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# 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* Benth, which are also known as 'Ashoka'. *S. asoka* is an important indigenous drugs 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'[1]. Another two species [*Saraca declinata* Linn and *Polyalthia longifolia* Benth (*Annonaceae*)] are also known as 'Ashoka' in India[2,3]. The drug is reported as astringent, refrigerant, alexiteric, anthelmintic, 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[4,5]. Pharmacognostical studies of *S. asoca*[6-8] and *P. longifolia*[8] 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.

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## 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[9]. 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[1]; sugar, starch[10] and total tannins according to AOAC method[11]. 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 x10 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

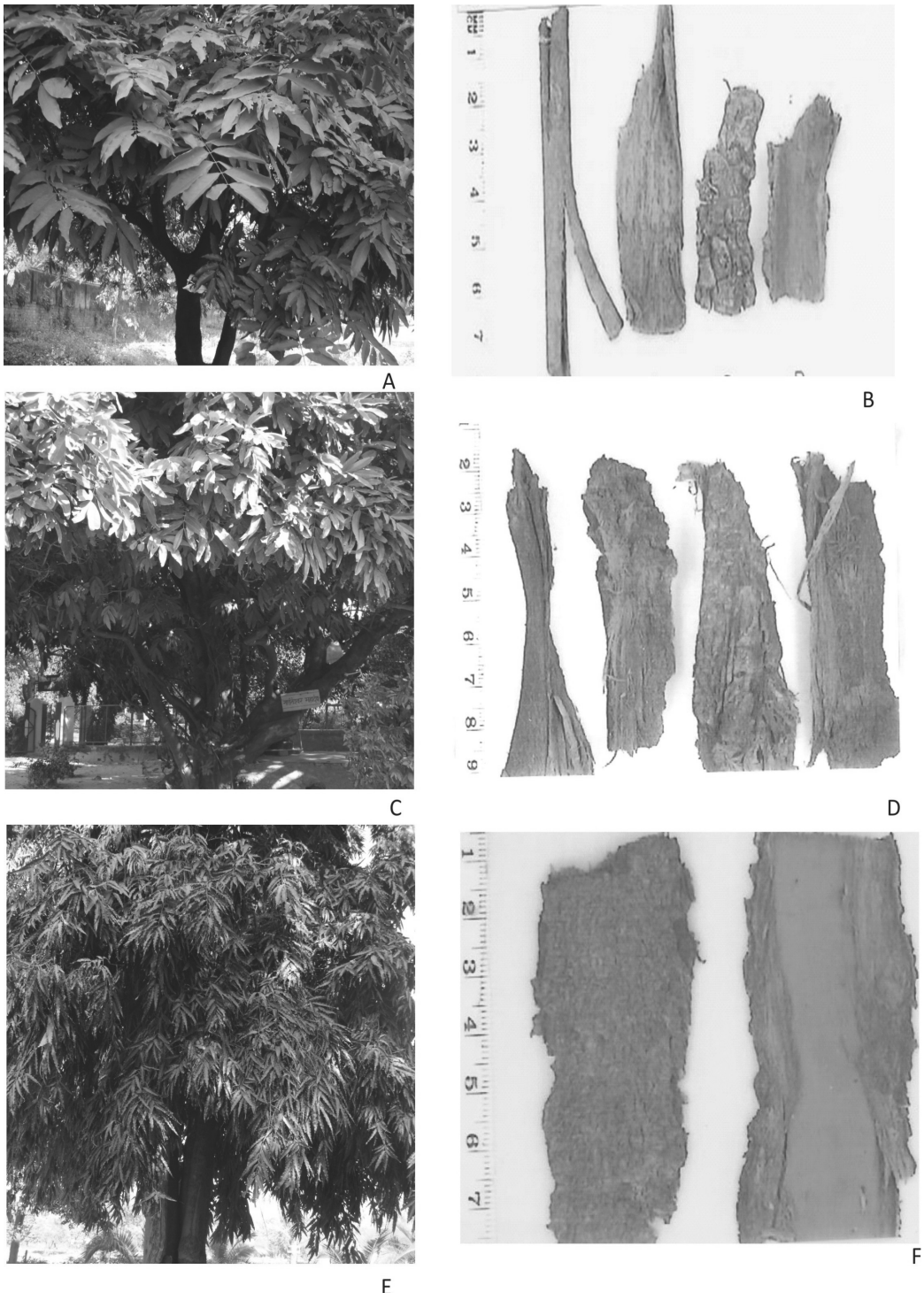



Fig. 1—Macroscopy of 'Ashoka' [A, *Saraca asoca* tree; B, *S. asoca* stem bark; C, *S. declinata* tree; D, *S. declinata* stem bark; E, *Polyalthia longifolia* tree; F, *P. longifolia* stem bark

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

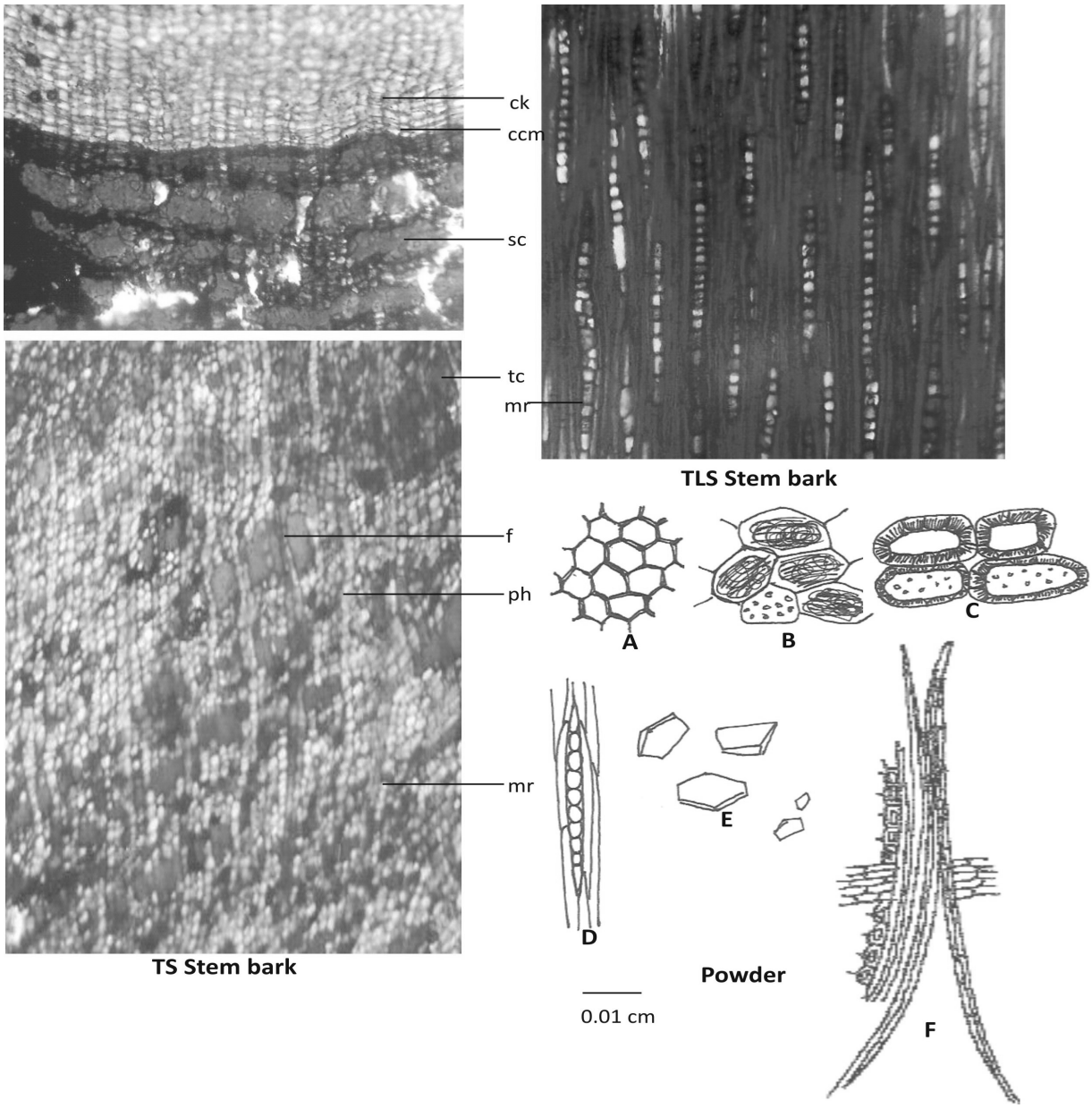



Fig. 2—Microscopy of *Saraca asoca* stem bark: A, cork cells in surface view; B, parenchymatous cells filled with reddish brown content; C, stone cells; D, tangentially cut medullary ray; E, prismatic crystals of calcium oxalate, F, radially cut medullary rays along with crystalloid fibres [ccm, cork cambium; ck, cork; f, fibre; mr, medullary rays; ph, phloem; sc, stone cells; tc, tanniniferous cells]

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

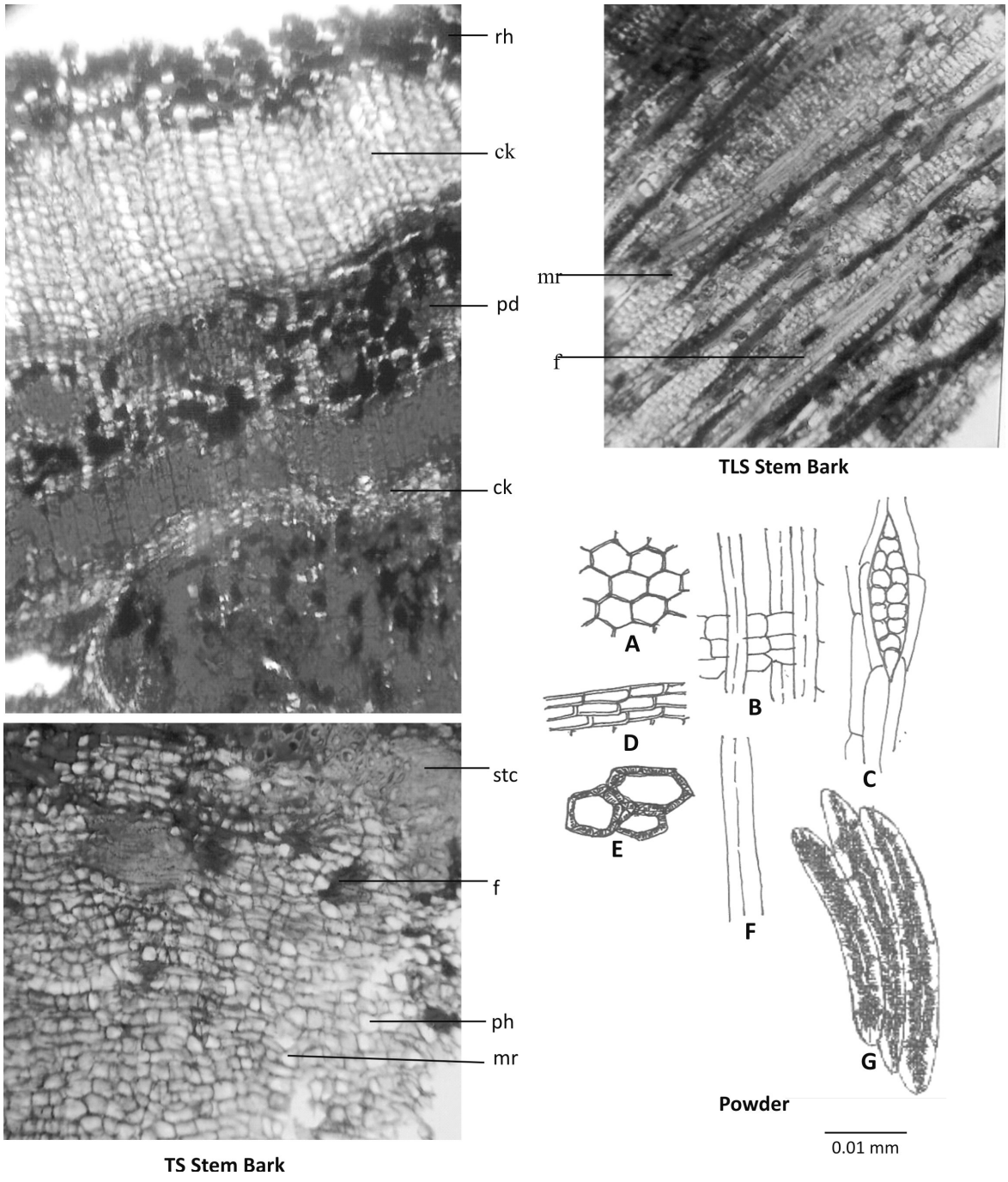



Fig. 3—Microscopy of *Saraca declinata* stem bark: A, cork cells in surface view; B, radially cut medullary rays; C, tangentially cut medullary rays; D, cork cells in transverse view; E, stone cells; F, fibres; G, fibre sclerieds [ck, cork; f, fibre; mr, medullary rays; pd, phelloderm; ph, phloem; rh, rhytidoma; stc, stone cells]

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

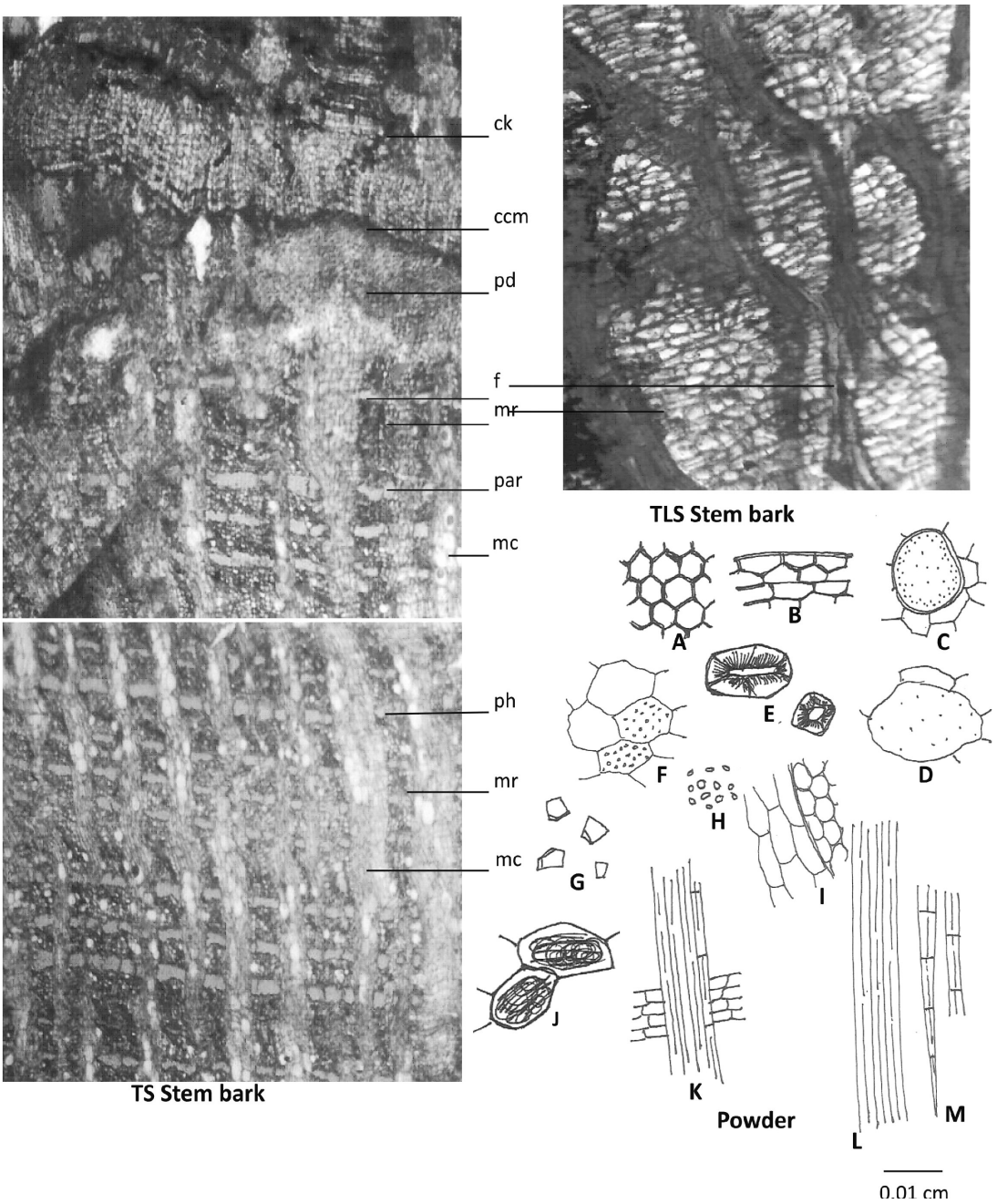



Fig. 4—Microscopy of *Polyalthea longifolia* stem bark: A, cork cells in surface view; B, cork cells in transverse view; C, schizogenous mucilage canal; D, lysigenous mucilage cavity; E, stone cells; F, parenchyma with starch grains; G, prismatic crystals; H, starch grain; I, tangentially cut medullary ray; J, tannin containing cells; K, radially cut medullary ray; L, fibre; M, septate fibre [ccm, cork cambium; ck, cork; f, fibre; mc, mucilage canal, mr, medullary rays; par, parenchyma; pd, phelloderm]

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

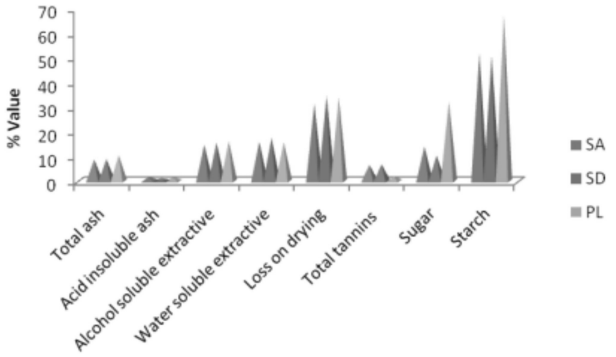



Fig. 5—Physico-chemical parameters of 'Ashoka' [SA, *Saraca asoca;* SD, *S. declinata*; PL, *Polyalthia longifolia*]

*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

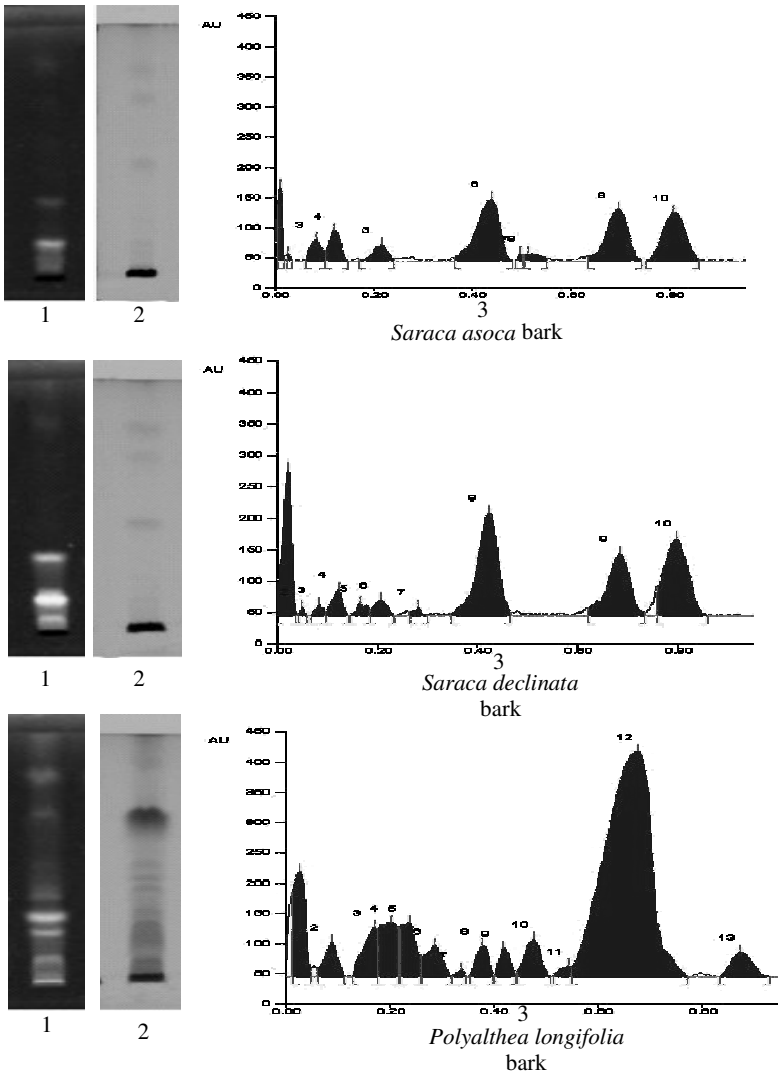
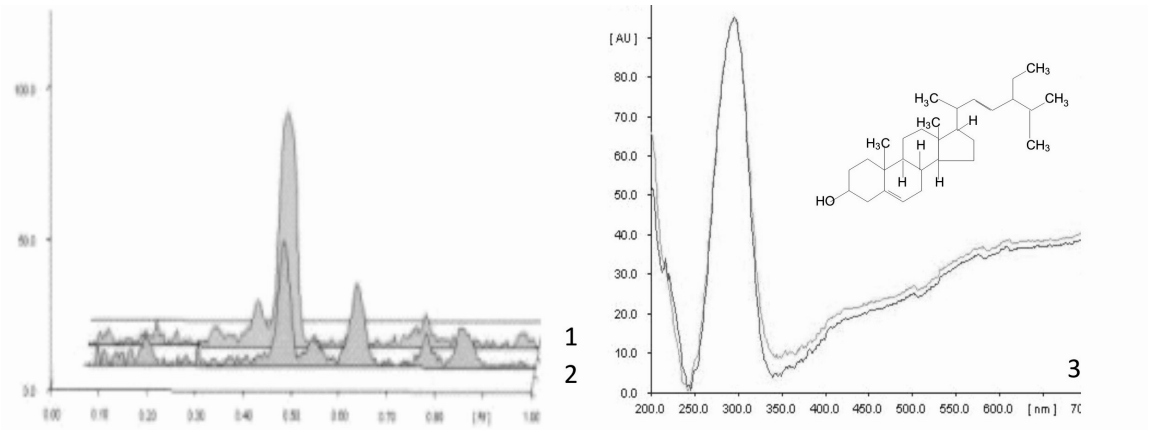
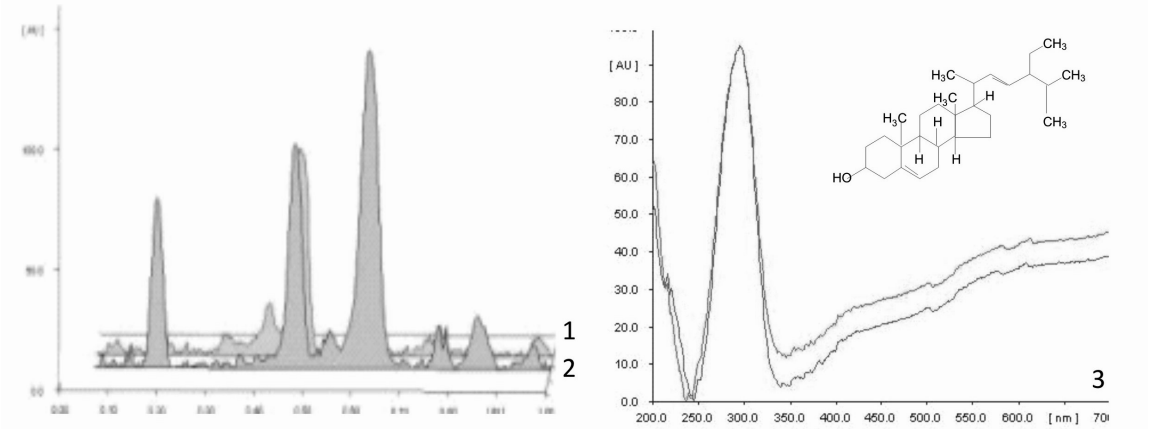


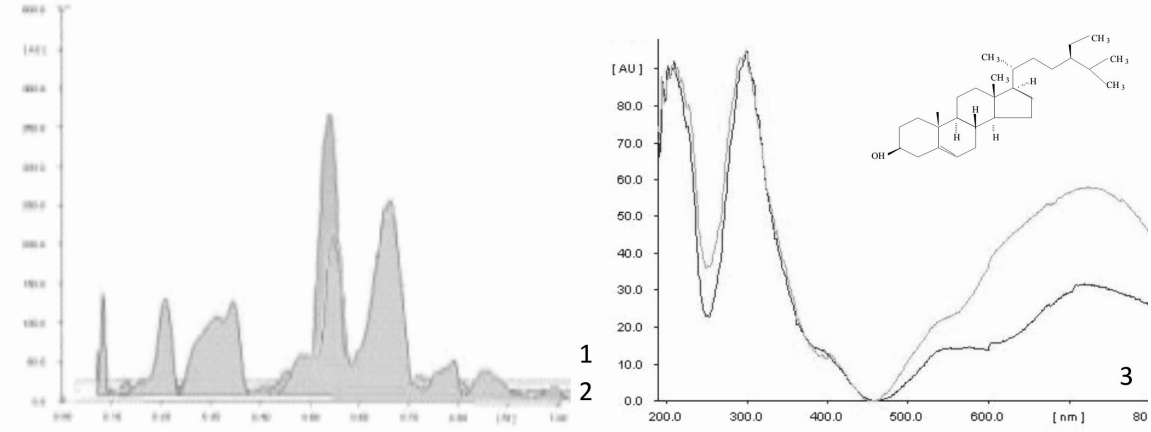

Fig. 6—HPTLC fingerprint profiles of methanolic extract of all three species attributed to 'Ashoka': 1, Under UV 366 nm; 2, under visible light after derivatization; 3, Densitometeric scanning profile at 560 nm




Methanol extract of *Saraca asoca* bark showing presence of Stigmasterol




Methanol extract of *Saraca declinata* bark showing presence of Stigmasterol




Methanol extract of *Polyalthea longifolia* bark showing presence of β-Sitosterol

Fig. 7—Densitometeric scanning of HPTLC plate showing presence of marker compound: 1, marker compound; 2, extract; 3, overlapping spectra

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 $\beta$-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.

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

| S No. | Characters | *Saraca asoca* Linn. | *Saraca declinata* Miq. | *Polyalthia longifolia* Benth. |
|---|---|---|---|---|
| 1 | Macroscopy | | | |
| | Size | 0.5 to 0.8 cm thick | 0.5 to 1.2 cm thick | 1.5 to 3.0 cm thick |
| | Outer surface | Blackish brown, rough due to warty protuberances and transversely arranged lenticels | Dark brown, rough due to presence of lenticels and small warts but the young bark greyish brown finely granulated along with fine uneven striations | Brown, rough, ridged, ridges and furrows faint, lenticels vertical |
| | Inner surface | Reddish brown | Longitudinally ridged and blackish brown | Yellowish brown |
| | *Rhytidoma* | Not present | Present | Present |
| | *fracture* | Medium hard and fibrous | Medium hard and fibrous | Hard and strongly fibrous |
| 2 | Powder | | | |
| | Colour | Brownish red | Reddish brown | Reddish brown |
| | Taste | Astringent | No | Mucilaginous |
| | Odour | No | No | Pleasant sweet |
| 3 | HPTLC of methanolic extract | 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 | 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 | 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 |

## 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.

2 Mehrotra S, Rawat A K S, Khatoon S & Pushpangadan P, Adulteration and substitution in herbal drugs – A review, in *Recent Progress in Medicinal Plant,* **vol 8,** edited by D K Majumdar, J N Govil & V K Singh (SCITECH Publications, USA) 2001 177-191.

3 Khatoon S, Rawat A K S & Mehrotra S, Commercial crude herbal drugs and their quality, *Indian J Develop Res Soc Action*, **2** (2006) 247–258.

4 Kirtikar K R & Basu B D, *Indian Medicinal Plants.* **2nd edn, vol 9,** edited by E Blatter, J Caius & K S Mhaskar (Bishen Singh Mahindra Pal Singh, Dehra Dun) 2001, 3068-3069.

5 Chopra R N, Nayar S L & Chopra I C, *Glossary of Indian medicinal Plants* (NISCAIR, New Delhi, India) 1956.

*6* *Quality Standards of Indian Medicinal Plant* , **vol 2,** edited by A K Gupta, N Tandon & M Sharma (ICMR, New Delhi, India) 2005, 201-208.

7 Iyer K S & Kolammal M, *Pharmacognosy of Ayurvedic Drugs,* **ser 1** (Deptt Pharmacognosy, University of Kerala, Trivendram. India) 1960, 1-7.

8 Prasad S, Natrajan P N & Wahi S P, Pharmacognostic studies on Ashoka bark: Barks of *Saraca indica* Linn and *Polyalthia longifolia* Benth Hook, *J Sci Ind Res*, **20** (1961) 125-132.

9 Johansen D A, *Plant Micro Technique 182* (Mc Grew Hill Book Co Inc., New York, London) 1940, 102-104

10 Mont Gomery R, Determination of glycogen. *Arch Biochem Biophys*, **67** (1957) 378-386.

11 *Official Methods of Analysis of the Association of Official Analytical Chemists* (AOAC) **14th edn** (Arlington, Virginia, USA) 1984, 187.