PHARMACOGNOSTIC STANDARDIZATION OF STEM BARK OF ERYTHRINA VARIEGATA L.

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_Erythrina variegata_ L. is an important plant employed in various indigenous systems of medicine against several diseases. Its stem barks are included in the single drug list of the Ayurvedic Pharmacopoeia of India and Ayurvedic Formulary of India. The current communication provides a detailed account of the pharmacognostic investigation carried out on _Erythrina variegata_ L. The study has immense value in the botanical identification and standardization of the drug in the crude form. Preliminary phytochemical analysis and HPTLC analysis was done along with loss on drying, extractive value, total ash values, acid insoluble ash which may serve as useful indices for the correct identification of the powdered drug. This study would be useful evidences for further investigations of this medicinal plant.

**Keywords:** _Erythrina variegata_ L., Pharmacognostic evaluation, HPTLC fingerprinting, Microscopic characterization, Physico-chemical tests, Phytochemical analysis

_Erythrina variegata_ L. (Family Fabaceae) is a highly valued plant, distributed widely in deciduous forests throughout India and in Andaman and Nicobar island, often planted on foot of Himalaya, Bihar, Orissa, Bengal, Konkan, North Karnataka, East coast of South India. In South India, it is used as a support for the betel and pepper vines. It is also grown in gardens as an ornamental plant (Anonymous 1999).

_Erythrina variegata_ L. is a moderate sized tree armed with black conical prickles arising from woody tubercles. Its bark is thin yellowish, leaves 3-foliolate; petioles unarmed; leaflets broadly ovate or rhomboid, acuminate, entire, lateral ones oblique. Flowers appearing before the leaves, in dense racemes, arranged in clusters at the end of leafless branches. Flowers are bright scarlet. Pods are 15-30 cm long, stalked, subcylindrical, torulose, glabrescent, 6-12 seeded (Verma _et al._ 1993).

In English it is known as 'The Indian Coral Tree', in Sanskrit 'Mandar', 'Parijata' and 'Paribhadra' and in Hindi 'Rakta madar', 'Dadap' and 'Mandara'. Traditionally the leaves of plant are considered to be laxative, diuretic, anthelmintic, galactagogue and emmenagogue; applied externally in venereal buboes, cough, ulcers and stem bark inflammations, conjunctivitis, earache, worm infestation, leprosy, skin diseases, cold, convulse, eye complaints, fever, menorrhoea, paralysis, pimples, rheumatism, skin diseases, snake bites. Bark decoction taken orally daily by women reduced infertility and induced capability of conception. Leaves are useful in urinary disorders, dysmenorrhoea, acid gastritis, toothache and helminthiasis. The leaf paste is used to treat fresh cuts and wounds (Narayana 1963, Ambasta 1986, Chopra _et al._ 1956, 1969, Kirtikar and Basu 1935, Jain 1991). _Erythrina variegata_ L. stem barks are used as single and compound formulations of Ayurveda such as Paribhadrawaleha, Nyagrodhadi Churna, Abhaya Lavana, and Narayana Taila. Since, there are no reports of systematic pharmacognostic studies on the stem bark of this plant, the present work was planned to study the detailed macroscopic, microscopic, powder microscopic, physicochemical constants, preliminary phytochemical screening and chromatographic characteristics of the bark of Paribhadra, which would serve as a standard reference for identification, authentication and for distinguishing the plant from its adulterants.

**MATERIALS AND METHODS**

**Collection of specimens**
The fresh plant stem bark was collected from the Bagdara Ghati, Chitrakoot forest, Satna...
(M.P.), India in the month of October. The plant was identified and authenticated. The voucher specimen (AD/AS/112/2013) maintained in the herbarium of Department of Pharmacognosy, Ayurveda Sadan (Research Laboratory), Deendayal Research Institute Chitrakoot for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for powder microscopy, physico-chemical, preliminary phyto-chemical and HPTLC studies.

**Macroscopy**

Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated.

**Microscopy**

Bark section were cut by free hand sectioning and numerous sections examined microscopically. Wherever necessary, the section was also stained with hydrochloric acid-phloroglucinol, Safranin and iodine-solution (Sharma et al. 2001, Meena et al. 2010, Sholapur and Patil 2013,). Photomicrographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX-211 with Digi-eye camera using Caliper plus version 4.2 software.

**Powder microscopy.**

The dried bark was subjected to powdered and completely passes through 355 μm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 μm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, was poured out without loss of material. Several slides were prepared as follows: mounted a small portion in glycerin, warmed a few mg with chloral hydrate solution, washed and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, about 1 g of powder warmed over water bath with 50% conc. Nitric acid till brown fumes appear, cooled and washed with water thoroughly and mounted a small portion in glycerin and seen under microscope at 40x X 10x magnification of the Trinocular Research Microscope (Anonymous 2001, Mukherjee 2002).

**Physico-chemical parameters**

Physico-chemical parameters such as moisture content (loss on drying at 105°C ), water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble ash value were calculated (Harborne 1984).

**Preliminary phytochemical screening**

Preliminary phytochemical screening were carried out on ethanolic and water extract for the presence/absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins and saponins (Ansari et al. 2013).

**HPTLC finger print profile**

For HPTLC, 5 gm of the powdered bark sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F254 (5x10 cm with 0.2 mm layer thickness Merck Germany) using Camag Linomat -5 sample applicator and a 100 μl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of Toluene: Ethyl acetate (7:3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with with Dragendorff’s reagent) at 254nm, 366nm and day light with Win cat software and
RESULTS AND DISCUSSION

Macroscopic characters
Mature dried stem bark slightly curved or flattened slightly curved pieces 0.5 to 1.2 cm in thickness, 2.5 to 4.5 cm in breadth and 4 to 9 cm in length were examined. Outer surface yellowish to yellowish grey colour, lenticels found at short intervals longitudinal lines on the outer surface. Inner surface is pale brown or yellowish colour and smooth. Whole bark differentiated into outer non-fibrous and inner fibrous zones. Odour characteristics and taste is slightly bitter or astringent (Fig.1 & 2).

Microscopic characters
The transverse section of the stem bark shows outer stratified and lignified cork cells consisting about 6-10 layered cells, alternating bands of narrow squarish to tangentially elongated thin walled, pinkish coloured and a few cells contain prismatic crystals of calcium oxalate. Secondary cortex consisting of polygonal to large tangentially elongated parenchymatous cells, some cells containing prismatic crystals of calcium oxalate, stone cells occur in singles or in groups. Phloem very broad zone, traversed with tangentially running groups of fibres of various shapes and shizes associated with idioblasts containing prismatic crystals of calcium oxalate alternating with ceratenchyma. Medullary rays are multisertate. Simple starch grains of various sizes traversed throughout parenchymatous cells of the section (Fig. 3 & 4).

Powder microscopic characters
The powder colour is creamish-yellow, odour characteristics and taste slightly bitter or astringent. Under microscope examined powder shows thick-walled lignified cork cells in surface view, prismatic crystals of calcium oxalate, simple and compound starch grains, tangentially longitudinally cut medullary rays associated with crystal fibres, crystal fibres numerous septate and each chamber contains a single prismatic crystals of calcium oxalate, radially-longitudinally cut medullary rays crossing the fibres, thick walled lignified fibres, groups of stone cells and sclereids in various shape and sizes (Fig. 5-12).

Physico-chemical analysis
The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in Table 1.

Preliminary phytochemical studies
Quantitative phytoconstituents were screened in the extracts taken in water, acetone, petroleum ether and ethyl alcohol. The screening exhibited presence of saponin, alkaloids, tannin and flavonoids.

HPTLC finger print profile
High performance thin layer chromatography (HPTLC) study of the ethanic extract two spots of the sample extracts applied in the TLC plate. Major spots Rf values with colour were recorded under 366nm, after derivatization at 366nm and UV light. Chromatogram profile and Rf values are given (Fig. 13 - 14 & Table 2).

The macroscopic, microscopic and powder microscopic diagnostic features have been established to identify *Erythrina variegata* L. stem bark. The pharmacognostic and physico-chemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify and isolate's important phyto-constituents. These findings could be helpful in identification and authentication.
Figures 1-12: 1 Plant; 2 Stem bark; 3 T. S. of stem bark with rhytidoma & cork cells; 4 T.S. of stem bark with cortex & phloem; 5 Cork cells in surface view; 6 Prismatic crystals of calcium oxalate; 7 Tangentially-longitudinally cut medullary rays associated with crystal fibres; 8 Starch grains; 9 Crystal fibres; 10 Radially-longitudinally cut medullary rays crossing the fibres 11 Fibres; 12 Stone cells & sclereids; 13 HPTLC finger print at 366nm; 14 HPTLC finger print at 366nm after derivatization; 15 HPTLC finger print in visible light after derivatization
Table 1: Physicochemical parameters of Paribhadra stem bark

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Values</th>
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<tbody>
<tr>
<td>Loss on drying</td>
<td>6%</td>
</tr>
<tr>
<td>Ethanol-soluble extractive</td>
<td>8%</td>
</tr>
<tr>
<td>Water-soluble extractive</td>
<td>17%</td>
</tr>
<tr>
<td>Total ash</td>
<td>11.5%</td>
</tr>
<tr>
<td>Acid-insoluble ash</td>
<td>0.1%</td>
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Table 2: Rf Values in test solution of Paribhadra stem bark

<table>
<thead>
<tr>
<th>Rf values</th>
<th>366nm (before derivatization)</th>
<th>366nm (after derivatization)</th>
<th>UV light (after derivatization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf1</td>
<td>0.28 (blue)</td>
<td>0.10 (yellow)</td>
<td>0.10 (black)</td>
</tr>
<tr>
<td>Rf2</td>
<td>0.54 (fluorescence)</td>
<td>0.60 (orange)</td>
<td>0.28 (light yellow)</td>
</tr>
<tr>
<td>Rf3</td>
<td>0.68 (pink)</td>
<td>0.72 (orange)</td>
<td>0.60 (black)</td>
</tr>
<tr>
<td>Rf4</td>
<td>0.72 (blue)</td>
<td>0.90 (orange)</td>
<td>0.72 (black)</td>
</tr>
<tr>
<td>Rf5</td>
<td>0.80 (red)</td>
<td>0.94 (orange)</td>
<td>0.94 (black)</td>
</tr>
<tr>
<td>Rf6</td>
<td>084 (red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rf7</td>
<td>0.90 (red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rf8</td>
<td>0.94 (red)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

CONCLUSION

*Erythrina variegata* L. has numerous uses in traditional medicine to treat several ailments like conjunctivitis, earache, worm infestation, leprosy, skin diseases, urinary disorders, dysmenorrhoea, acid gastritis, toothache, helminthiasis, cuts and wounds. Due to its wide therapeutic importance it is worthwhile to standardize it for use as drug. The present study reveals standardization profile of drug *Erythrina variegata* L., which would be of immense value in botanical identification and authentication of plant drug and may help us in preventing its adulteration.

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