Pharmacognostical studies on *Oroxylum indicum* (Linn.)Vent. stem bark

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*Oroxylum indicum* (Linn.)Vent. is one such plant which is extensively used in the Indian systems of medicine as an important ingredient of “Dashmula” and in the treatment of many diseases. Proper identification of the drug is desired for obtaining its complete therapeutic effects. It is with this aspect in view the present study dealing with pharmacognostical study and some other related studies of the stem bark of the species *O. indicum* (Linn.)Vent. were done. The stem bark is characterized by the well developed cork region; phellem region consisting number of stone cells and fibres, ceratenchyma is also present in inner phloem region, medullary rays are heterogenous and multiseriate, minute starch grains up to 5 µm in diam. are present in secondary phloem region. Powder of stem bark shown fragments of cork cells, stone cells and sclereids, fragments of fibres, parenchymatous cells, filled with black brown content, acicular crystals and starch grains were also found present. TLC of various extracts showed spots in different solvent system and phytochemically the plant was found to contain flavonoids, saponin, phenolic and tannins.

**Keywords:** Ceratenchyma, Flavonoids, *Oroxylum indicum*, Bignoniaceae, Dashmula.

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**Introduction**

The Bignoniaceae is a family of about 100 genera and 800 species, having mainly climbing plants. *Oroxylum* Vent. is a genus of trees found in Indo–Malaysian region and China. One species *Oroxylum indicum* (Linn.)Vent. (Shyonaka) is found in India1. It is a small to medium sized deciduous tree, up to 12 m in height, branched at top, bark light brown, soft and often with numerous corky lenticles. Leaves large, up to 1.5 m long, pinnate and bipinnate. Flowers numerous in large erect racemes, fleshy, dark lurid reddish purple outside, dull or pale pinkish yellow within. Fruit is large, flat sword shaped, 1 m long, seeds many, flat, with broad silvery wings2,3. It grows in the foothills of tropical India. Most parts of this tree are used as medicine. The Ayurvedic drug *O. indicum* or Shyonaka is mentioned in various classical texts of Ayurvedic System of Medicine, viz. Charak, Sushrut and other treaties like Bhava Prakash. The root, bark, stem and leaf are prescribed for snake-bite. The stem and wood for scorpion-sting (Sushruta)4. *O. indicum* is extensively used in Indian System of Medicine as an important ingredient of Dashmula which is a compound decoction of 10 roots. It is a medicine of repute in the treatment of remittent fever, otorrhoea, bronchitis, leucoderma, diarrhoea, inflammation and in acute rheumatism. Powdered stem bark or its infusion is diaphoretic4. Phytochemical studies on leaves shown presence of baicalein-6-glucuronide, baicalein-7-glucuronide, scutellarein, scutellarein-7-glucuronide5, alo-emodin6 and stem bark contains oroxylin-A, baicalein, scutellarein, ρ-coumaric acid7. Root bark contains oroxylin-A and ellagic acid8; heart wood contains prunetin and β-sitosterol9. Seeds contain oxorindin, baicalein-6-glucoside, tetuin, a glucoside and fixed oils10-12. Studies have shown that the oral administration of concentrated aqueous extract of the root bark provided symptomatic relief as well as absence of *Entamoeba histolytica* cysts in stool of patients13. The aqueous extract of the stem bark possesses anti-inflammatory activity14 and dichloromethane extract exhibited antifungal activity against dermatophytes and wood rot15. Literature survey revealed that the pharmacognostic details on root bark has been already worked out16,17.

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but the information on the stem bark is minimal. Therefore, the present study is focused on the structural features of stem bark of *O. indicum* and preliminary phytochemical screening, fluorescence analysis and TLC fingerprinting, etc. of the stem bark were done for the purpose of standardization of the crude drug.

**Materials and Methods**

**Plant material**

The stem bark was collected from Dibrugarh University campus, Assam and was identified by Dr. Vivek Kumar, Taxonomist, Pharmacognosy & Ethnopharmacology Division, National Botanical Research Institute, Lucknow. A herbarium No.DU/PSc/HRB-03/2005 of this plant was deposited in the Department of Pharmaceutical Sciences, Dibrugarh University, Assam. The bark material was dried in shade, powdered and stored in air-tight container for further study.

**Macroscopy**

Macroscopy i.e. evaluation of the drug for the confirmation of its identity, determination of quality and purity and detection of adulteration was done by visual appearance by the naked eyes. Other sensory characteristics like odour, taste, and feel of the drug to the touch were also observed.

**Microscopic features**

For microscopic studies the plant material was fixed in the solution of formaldehyde, glacial acetic acid and ethanol (90 ml of 70% ethanol + 5 ml of glacial acetic acid + 5 ml of formaldehyde). The section was cut transversely and stained with safranin and mounted following the usual plants microtechniques\(^ {19}\). Photomicrographs were taken with LeicaUSA (Leica2000ATCmodel) Digi 3 Photomicrographic unit at 10 × 10 × magnification. For powder drug study, the dried stem bark was grinded in pestle–mortar then passed through 60 mesh sieve. The sieved powder was mounted in chloral hydrate and Glycerin solution (1:1) and seen under microscope at 15 × 10 × magnification. Majority of the cell structures were studied and drawn directly from the microscope by the help of Camera lucida.

**Physicochemical study**

Physico-chemical constants like ash values, extractive values, crude fibre content and moisture content were determined as per methods given in IP 1996\(^ {19}\).

**Fluorescence analysis**

For fluorescence analysis of the powder sample it was treated with different chemical reagents to observe various colour reactions which may help to ascertain the purity of the drugs\(^ {20}\).

**Preliminary phytochemical screening**

Dried, coarsely powdered stem bark was extracted successively with petroleum ether (60-80°C), chloroform, acetone, methanol and water using Soxhlet apparatus. All the four extracts were screened for the presence of phytoconstituents\(^ {21}\).

**TLC finger print profile**

For TLC fingerprinting, methanolic extract was prepared by extracting 2g. of the powdered stem bark with methanol (3 × 10 ml) at room temperature, the extracts were combined, filtered and evaporated on water bath. 10 mg of this dried extract was re-dissolved in 1 ml of methanol and 15µl of it was implicated with Camag Linomet IV applicator on a precoated silica gel G 60 F\(_{254}\) TLC plate (Merck) of uniform thickness of 0.2 mm. The plate was developed in solvent system Chloroform: Methanol: Formic acid (8.8:0.5:0.2) and visualized under UV 254 nm, UV 366 nm and under visible light after spray with Vanillin-sulphuric acid reagent followed by heating at 110°C for 5-10 minutes in order to develop the chromatogram. The finger print profiles were documented with the help of CAMAG Switzerland photo documentation unit Reprostar 3\(^ {22}\).
and are arranged in radial rows, suberized, some cells become lignified and appear as stone cells. Cork followed by 2-4 layered cork cambium which is 19.0-28.5 µm thick. Phelloderm region vary narrow, 15-25 cells broad. The phelloderm is 285 µm-380 µm thick. Number of stone cells and fibres were embedded in this region. Stone cells are much smaller in size as compared to phloem region. Rest of the section is made of secondary phloem. The phloem is divided into two parts, outer and inner region. In outer phloem plenty of stone cells are present in large groups and some fibres. While in inner phloem the condition is vice versa that is more fibre groups are presents and stones cells are very less in number. Ceratenchyma is also observed in inner phloem region. Most of the parenchymatous cells are filled with brownish-black content. Medullary rays are multiseriate and heterogenous and the cells of medullary rays are much smaller compared to the other phloem parenchymatous cells. Acicular crystals are embedded in medullary rays cells and parenchymatous cells of phloem. Starch grains, very minutes up to 5 µm in diam. are present in the secondary phloem region (Plates 3, 3a-f).

Diagnostic character of powder
The powder was yellowish-green in colour, without any characteristic odour, astringent in taste. Under microscopic observation the powder shows fragments of cork cells in surface and tangential view. In surface view the cells are hexagonal and polygonal in shape. Stone cells and abundant sclereids, isolated or fairly in large groups, thick walled, pitted showing a considerable variation in size and shape. Few are elongated (fibre sclereids). Parenchymatous cells filled with black–brown content, patches of parenchymatous cells. Fragments of fibres in groups, isolated bast fibre, thick walled with uneven lumen with tapering ends, sepatate and non-septate fibre with varying shapes and sizes. The abundant acicular crystals were found scattered as such and embedded in medullary ray cells. Very minute starch grains were scattered into the powder. Parenchymatous cells were attached to the medullary rays (Plate 4).

Physicochemical properties
Various physico-chemical constants like loss on drying, total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble extractive and alcohol soluble extractive were determined and are given in Table 1.

Fluorescence analysis
Powdered drug was treated with different reagents and was examined under UV light (254 & 366 nm) emitted various colour radiations which help in identifying the drug in powder form are given in Table 2.

Preliminary phytochemical studies
Phytochemical test shows the presence of carbohydrates in alcohol and water extracts. Phenolic compounds and tannins in acetone, alcohol and water extracts. Flavonoids in chloroform, acetone, alcohol and water extracts whereas saponins were observed in water extracts and sterols in petroleum ether extracts only.

TLC finger print profile
Thin layer chromatography of the methanol extract was done using Chloroform: Methanol: Formic acid (8.8:0.5:0.2) as mobile phase and the Rf value were recorded (Plate 5 a-c and Table 3).
Plate 3—Microscopic features of stem bark of *Oroxylum indicum* (at 10×10× magnification): Plate 3a—T.S. of stem bark showing cork, cork cambium, phelloderm, stone cells, phloem, medullary rays; Plate 3b—T.S. of stem bark showing outer phloem region; Plate 3c—T.S. of stem bark showing fibers and phloem; Plate 3d—T.S. of stem bark showing ceratenchyma, fibers and medullary rays; Plate 3e—T.S. of stem bark showing ceratenchyma, fibers, stone cells and medullary rays.
Table 1—Quantitative standards for the stem bark of *Oroxylum indicum*

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Parameter</th>
<th>Value* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ash Values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Total ash</td>
<td>17.38</td>
</tr>
<tr>
<td></td>
<td>b) Acid-insoluble ash</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>c) Water-soluble ash</td>
<td>4.21</td>
</tr>
<tr>
<td></td>
<td>d) Sulphated ash</td>
<td>18.01</td>
</tr>
<tr>
<td>2</td>
<td>Extractive Values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Alcohol soluble</td>
<td>26.09</td>
</tr>
<tr>
<td></td>
<td>b) Water soluble</td>
<td>28.38</td>
</tr>
<tr>
<td>3</td>
<td>Loss on drying</td>
<td>18.91</td>
</tr>
<tr>
<td>4</td>
<td>Crude fibre content</td>
<td>25.5</td>
</tr>
</tbody>
</table>

*Average of three readings

Table 2—Fluorescence analysis of the powdered stem bark of *Oroxylum indicum*

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Treatment</th>
<th>Day Light</th>
<th>Short UV Light 254 nm</th>
<th>Long UV Light 365 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Powder as such</td>
<td>Yellow</td>
<td>Dark brown</td>
<td>Yellow</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1N NaOH (aqueous)</td>
<td>Yellowish–green</td>
<td>Dark brown</td>
<td>Fluorescent green</td>
</tr>
<tr>
<td>3</td>
<td>Powder + 1N NaOH (alcoholic)</td>
<td>Straw colour</td>
<td>Chocolate brown</td>
<td>Greenish–yellow</td>
</tr>
<tr>
<td>4</td>
<td>Powder + 1N HCl</td>
<td>Straw colour</td>
<td>Chocolate brown</td>
<td>Green</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 50% H₂SO₄</td>
<td>Yellow</td>
<td>Dark brown</td>
<td>Yellowish–green</td>
</tr>
<tr>
<td>6</td>
<td>Powder + Conc. HNO₃</td>
<td>Light Orange</td>
<td>Dark brown</td>
<td>Green</td>
</tr>
</tbody>
</table>
**Discussion**

Previous work done on this bark reported that the stem bark is rough, greyish yellow in outer surface and smooth golden yellow in inner surface with short and fibrous fracture, mucilaginous and sweetish taste. T.S. of stem bark showed cork is 12-20 layered, arranged in tangentially elongated rectangular cells with wavy walls. Phelloderm consists of parenchyma and large number of groups of stone cells. In phloem sieve plates are prominent, showing callus deposition. In inner phloem region medullary rays are bi to tetraseriate while in outer phloem region rays are multiseriate\(^\text{24}\). In our studies stem bark is curved, warty, buff or whitish black in colour, fracture medium and coarse, odourless, astringent in taste. The T.S of stem bark showed that the cork is 10-15 layered, arranged in radial rows, suberized. Cork is followed by 2-4 layered cork cambium. 15-25 cells broad phelloderm region consisting number of stone cells and fibre. The stone cells are much smaller in size as compared to phloem region; secondary phloem consist number of stone cells and fibres; presence of ceratenchyma in inner phloem region; heterogenous and multiseriate medullary rays, acicular crystals embedded in medullary rays cells and parenchymatous cells of phloem and minute starch grains up to 5 \(\mu\)m in diam. are present in secondary phloem region.

In our finding ceratenchyma was observed but no callus deposition was found in sieve plates, cells of multiserriate medullary rays consist starch grain and acicular calcium oxalate crystals. Parenchymatous cells were filled with black brown content. Under microscopic observation powder shows fragments of cork cells, stone cells and sclereids, fragments of fibres in groups, parenchymatous cells filled with black brown content, acicular crystals, medullary rays attached with parenchymal cells and starch grains. Medullary rays attached with parenchymal cells and starch grains. Various physicochemical parameters, viz. loss on drying, ash values, extractive values, TLC of different extracts, fluorescence analysis and phytochemical screening of various extracts were performed to substantiate standardization data on \(O.\) indicum.

**Conclusion**

The present study concludes that the complete pharmacognostic parameters of the stem bark of \(O.\) indicum would be useful in identifying the stem bark for safe applications in drug manufacturing.

**Acknowledgements**

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**References**