Pharmacognostical studies on the root of *Nothosaerva brachiata* Wt. – A botanical source of the Ayurvedic drug, *Pashanabheda*

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**Pashanabheda** is an important Ayurvedic drug. Several species belonging to different families are used as the botanical source of *Pashanabheda* while the accepted source is *Bergenia ciliata* (Haw.) Sternb. The roots of *Nothosaerva brachiata* Wight is used in South India as one of the sources of *Pashanabheda*. The study comprising taxonomy of the species, macro- and microscopical characters, physicochemical and ultra-violet analysis besides chromatographic details of the root of *N. brachiata*, helps in the identification of the plant and the drug but also contribute towards establishing pharmacopoeial standards. HPTLC studies helps to identify the species in drug form and to establish the biomarker compound.

**Keywords:** *Nothosaerva brachiata*, Pharmacognosy, *Pashanabheda*, Ayurvedic drug, Medicinal plant

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Pashanabheda is an Ayurvedic drug used in the treatment of urinary calculi (ashmari), dysuria (mootrakrichchhra), polyuria (atimutra), fevers (jwara), piles (arsha), dysentery (pravahika) and uterine disorders (garbhashaya vikara)⁴. The main Ayurvedic formulations of the drug are *Ashmarihara kashaya*, *Mootra virechaniya kashaya*, *Pashanabhedadi kvatha*, *Pashanabhedadya ghrita*, *Sarasvata ghrita*, *Varunadi kvatha* and *Vidari ghrita*, to mention a few². The accepted botanical source of *Pashanabheda* is *Bergenia ciliata* (Haw.) Sternb. belonging to family Saxifragaceae³. Different botanical sources are used in the name of *Pashanabheda* in different parts of the country, viz. *Aerva lanata* (L.) Juss. ex Schult., *A. persica* (Burm.f.) Juss. ex Schult., (both of Amaranthaceae), *Ammania baccifera* L. (Lythraceae), *Bauhinia racemosa* Lam. (Caesalpiniiaceae), *Bergenia stracheyi* (Hook.f. et Th.) Engl. (Saxifragaceae), *Bridelia crenulata* Roxb., B. retusa (L.) Spreng., B. stipulare Blume, *Homonoia riparia* Lour. (all Euphorbiaceae), *Didymocarpus pedicellata* R.Br., (Gesneriaceae), *Gentiana kurroo* Royle, G. lutea L. (both Gentianaceae), *Iris pseudacorus* L. (Iridaceae), *Kalanchoe pinnata* (Lamk.) Pers., K. integrá (Medic.) kuntze, (both Crassulaceae), *Lepidagathis trinervis* Wall. ex Nees, (Acanthaceae), *Nothosaerva brachiata* Wight (Amaranthaceae), *Ocimum tenuiflorum* L., *Plectranthus amboinicus* (Lour.) Spreng. (both Lamiaceae), *Rotula aquatica* Lour. (Boraginaceae) and *Trianthema triquetra* Rottl. ex Willd. (Aizoaceae)⁴-⁶. Of these, *A. lanata*, B. crenulata, B. stipulare, H. riparia, N. brachiata and R. aquatica are used as *Pashanabedha* in South India while B. ciliata, D. pedicellata, *Iris pseudacorus*, K. pinnata and O. tenuiflorum are used as *Pashanabheda* in North India⁷,⁸. The part used in *Pashanabheda* as per Ayurvedic literature is the root¹. Pharmacognostical investigation with macerate details and powder analysis besides HPTLC studies on the roots of *N. brachiata* which help in the identification of crude drug is not available in the literature and hence the study was undertaken⁹,¹⁰.

**Methodology**

Fresh roots were collected from the vicinity of Tirunelveli, Tamil Nadu, during February, 2007, preserved in 70% ethyl alcohol for histological studies. Botanical identification of the plant was carried out¹¹,¹². Voucher herbarium specimen (*Goswami Priyanka Kantivan*) is preserved along with crude drug sample at the herbarium of MS Ramaiah College of Pharmacy, Bangalore¹³. Pharmacognostical evaluation including histochemical, macerate and powder studies were carried out¹⁴-¹⁶. The vernacular names are provided¹⁷.

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Photomicrographs were obtained by observing free hand sections of drug under compound binocular microscope with built in analogue and computer images were captured. Measurements of cells and tissues were carried out using Micro Image Lite Image Analysis Software. Physicochemical constants, organic analysis, ultra-violet analysis and chromatographic studies were carried out from shade dried powder following prescribed methods18-22. HPTLC studies were carried out on alcohol extract for interpretation of data. An aluminium plate (5 x 10 cm) precoated with silica gel 60F254 was used as adsorbent. The plates were developed using chloroform: acetone: glacial acetic acid: water (7:2.4:0.6) for alcohol extract and n-butanol: glacial acetic acid: water (7:1:2) for aqueous extract in a Camag twin trough chamber to a distance of 8 cm each. The time of saturation for alcohol and aqueous extracts were 3 hrs and 2 hrs, respectively. The development time for alcohol extract was 25 min whereas for aqueous extract, it was 1h 45 min.

Results and discussion


Annual erect herbs. Leaves opposite or alternate, entire. Inflorescence of axillary, solitary or clustered spikes. Utricle rupturing irregularly. Seeds black (Figs. 1&2). It is a monotypic genus distributed from Tropical Africa to Asia23,24. Roots are slender, brownish, measuring 7-10 cm long. Several lateral filiform roots arise from the tap root. Taste is not characteristic, smell is pleasant. The roots are sold in the market in small bundles, which often consists of portions of aerial stem (Fig. 3).

Microscopical characters of the root

Transverse section of the root is circular in outline. It shows cork, secondary cortex and a wood region showing anomalous secondary growth. The cork is 4-7 layered and is made up of thick walled cells, measuring 20-50-37 x 8-15-12µ. Next to the cork lies a single layer of cork cambium. Secondary cortex occupies a large region, made up of several layers of parenchymatous cells measuring 23-46-70 x 24-29-38µ. Some cells are tanniferous, some others contain druses types of calcium oxalate crystals. Endodermis and pericycle are indistinct. Next to the secondary cortex, is situated 3-4 layers of secondary phloem consisting of thin walled parenchymatous cells, measuring 10-23-23 x 4-8-12µ. Secondary phloem cell measure 11-18-26 x 6-9-14µ and is demarcated by secondary xylem by 3-4 layers of vascular cambium. The vascular bundles are arranged in 3 rings exhibiting anomalous feature. It can be differentiated into an outer ring of vascular bundle showing secondary growth, traversed by interfascicular sclerenchyma and patches of intra-xylary parenchyma, cells measuring 5-6-8 x 2-5-7µ; middle ring of vascular bundle is conjoint, collateral and is traversed by parenchymatous conjunctive tissue, cells measure 16-27-45 x 9-11-15µ; the inner ring of vascular bundle exhibit secondary growth and is traversed in between by sclerenchymatous conjunctive tissue similar to that found in outer ring, cells measure 12-16-19 x 7-13-18µ; xylem cells measure 4-7-17 x 2-4-9µ. Druses type of calcium oxalate crystals are found in cells of conjunctive tissue, measure 28-29-30x24-25-26µ. The central portion is occupied by sclerenchymatous pith (Figs. 4-10).

Macerate

Macerate of the root exhibit the following elements: cork cells which are thick walled, rectangular or polyhedral, measure 65-67-70 x 38-48-57µ (Figs.11&12); parenchyma cells of different size and shape, globose or elliptical, measure 140-160-186 x 66-32-38µ (Fig. 13); xylem parenchyma with simple pits, measure 115-116-117 x 65-73-80µ (Fig. 14); fibres of different size and shape with broad lumen and narrow pointed ends, measure 220-250-300 x 11-15-17µ (Figs. 15); druses type of calcium oxalate crystals (Fig. 16); and vessels of different size and shape – narrow, cylindrical, barrel shaped or with pitted thickenings, measure 59-108-178 x 16-30-58µ (Fig. 17).

Powder study

Root powder is greenish, odourless, bitter, fibrous; when treated with chloral hydrate solution, stained in 1% safranin for 5 -10 minutes, mounted in 50% glycerine exhibits fragments of cortical parenchyma and druses type of calcium oxalate crystals. Histochemical tests

The root sections of *N. brachiata* when treated with phloroglucinol and dilute HCl gave red colour indicating the presence of lignin; with Millons’s reagent turned red indicating presence of proteins, with ferric chloride turned black showing presence of
tannins and with concentrate HCl effervescence was observed showing the presence of crystals; when treated with iodine no blue colour, with ruthenium red and Sudan III, no red colour, with Dragendorff’s reagent, no brown colour was observed indicating the absence of starch, mucilage, fixed oil and alkaloids, respectively.

**Physicochemical studies**
The percentage of moisture content was 8.5%, total ash 5.07%, acid insoluble ash 1.33%, water soluble ash 4.5%, alcohol soluble extractive 19.2% and water soluble extractive 28%; the colour, consistency and percentage of successive extractive values of extracts were: petroleum ether (60-80°C) (tata mimosa, sticky mass, 1.4%), benzene (olive green, sticky mass, 1.16%), chloroform (sand stone, sticky mass, 4.8%), acetone (brown, sticky mass, 3.24%), ethanol (brown, semi solid, 4.5%) and water (brown, semi solid, 4.72%).

**Preliminary organic analysis**
A known quantity of dried powder was extracted in a Soxhlet with petroleum ether (60-80°C), benzene, chloroform, acetone and ethanol (95%) and finally macerated with chloroform-water (2%) for 24 hrs successively and tested for different constituents. It revealed the presence of carbohydrates, glycosides, phenols, tannins, saponins, flavonoids, proteins and amino acids in ethanol and water extracts, whereas gums and mucilage were found in water extract.

**Chromatographic studies**
HPTLC profile of alcohol extract of roots revealed when scanned at 254 nm, 9 phytoconstituents at Rf 0.11, 0.14, 0.24, 0.28, 0.56, 0.78, 0.82, 0.86, 0.88 (Fig. 18), out of these, spots at Rf 0.56, 0.88 were pronounced, spots at Rf 0.24, 0.78, 0.86 were less pronounced, whereas spots at Rf 0.11, 0.14, 0.28, 0.82 were least pronounced; at 366 nm, 7 phytoconstituents at Rf 0.10, 0.13, 0.22, 0.26, 0.50, 0.80, 0.92 were observed (Fig. 19), out of these, spots at Rf 0.10, 0.13, 0.80, 0.92 were pronounced, spot at Rf 0.26 was less pronounced, whereas spots at Rf 0.22, 0.50 were least pronounced. All spots in alcohol extract quenched green fluorescence under 254 nm, showed blue fluorescence under 366 nm whereas under 425 nm all spots showed yellow fluorescence.

**Ultra-violet analysis**
Powdered drug under ultra-violet and ordinary light when treated with different reagents emitted various colour radiations (Table 1) which help in identifying the drug in powder form.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visible light</th>
<th>Ultra-violet light</th>
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<tr>
<td></td>
<td></td>
<td>Short wave (254 nm)</td>
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<tr>
<td>Powder as such</td>
<td>Pista Opaline green</td>
<td>No fluorescence</td>
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<tr>
<td>In methanol</td>
<td>Tata mimosa</td>
<td>Opaline green</td>
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<tr>
<td>In 1N methanolic</td>
<td>Tata mimosa</td>
<td>Mint green</td>
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<tr>
<td>NaOH</td>
<td>Mid buff</td>
<td>Water green</td>
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<tr>
<td>In ethanol (70%)</td>
<td>Tata mimosa</td>
<td>Mint green</td>
</tr>
<tr>
<td>In 1N ethanolic NaOH</td>
<td>Mid buff</td>
<td>Water green</td>
</tr>
<tr>
<td>In 1N NaOH</td>
<td>Mid buff</td>
<td>Water green</td>
</tr>
<tr>
<td>In 1N HCl</td>
<td>Mid buff</td>
<td>Water green</td>
</tr>
<tr>
<td>50% H2SO4</td>
<td>Pale cream</td>
<td>Water green</td>
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<tr>
<td>50% HNO3</td>
<td>Mid buff</td>
<td>Mint green</td>
</tr>
<tr>
<td>5% KOH</td>
<td>Mid buff</td>
<td>Water green</td>
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**Discussion**
*Pashanabedha* is considered as one of the controversial drugs in Ayurveda. Different botanical sources are used in various parts of the country as *Pashanabedha* and *Nothosaerva brachiata* is distinguished from other botanical sources by the following diagnostic characters. The plant is identified by the presence of flowers in sessile spikes; the root drug is characterised by the presence of anomalous secondary growth, secondary xylem arranged in three rings, presence of druses, intraxylary parenchyma and sclerenchymatous pith.
Conclusion

The pharmacognostical and phytochemical studies of the root of *N. brachiata* were carried out. Preliminary phytochemical analysis revealed presence of carbohydrates, glycosides, phenols, tannins, saponins, flavonoids, proteins and amino acids in ethanol and water extracts, whereas gums and mucilage were present in water extract. HPTLC studies help in identification of the drug and provide leads for establishing the biomarker compound.

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