, unicellular trichomes, calcium oxalate crystals, oil globules, tannins etc. The quantitative surface microscopy study showed 174 numbers of epidermal cells, each epidermal cell measured about 22.48µm², through veins 29.36µm² and stomatal index 25. The purity test showed loss on drying (9.14%w/w), total ash (9.68%w/w), water soluble extractive (30.91%w/w), alcohol soluble extractive (5.73%w/w) and pH (6.5).

**ACKNOWLEDGEMENTS**

The authors are thankful to Director IPGT&RA, Gujarat Ayurved University, Jamnagar for providing facilities to carry out the research work. We express our thankfulness to Mr B N Hota, Rtd. DFO, Govt. of Odisha; Mr Pareswar Sahu, Pharmacognosy expert; Dhala Bhai Plant collector, Mr Malaya Das, Forest Range Officer, Govt. of Odisha and other tribal practitioners who helped us during drug collection at Gurudongar, Nuapada, Odisha.
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Source of Support: Nil
Conflict of Interest: None Declared