Authentication and quality evaluation of an important Ayurvedic drug - Ashoka bark

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This paper presents authentication and quality evaluation of stem bark of Saraca asoca (Roxb.) Wilde, officially considered as ‘Ashoka’, in comparison with stem barks of S. declinata Linn and Polyalthia longifolia, which are also known as ‘Ashoka’. S. asoca is an important indigenous drug for treatment of various female disorders. HPTLC profile shows characteristic band under UV 366 nm as follows: S. asoca, Rf 0.53; S. declinata, Rf 0.18; and P. longifolia, Rf 0.13 0.21, 0.27, 0.38, 0.49. Presence of stigmasterol in S. asoca and S. declinata and its β-sitosterol in stem bark of P. longifolia was observed.

Keywords: Ashoka, Pharmacognosy, Polyalthia longifolia, Saraca asoca, Saraca declinata, TLC

Introduction
Unprecedented demand for raw materials of herbal drugs, which are mostly collected from wild sources, has led to adulteration and substitution of genuine drug. Stem bark of Saraca asoca De Wilde (Fabaceae) is official drug of ‘Ashoka’. Another two species [Saraca declinata Linn and Polyalthia longifolia Benth (Annonaceae)] are also known as ‘Ashoka’ in India. The drug is reported as astringent, refrigerant, alexiteric, anthelminthic, demulcent and emollient and also employed in treatment of dyspepsia, enlargement of abdomen, colic, piles, ulcers, and is considered as an important indigenous drug for the treatment of various female diseases especially menorrhagia. Pharmacognostical studies of S. asoca and P. longifolia are on record. During survey of major Indian crude herbal drug market, it was found that almost all the samples were mixture of two or three species, may be because S. asoca is now considered as an endangered species, whereas P. longifolia is abundantly available.

This study presents macro-microscopic description, physico-chemical parameters and high performance thin layer chromatographic (HPTLC) profiles of S. asoca, S. declinata and P. longifolia to lay down standard parameters for authentication and quality evaluation of commercial samples.

Materials and Methods
Stem bark of S. asoca, S. declinata and P. longifolia were collected from plants growing in premises of NBRI, Lucknow, India. For microscopic studies, transverse sections (TS) and longitudinal sections (LS) were prepared and stained. Samples were dried at 50°C in a hot air oven, stored at 25°C in air tight container. Stem bark of all species was powdered and sieved through 85 mesh. A small quantity of powdered material was washed with water to remove sugar and then cleared by heating gently with saturated chloral hydrate solution, cooled and mounted in glycerin for microscopic observation.

Physicochemical and HPTLC Studies
Physicochemical values (Fig. 1) were calculated as per Ayurvedic pharmacopoeial methods; sugar, starch and total tannins according to AOAC method. Powdered material (5 g) was extracted with methanol on a water bath for 25 min, consecutively three times, and extract was concentrated and dried. Known quantity of extract was dissolved in methanol for HPTLC. 1.0 mg of β-sitosterol and stigmasterol (Sigma) as reference markers were also dissolved separately in 1.0 ml methanol.

Chromatographic Conditions
Known quantity of methanolic extracts were applied on to Higlachrosep nano silica UV 254 HPTLC plates (10 cm x 10 cm) with 0.2 mm nano silica with fluorescent indicator (S.D. Fine-Chem. Ltd. India) using CAMAG Linomat Applicator V, positioned 15 mm from side and
15 mm from bottom of plate general HPTLC profiles. Plate was eluted to a distance of 8.0 cm at room temperature (21°C) in a solvent system – toluene: ethyl acetate (9.3: 0.7) in previously saturated twin trough chamber (CAMAG). Photographs were taken under ultra violet (UV) light 366 nm using CAMAG Reprostar 3. Plate was derivatized by spraying with anisaldehyde sulphuric acid reagent and after heating plate at 110°C.
for 10 min documented under visible light. Plate was scanned at wavelength 560 nm using CAMAG TLC Scanner 3 with software CATS4.3.

Known quantity of methanolic extracts was taken as test solutions along with chemical markers (stigmasterol and β-sitosterol) and similar application conditions were applied. Plate was eluted to a distance of 8.0 cm at room temperature (21°C) in a solvent system – toluene: ethyl acetate (8: 2) in previously saturated twin trough chamber (CAMAG). Plate was derivatized by spraying with anisaldehyde sulphuric acid reagent and after heating the plate at 110°C for 10 min documented under visible light and scanned at wavelength 600 nm using CAMAG TLC Scanner 3 with software CATS4.3.

**Results and Discussion**

Three plant species attributed to ‘Ashoka’ can be differentiated on the basis of macro-microscopic characters (Table 1, Figs 1-4). Stem bark of *S. asoca* is channeled and that of *S. declinata* and *P. longifolia* are
curved. Stone cells are present in cork region of *P. longifolia* and *S. declinata* and phellogen only in *P. longifolia*. Phelloderm differentiated in outer and inner zones with tangential clusters of 6-7 rows of stone cells interrupted by crushed suberized cells in outer zone and scattered patches of stone cells in inner zone of *S. declinata* only and throughout phelloderm region in *P. longifolia* while in *S. asoca* it is represented by 2-3 continuous tangential bands of stones cells. Similarly, phloem region shows characteristic distribution pattern of fibres, stone cells, oil cells and mucilage canals; fibres in group of 3-24 in *S. asoca*, solitary or in groups

Fig. 3—Microscopy of *Saraca declinata* stem bark: A, cork cells in surface view; B, radially cut medullary rays; C, tangentially cut medullary rays; D, cork cells in transverse view; E, stone cells; F, fibres; G, fibre sclerieds [ck, cork; f, fibre; mr, medullary rays; pd, phelloderm; ph, phloem; rh, rhytidoma; stc, stone cells]
of 3-6 in *S. declinata* and in broad concentric bands alternating with parenchymatous bands and interrupted by 2-12 cells broad radiating medullary rays in *P. longifolia*. Mucilage canals and oil cells are present only in the latter. Broadness of medullary rays also varies: uni-biseriate in *S. asoca*; uni-to triseriate in *S. declinata*; and multiseriate in *P. longifolia*.

Under physico-chemical values (Fig. 5), total tannins are found almost three times higher in *S. asoca and S. declinata*. (6.55 and 6.75 respectively) as compare to
P. longifolia (1.9), whereas sugar and starch content was higher in P. longifolia.

Likewise, HPTLC fingerprint profiles (Table 1, Fig. 6) also showed similar and differentiating bands. Three common bands at Rf 0.31, 0.69 and 0.84 under UV 366 and five bands at Rf 0.08, 0.12, 0.16, 0.22, 0.45 after derivatization were present in all three species. S. asoca and S. declinata showed almost similar general profiles except presence of one minor spot in each at Rf 0.53 and Rf 0.18 was absent in S. declinata and S. asoca respectively. HPTLC profile of P. longifolia showed some characteristic bands at Rf 0.13 (orange), 0.21, 0.27 (both

**Fig. 5**—Physico-chemical parameters of ‘Ashoka’ [SA, *Saraca asoca*; SD, *S. declinata*; PL, *Polyalthia longifolia*]

**Fig. 6**—HPTLC fingerprint profiles of methanolic extract of all three species attributed to ‘Ashoka’: 1, Under UV 366 nm; 2, under visible light after derivatization; 3, Densitometric scanning profile at 560 nm
fluorescent blue), 0.38, 0.49 (both blue). Comparative HPTLC profile with marker components showed presence of stigmasterol in *S. asoca* and *S. declinata* and β-sitosterol in *P. longifolia* (Fig. 7).

**Conclusions**

All three species used or sold as ‘Ashoka’ can easily be differentiated based on morphological as well as chromatographic profile.
Acknowledgements
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References
1. The Ayurvedic Pharmacopoeia of India, part I - vol I (Govt of India, Ministry of Health and Family Welfare, Department of AYUSH, New Delhi) 1986.

Table 1—Comparative distinguishing characters of possible adulterants/substitutes of Asoka

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<tr>
<td>1</td>
<td>Macroscopy</td>
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<tr>
<td></td>
<td>Size</td>
<td>0.5 to 0.8 cm thick</td>
<td>0.5 to 1.2 cm thick</td>
<td>1.5 to 3.0 cm thick</td>
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<td></td>
<td>Outer</td>
<td>Blackish brown, rough due to warty protuberances and transversely arranged lenticels</td>
<td>Dark brown, rough due to presence of lenticels and small warts but the young bark greyish brown finely granulated along with fine uneven striations</td>
<td>Brown, rough, ridged, ridges and furrows faint, lenticels vertical</td>
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<tr>
<td></td>
<td>Inner surface</td>
<td>Reddish brown</td>
<td>Longitudinally ridged and blackish brown</td>
<td>Yellowish brown</td>
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<tr>
<td></td>
<td>Rhytidoma fracture</td>
<td>Not present</td>
<td>Present</td>
<td>Present</td>
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<tr>
<td>2</td>
<td>Powder</td>
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<td>Reddish brown</td>
<td>Reddish brown</td>
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<td></td>
<td>Colour</td>
<td>Astringent</td>
<td>No</td>
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<td></td>
<td>Taste</td>
<td></td>
<td>No</td>
<td>Pleasant sweet</td>
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<td></td>
<td>Odour</td>
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<td>3</td>
<td>HPTLC of methanolic extract</td>
<td>Spots at Rf 0.08, 0.14 (blue), 0.31 (dark blue), 0.53 (faint blue), 0.69, 0.84 (blue) under UV 366 nm and at Rf 0.08, 0.12, 0.16, 0.22, 0.45, 0.73, 0.86 (all blue) after derivatization</td>
<td>Spots at Rf 0.14 (blue), 0.18, 0.31 (fluorescent blue), 0.69 (faint blue), 0.84 (blue) under UV 366 nm and at Rf 0.08, 0.12 0.16, 0.22, 0.45, 0.73, 0.81, 0.86 (all blue) after derivatization</td>
<td>Spots at Rf 0.13 (orange), 0.21, 0.27 (both fluorescent blue), 0.31 (ink blue), 0.38 (faint blue), 0.49 (blue), 0.69 and 0.84 (both blue) under UV 366 nm and at Rf 0.08, 0.12 0.16 (all blue), 0.22 (purple), 0.31, 0.39 (both purple), 0.45 (pinkish blue), 0.69 (purple), 0.89 (blue) after derivatization</td>
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