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Manosi Das
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Avijit Banerji
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Amit Kumar Dixit
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Mruthyumjaya Meda Rao
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Correspondence
Avijit Banerji
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Phytochemical analysis and HPTLC finger-printing profile of roots of *Alstonia scholaris* (L.) R.Br (Saptaparṇa)

Manosi Das, Avijit Banerji, Amit Kumar Dixit and Mruthyumjaya Meda Rao

Abstract
Plants are rich sources of bioactive compounds, and have been used in traditional medicine systems for the past few millennia; even now plant-based drugs continue to provide new remedies to mankind. The medicinal plant *Alstonia scholaris* (L.) R. Br. (Saptaparṇa, Chhatim) is widely used in the Indian school of traditional medicine of Ayurveda. The present study reports phytochemical studies and HPTLC finger-printing profile of *Alstonia scholaris* roots. These results provide referential parameters for identification of this drug.

Keywords: *Alstonia scholaris* root, phytochemical analysis, extractive values, HPTLC

Introduction
In recent years there has been a global resurgence of interest in Ayurveda, the traditional Indian system of medicine, which depends on the use of plant based materials and their formulations. Medicinal plants contain an array of chemical compounds which play a pivotal role in health care. Hence, it is of vital importance to analyse, isolate and characterise the chemical constituents present in any drug or drug formulation. Professor Asima Chatterjee's research group at the Chemistry Department, University of Calcutta and those of her associates have done pioneering work on different families of Indian medicinal plants. Fruitful collaborative work was initiated with the unit of Central Council for Research in Ayurveda and Siddha based in Calcutta, the predecessor of the present Central Ayurveda Research Institute for Drug Development, on various aspects of the chemistry, pharmacognosy, pharmacology and biomedical uses of Indian medicinal plants. {see ref. [1] for selected references in this regard}.

In continuation of the earlier studies at the University of Calcutta, a thorough re-investigation has been initiated of different parts of *Alstonia scholaris* (L.) R. Br. (Family Apocynaceae; tribe - Plumeriae; Subtribe - Alstoniinae), a reputed medicinal plant in the Indian traditional system of Ayurveda. *Alstonia scholaris* [2-5], a tall evergreen tree grows in sub-Himalayan tracts in north India and in the western peninsula; it also occurs in several other countries in Eastern Asia. It is referred to as Saptaparṇa (sapta: seven, parṇa: leaves) in Sanskrit, on account of the arrangement of its leaves which occur in whorls of five to ten (not necessarily seven, which is the most usual arrangement). It is also referred to as devil tree or dita bark in English, and Chhatim in Bengali [2-5]. The tree is fairly widespread in the city of Kolkata [Fig. 1], being planted in parks and street verges. In Kolkata, the tree flowers in late October to early March, and fruiting may initiate as early as January, though more usually in February-April. The flowers are greenish-white, fragrant, borne in compact, many-flowered umbellate cymes [2]. Different parts of the plant are used as drugs [2]: the bark is reported to be alterative, antimalarial, antidysentric, anthelmentic and astringent; decoction of the leaves are used in beriberi. The stem-bark is used in the antimalarial drug formulation Ayush-64, developed by Professor A. Chatterjee's group working in collaboration with the CCRAS Institute. The roots show antibacterial property [6]. A number of publications list the other uses of the roots of *Alstonia scholaris* [7]: Anthelmintic [7a]; treatment of – leprosy [7b], enlarged liver with pain [7c]; antimalarial activity [7d]. A large number of compounds, particularly indole alkaloids have been isolated from different parts of the plant [2-5, 8-10]. Pioneering work on the alkaloidal constituents of *Alstonia scholaris* and *Alstonia macrophylla* have been carried out by Professor A. Chatterjee's group at the University of Calcutta [9]. Less phytochemical work has been done on the roots of *Alstonia scholaris* [10], in contrast to the leaves and stem bark of this plant [8, 9]; this enthused us to take up further work on the roots.

The present communication reports preliminary phytochemical analysis and the HPTLC finger-printing profile of the roots of *Alstonia scholaris* (L.) R. Br. (Saptaparṇa). Details of the pharmacognosy of the stem-bark are recorded in the Ayurvedic Pharmacopoeia of India [5].

As no pharmacognostical studies on the root had been reported, we recently investigated this aspect, and reported the results to provide referential information for identification parameters of this herbal drug [11].

Fig 1a: *Alstonia scholaris* (L.) R. Br. tree at flowering season in Kolkata

Materials and Methods

Plant Material collection and authentication

Alstonia scholaris (L.) R.Br. roots were collected from Sector 5, Salt Lake City, Kolkata in January 2018 (Figs. 1, 2). The tree was identified by Professor N. D. Paria, Indira Gandhi National Open University, and formerly of Botany Department, University of Calcutta. A voucher sample of the roots, stem bark, leaves and flowers of *Alstonia scholaris* have been deposited (Voucher number - CRD/Chem/AS/R) at CARIDD, Kolkata.

Fig 2: (a) Roots of *Alstonia scholaris*; (b) Coarsely powdered roots of *Alstonia scholaris*

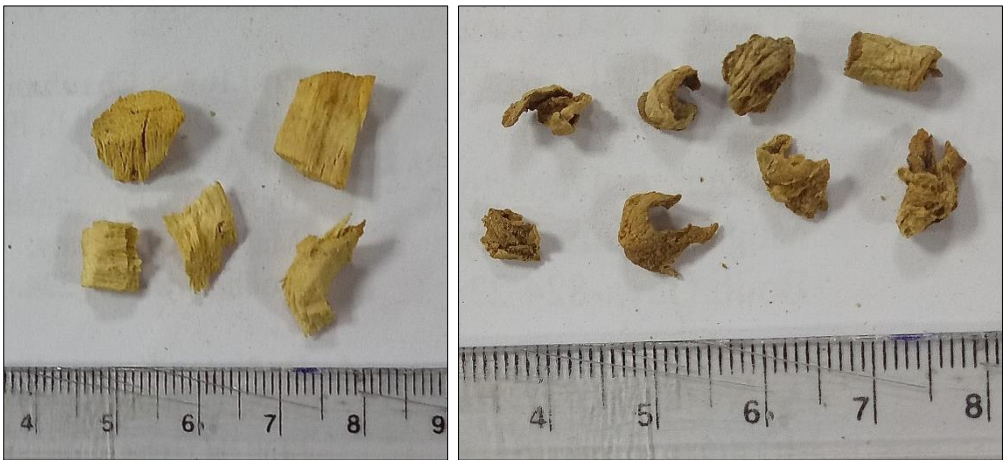


Fig 2: (c) Root Wood Core of *Alstonia scholaris*; (d) Root Bark of *Alstonia scholaris*

Plant sample processing

The roots were dried at ambient temperature in the shade for 7 days – approximately 170g of the dried roots were obtained. From a small portion of the roots (Figs. 2a-d), the root bark was carefully stripped and isolated from the root wood core. These were ground separately with a grinder (National SM 2000) to obtain 60 mesh size root powder. Rest of the roots were chopped to small pieces and ground to a coarse powder – this was used for determining extractive values, preliminary phytochemical studies and HPTLC analysis. HPTLC analysis were separately carried out for powdered samples of the total roots (Rt), root wood core (Rw) and root bark (Rb).

Results

Physicochemical Parameters: Water soluble extractive, 90% alcohol soluble extractive.

These were initially determined in accordance with standard protocols as mentioned in Ayurvedic Pharmacopoeia of

India/WHO protocols [12, 13] – these results have been presented in our recent communication [11] along with other physicochemical parameters. An alternative innovative procedure utilising ultra-sonication has been developed to determine these extractive values.

The comparative results are given in Table-1. The ultra-sonication procedure appears slightly superior to the conventional API protocol for the water extractive, but worse for the 90% alcoholic extract.

Table 1: Physicochemical Evaluation – Extractive values of *Alstonia scholaris* roots

S. No.	Parameters	Result (in w/w %)	
		API/WHO protocol	Ultra-sonication procedure
1.	Water soluble extractive	9.90	10.40
2.	90% Alcohol soluble extractive	9.40	8.40

API/WHO protocol [12, 13]

4g plant material and 100ml distilled water were taken in a 250ml round bottom flask, well-stoppered and shaken on a VDRL mechanical shaker at laboratory temperature. The whole was then allowed to stand for 18hr, and then filtered through Whatman No. 40 filter paper – a yellow-buff solution was obtained. 25ml of this filtrate was evaporated to dryness in a Petri dish to constant weight at 105^0 (6hr) in an air-oven. The amount of residue left was determined, and the water extractive value calculated from this.

A similar procedure was followed for the 90% alcohol extractive – a pale yellow solution.

Ultra-sonication procedure

4g plant material and 100ml distilled water was taken in a 150ml conical flask and ultra sonicated in a Citizon Ultrasonic cleaner for 30min, allowed to stand for one hour, again ultra-sonicated for 30min, allowed to stand for one hour, then filtered through Whatman No. 40 filter paper – a yellow-buff solution was obtained. The subsequent steps were identical with the API/WHO protocol. 25ml of this filtrate was evaporated to dryness in a Petri dish to constant weight at 105^0 (6hr) in an air-oven. The amount of residue left was determined, and the water extractive value calculated from this.

A similar procedure was followed for the 90% alcohol extractive – a pale yellow solution.

Preliminary phytochemical analysis

The HPTLC chromatograms of the methanolic extracts (see below) showed that the components present in the root wood core and root bark were very similar to each other (and hence of the total root) though differing in relative proportions of some of the components – see below. Preliminary phytochemical investigations were therefore done on extracts of the whole roots only.

Table 2: Qualitative Phytochemical Tests of Water and 90% Alcoholic Extracts of *Alstonia scholaris* Roots

S. No.	Test/Reagent used	Water extract	90% Alcoholic extract
1a.	Alkaloids - Mayer's test	(+++) Copious pale-yellow ppt.	(+++) Copious white ppt.
1b.	Alkaloids - Dragendorff's test	(+++) Heavy orange ppt.	(+++) Heavy orange ppt.
2a.	Carbohydrates - Molisch test	(++) Purple ring at juncture of two layers	(++) Purple ring at juncture of two layers
2b.	Reducing sugars-Barfoed test	(+) Slight yellow ppt.	(+) Slight yellow ppt.
3.	Flavonoids-Shinoda test	(-)	(-)
4.	Terpenoids - (Liebermann-Burchardt Test)	(-)	(+) Magenta colour
5.	Steroids - (Liebermann-Burchardt Test)	(-)	(-)
6.	$FeCl_3$ test - Phenolic compounds	(-)	(+) Dull magenta colour
7.	Amino Acids - Ninhydrin test	(-)	(-)
8.	Proteins - Biuret test (Pietrowski's test)	(-)	(-)
9.	Saponins - Frothing test	(+)	(-)

Note:- (+) Trace amount, (++) Higher amount, (+++) profuse amounts; (-) Absent.

HPTLC Finger-printing profile of *Alstonia scholaris* Roots

Chromatography experiment

Dried and powdered total root (Rt), root wood core (Rw) and root bark (Rb) were separately extracted with refluxing methanol; the extracts were subjected to HPTLC analysis.

Sample preparation

General procedure: 1g of the dried and powdered plant part of *Alstonia scholaris* was taken in a 50ml round bottom flask and refluxed with methanol (GR grade, Merck, India, 25ml) for 1h. The extract was filtered through fluted filter paper (Whatman No. 40). The filtrate was concentrated to 10 ml, and taken for HPTLC profiling.

Stationary Phase: Precoated (support on Aluminum Sheets) Silica Gel Plate. Specification: TLC Silica Gel $60F_{254}$, Merck, Batch No. 1.05554.0007.

Mobile Phase: Ethyl acetate: chloroform: methanol: formic acid (5:3.5:1.5:0.5) v/v [GR grade solvents, Merck, India]

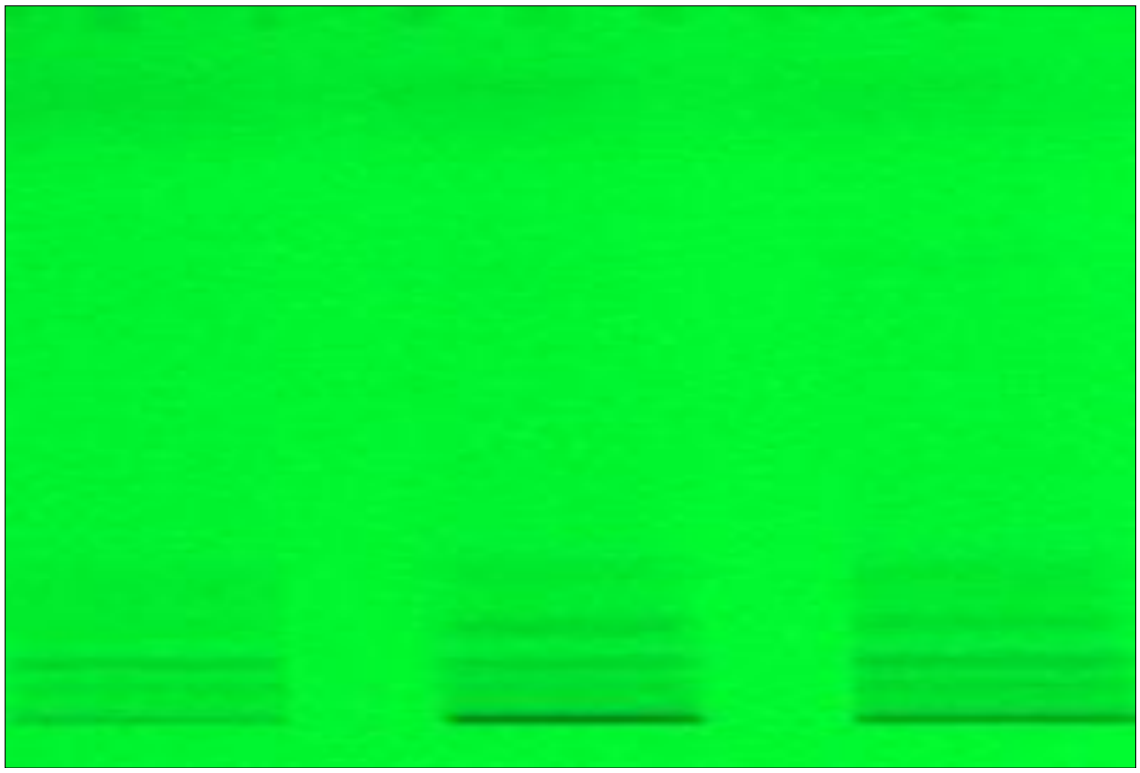
Sample application: Applied volume – 5µL as 8mm band and applied at 12mm from the base of the plate, with a CAMAG ATS4. Plate size was 10 x10 cm.

Development: Developed up to 80mm in CAMAG Twin trough chamber, Plate preconditioning – temperature 27°C; relative average humidity was 48%.

Observation: The chromatograms were visualised in CAMAG TLC visualiser, and scanned using a CAMAG TLC Scanner 4. The HPTLC chromatograms are given in Fig. 3 (visualised at 254nm), and Fig. 5 (visualised at 366nm).

General Comments

In each case, a large number of bands were obtained – some of which were overlapped. Figs. 4a-c and Figs. 6a-c depict the densitometric HPTLC finger print profiles observed at 254nm and 366nm respectively. The more intense and sharper bands have been listed, along with their R_f values, absolute intensities and relative areas – Tables 3a-c for visualisation at 254nm; Tables 4a-c for visualisation at 366nm. Some of the listed bands could be composite bands for different phytoconstituents. The chromatograms showed that the components present in the root wood core and root bark were very similar to each other (and hence of the total root) though differing in relative proportions of some of the components.



Als (Rb) Als (Rw) Als (Rt – total root)

Fig 3: Photography of HPTLC Plate – Visualisation at 254 nm - Plate 1

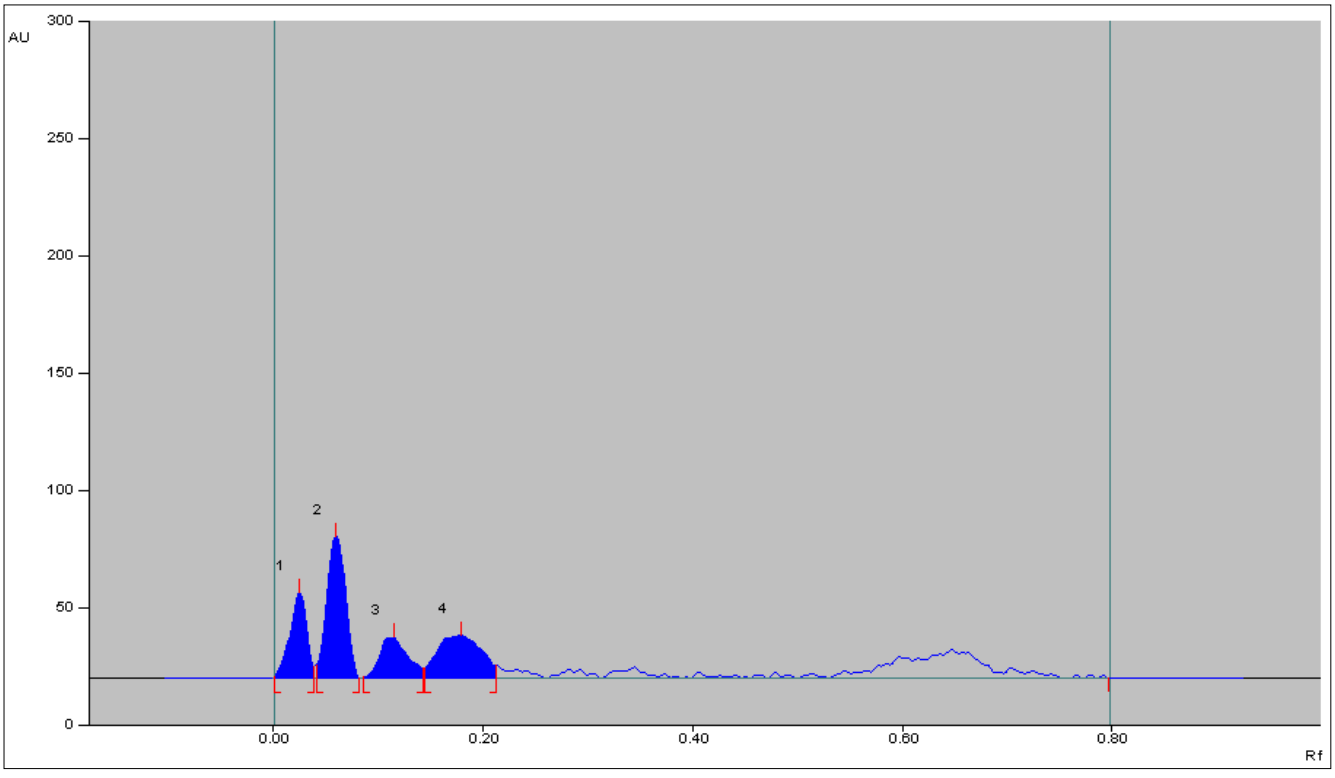


Fig 4(a): *Alstonia scholaris* Root bark (Rb)

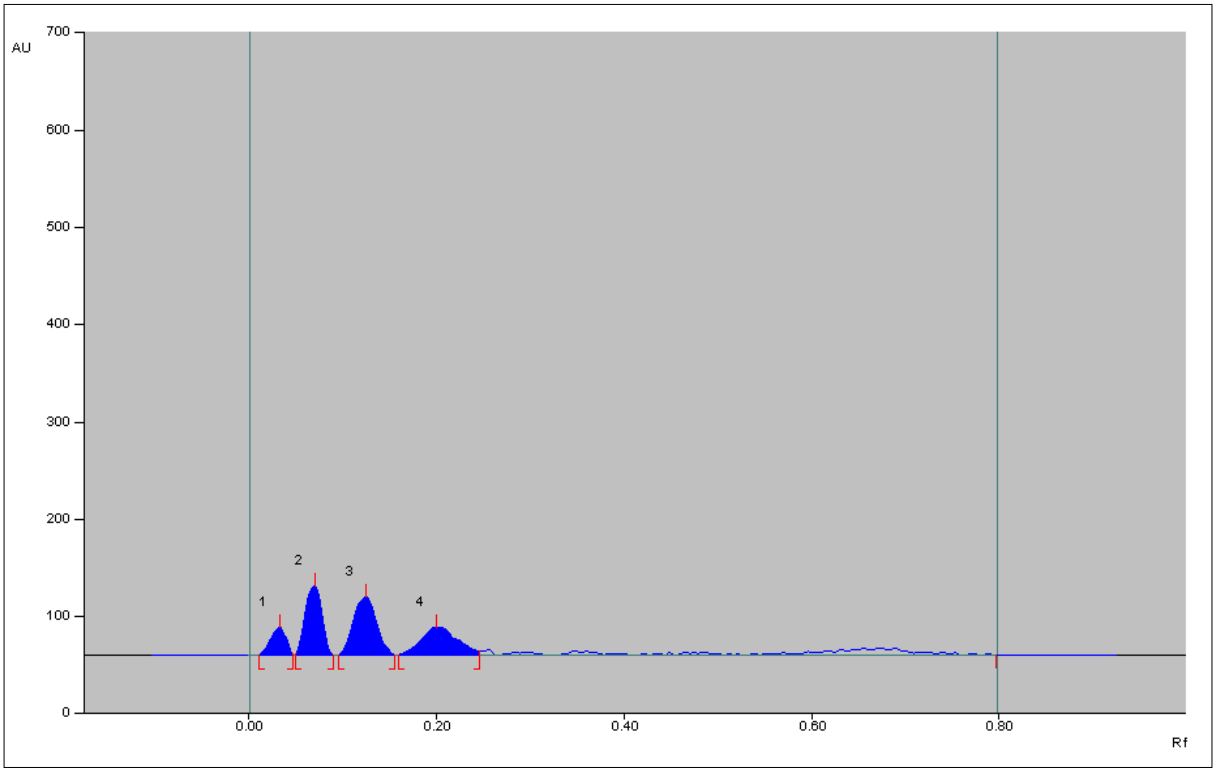


Fig 4 (b): *Alstonia scholaris* Root wood core (Rw)

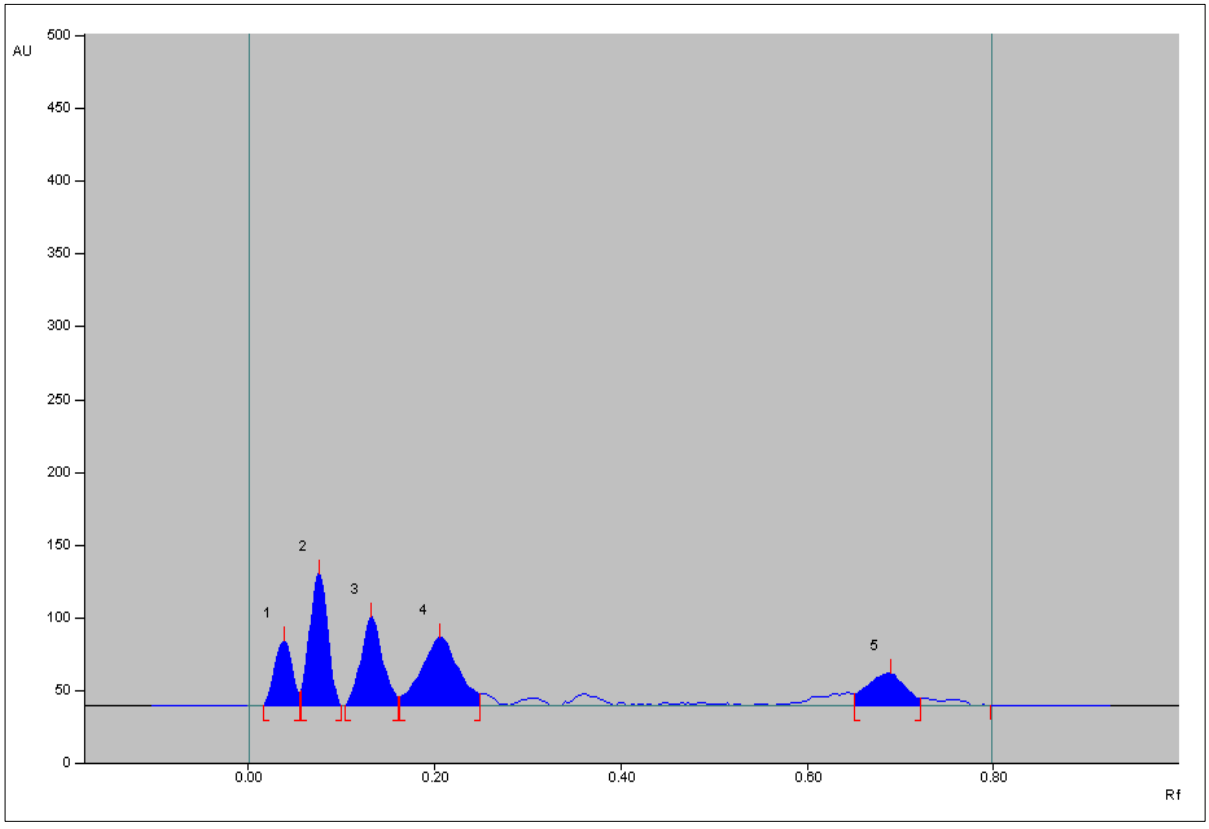


Fig. 4 (c): *Alstonia scholaris* Root - total (Rt)

Fig 4: Densitometric finger print profiles at 254 nm of *Alstonia scholaris* Root bark (Rb), Root wood core (Rw) and Root - total (Rt, *i.e.* Root bark + Root wood core)

Table 3a: R_f values and relative areas of the HPTLC peaks of *Alstonia scholaris* Root bark (Rb) visualised at 254 nm

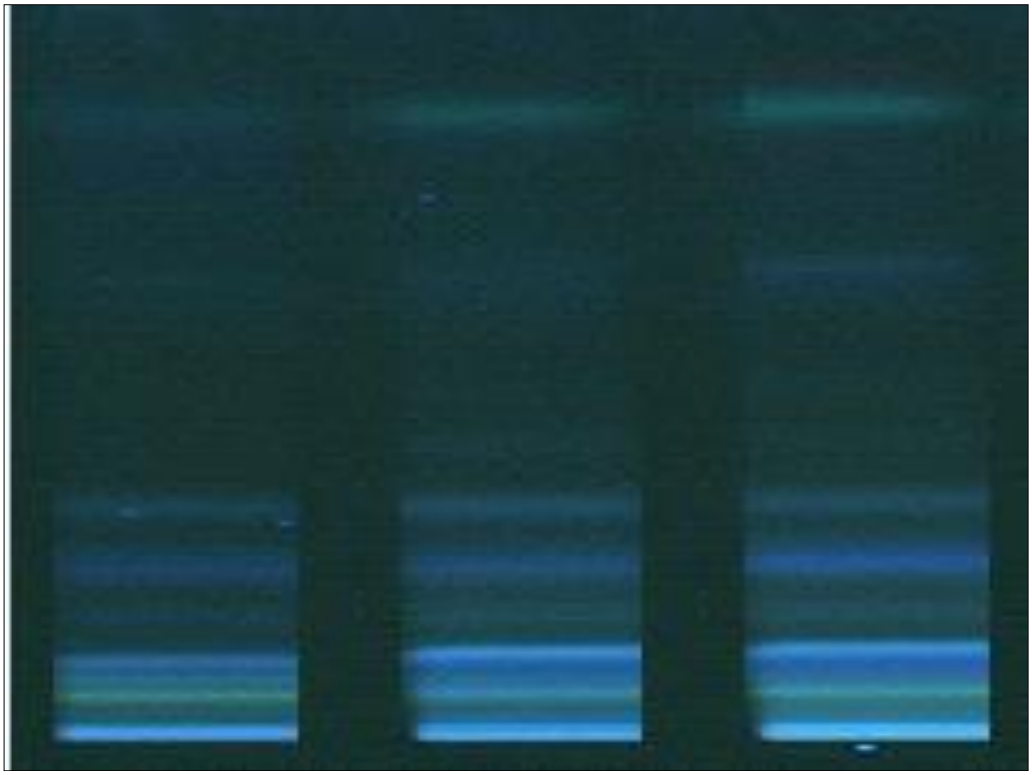
S. No.	R_f	Height of the Peak	RelativeArea (%)
1	0.03	36.4AU	20.36
2	0.07	60.7AU	37.09
3	0.12	17.4AU	16.07
4	0.18	18.1AU	26.47

Table 3b: R_f values and relative areas of the HPTLC peaks of *Alstonia scholaris* Root wood core (Rw) visualised at 254 nm

S. No.	R_f	Height of the Peak	Relative Area (%)
1	0.03	28.7AU	10.91
2	0.07	71.1AU	28.96
3	0.13	60.2AU	34.09
4	0.20	28.7AU	26.05

Table 3c: R_f values and relative areas of the HPTLC peaks of *Alstonia scholaris* Root - total (Rt) visualised at 254 nm

S. No.	R_f	Height of the Peak	Relative Area (%)
1	0.03	44.5AU	12.27
2	0.07	90.8AU	25.72
3	0.13	60.7AU	20.92
4	0.20	47.1AU	27.65
5	0.69	21.9AU	13.43



Als (Rb) Als (Rw) Als (Rt - total root)

Fig 5: Photography of HPTLC Plate – visualization at 366 nm - Plate 2

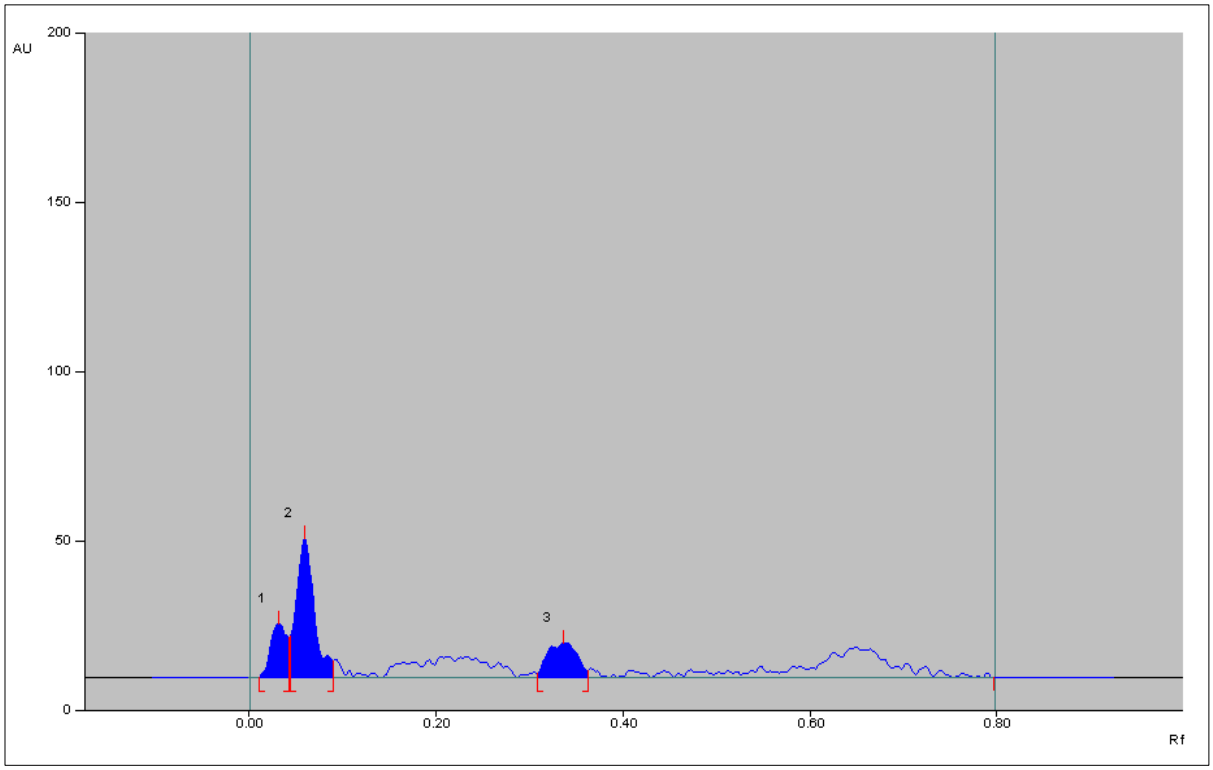


Fig 6 (a): *Alstonia scholaris* Root bark (Rb)

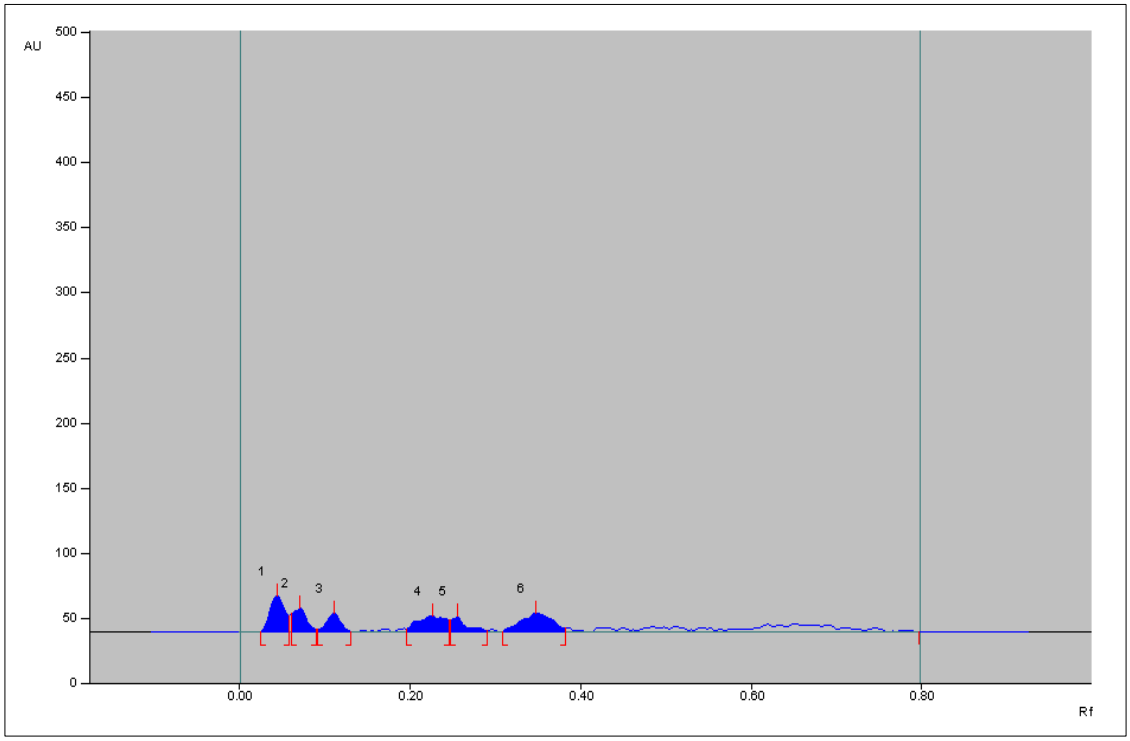


Fig 6 (b): *Alstonia scholaris* Root wood core (Rw)

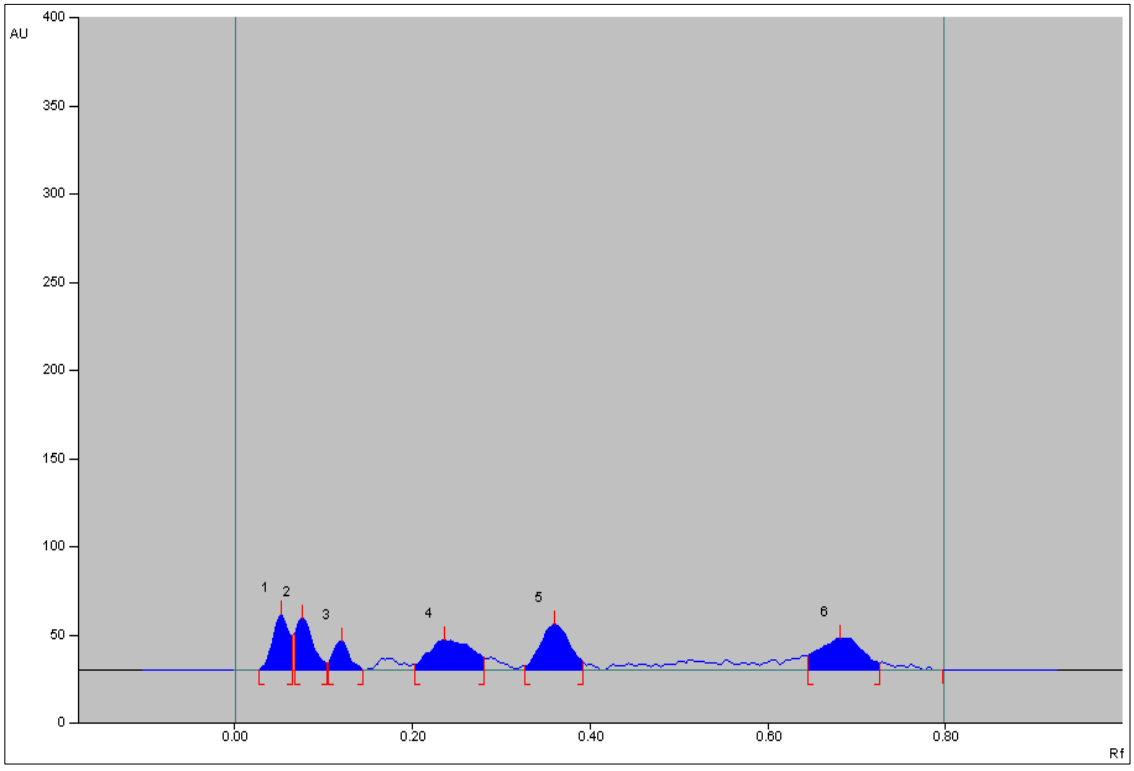


Fig. 6 (c): *Alstonia scholaris* Root - total (Rt)

Fig 6: Densitometric finger print profiles at 366 nm of *Alstonia scholaris* Root bark (Rb), Root wood core (Rw) and Root - total (Rt, *i.e.* Root bark + Root wood core)

Table 4a: R_f values and relative areas of the HPTLC peaks of *Alstonia scholaris* Root bark (Rb) visualised at 366 nm

S. No.	R_f	Height of the Peak	Relative Area (%)
1	0.03	16.0AU	19.61
2	0.06	41.1AU	56.89
3	0.34	10.3AU	23.05

Table 4b: R_f values and relative areas of the HPTLC peaks of *Alstonia scholaris* Root wood core (Rw) visualised at 366 nm

S. No	R_f	Height of the Peak	Relative Area (%)
1	0.04	27.7AU	22.4
2	0.07	18.0AU	13.65
3	0.11	14.6AU	11.15
4	0.23	12.7AU	19.02
5	0.26	11.9AU	9.26
6	0.35	13.8AU	24.50

Table 4c: R_f values and relative areas of the HPTLC peaks of *Alstonia scholaris* Root - total (Rt) visualised at 366 nm

S. No.	R_f	Height of the Peak	Relative Area (%)
1	0.04	31.5AU	14.70
2	0.07	29.5AU	14.41
3	0.12	16.5AU	7.27
4	0.24	17.1AU	20.42
5	0.35	25.9AU	21.10
6	0.68	18.2AU	22.09

Discussions

The present study on preliminary phytochemical analysis and HPTLC finger-printing profile of *Alstonia scholaris* (L.) R.Br (Saptaparṇa) roots will serve as ready reference for authentication of this drug. HPTLC is a valuable assessment tool for the identification of chemical constituents present in plant drugs. Indications have been obtained about the presence of large number of secondary metabolites, *viz*. alkaloids, carbohydrates, steroids, phenols and saponins in aqueous and 90% alcohol extracts. Thus the presence of so many secondary metabolites in the roots of *Alstonia scholaris* makes this drug effective against several ailments. Comparatively less phytochemical work has been done on the roots of *Alstonia scholaris*, in contrast to the leaves and stem bark of this plant. The results showing the presence of a large number of secondary metabolites indicate that further phytochemical work on the roots is warranted.

Conclusions

This communication furnishes preliminary phytochemical analysis of classes of secondary metabolites and HPTLC finger-printing profile of roots of *Alstonia scholaris* (L.) R.Br. (Saptaparṇa). These data can be used for the authentication and identification of the roots of this medicinally important plant.

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