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Pharmacognostical evaluation of *Alstonia scholaris* (L.) R.Br (Saptaparṇa), roots

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Abstract
Plants are among the richest sources of bioactive compounds throughout the world and have been used in traditional medicine systems for thousands of years, and still continue to provide new remedies to mankind. The medicinal plant *Alstonia scholaris* (L.) R. Br. (Saptaparṇa, Chhatim) is extensively used in Ayurveda, the Indian school of traditional medicine. The present study reports the physicochemical characteristics and pharmacognostical investigation of roots of *Alstonia scholaris*. Organoleptic, macroscopic and microscopic studies have been performed. These give referential information for identification parameters of the drug.

Keywords: *Alstonia scholaris* root, physicochemical, macroscopic studies, microscopy

Introduction
In recent years there has been a global resurgence of interest in the traditional Indian system of Ayurveda, which relies on plant based crude materials and their formulations. This is due to several reasons - specifically, incorrect use of synthetic drugs may result in deleterious side effects; whereas drugs of natural plant origin in general have lesser side effects and are cost effective. Medicinal plants contain wide array of chemical compounds which play an important role in health care.

The Chemistry Department, University of Calcutta and the Calcutta unit of the Central Council for Research in Ayurveda and Siddha, which has developed to the present Central Ayurveda Research Institute for Drug Development (CARIDD) have been carrying out investigations on different aspects of chemistry, pharmacognosy, pharmacology and biomedical uses of Indian medicinal plants for several years {see ref. [1] for selected references}.

As a continuation of these studies, we took up the reinvestigation of the roots of *Alstonia scholaris* (L.) R. Br., a reputed medicinal plant in the Indian traditional system of Ayurveda. *Alstonia scholaris* (L.) R. Br. (Family Apocynaceae; tribe – Plumeriae; Subtribe – Alstoniinae) is a tall evergreen tree growing in sub-Himalayan tracts ascending to 600m. From Jammu eastwards and also in the western peninsula mostly in deciduous forests. It is called devil tree or dita bark in English, Saptaparṇa (sapta: seven, parna: leaves) in Sanskrit, Chhatim in Bengali; Palagaruda in Telegu [2, 3, 4, 5]. The leaves occur in whorls of five to ten; the leaves are narrowly obovate to very narrowly spathulate, base cuneate, apex usually rounded [3]. The tree is fairly widespread in the city of Kolkata, being planted in several places on street verges and parks. In Kolkata, the tree flowers in late October to February, and fruits in January-May. Flowers are greenish-white, fragrant, borne in compact, many-flowered umbellate cymes [3]. All the parts of the plant are used as drugs [3]: the bark is alterative, anthelmentic, antidysentric, antimalarial, astringent, and cures gastro-intestinal troubles; decoction of the leaves are used in beriberi; wood-paste with water is applied in rheumatism and wounds. The roots show antibacterial property [6].

A large number of compounds, particularly indole alkaloids have been isolated from different parts of the plant [3, 4, 7, 8]. Extensive 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 [8]. The stem-bark is used in the antimalarial drug formulation Ayush-64, developed by this research group working in collaboration with the CCRAS Institute.

Details of the pharmacognosy of the stem-bark is recorded in the Ayurvedic Pharmacopoeia of India [2]. As no pharmacognostical studies on the root and root bark have been reported, we investigated the same to give referential information for identification parameters to improve confidence levels of acceptability of this herbal drug.

2. Materials and Methods

Plant material collection and authentication

Roots of *Alstonia scholaris* (L.) R.Br were collected from Sector 5, Salt Lake City, Kolkata – in January 2018 (Fig.1). 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 preserved (Voucher number - CRD/Chem/AS/R). The reference sample has been deposited at CARIDD, Kolkata.

Fig 1: Alstonia scholaris tree

Plant sample processing

The roots were dried at ambient temperature in the shade for 7 days, approximately 170g. of the dried roots were obtained. About 33g. of the roots were used for organoleptic and pharmacognostical studies. From this part, the root bark was carefully stripped and isolated from the root core. These were separately washed under running tap water, and then three times by sterile distilled water. These samples were air-dried in the shade at ambient temperature for 7 days, and stored in air-tight containers to avoid any contamination. Small portions of the air-dried plant samples were used for macroscopic and organoleptic studies, while the rest of the plant materials were pulverised with a grinder (National SM 2000) to obtain 60 mesh size root powder. The whole and powdered plant samples were stored at room temperature in airtight, light-resistant containers as per standard guidelines [9, 10].

The rest of the roots were chopped to small pieces and ground to a coarse powder. This was used for determining physicochemical parameters according to the standard methods as prescribed in API/ WHO protocols [9, 10].

Physicochemical parameters

Determination of the following physicochemical parameters were determined in accordance with standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/WHO protocols [9, 10].

Macroscopy of plant material

The organoleptic parameters *viz.* texture, shape, size, colour, odour etc. of the plant material were noted by naked eye observation [9, 10] with a simple microscope Olympus OIC DM.

Cytomorphology of plant material

The selected sections were mounted on slides in 50% glycerin and observed under a binocular compound microscope Olympus OIC-07964, at 10× and 40× magnifications [9, 10]. The Photomicrographs of cellular details were obtained using a Leica DM 1000 LED microscope attached with Leica EC3 camera.

For powder microscopy, finely powdered samples (~2 g) were separately treated with different solutions, *viz.* aqueous saturated chloral hydrate (for maceration), 50% glycerin, phloroglucinol in concentrated HCl (for staining lignified tissues) and 0.02 N iodine reagent (for starch grains), mounted on slides with glycerin following a standard protocol and observed under the binocular compound microscope (Olympus OIC- 07964) at 10× and 40× magnifications [9, 10]. The Camera Lucida drawing of cellular details was done. Photomicrographs of the different cellular structures and inclusions were taken using Magcam DC14 camera attached with Olympus CX21i trinocular compound microscope.

3. Results and Discussions

Physicochemical Parameters

Determination of the following physicochemical parameters were carried out - total ash, acid insoluble ash, 90% alcohol soluble extractive, water soluble extractive, pH of 10% aqueous suspension. These were determined in accordance with standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/ WHO protocols [9, 10].

Table 1: Physicochemical evaluation of *Alstonia scholaris* roots

***Alstonia scholaris* roots**		
Sl. No.	**Parameters**	**Result (in w/w %)**
1.	Loss on Drying	6.10
2.	Total Ash	5.88
3.	Acid insoluble ash	0.50
4.	90% Alcohol soluble extractive	9.40
5.	Water soluble extractive	9.90
6.	pH (10% Aq. suspension)	5.2

Pharmacognostical Study

(a) Macroscopic

(**i) Root bark:** Root bark of *Alstonia scholaris* appears in uneven, rough, sometimes with carved channels and occasionally quilled pieces, 2-3mm thick, curved or flat fragments, about 7 mm thick from stem, externally yellowish brown to grayish brown, internally dark grayish-brown; older bark very rough, fissured transversely and longitudinally, striated, fracture, short and smooth, fractured surface traversed by numerous, fine, medullary rays; taste bitter, odour not characteristic (Fig. 2).

Fig 2: Root Bark of *Alstonia scholaris*

ii) Root: Roots (Fig.3) are fragmented, solid, woody, with rootlets, occasionally branched, thick and hard, rigid, elongated, cylindrical with rough and wavy outer surface,

externally dark tortuous with transversely cracked and longitudinally fissured bark portion and internally bright creamish yellow central solid core, rootlets thin and wiry and curved; cork is thin, separates easily and peels off in flakes; root fragment is 0.8 cm to 2.3 cm in diameter; central core solid, 0.5 cm to 1.2 cm in diameter and outer surrounding cylindrical portion is 0.4 cm to 0.6 cm in breadth; length of fragment of root varies from 1.3 cm to 3.5 cm; fracture longitudinal, soft and short, splintery; taste bitter; odour not characteristic.

Fig 3: Roots of *Alstonia scholaris*; central solid core on the right

b) Powder microscopy

i) Root bark: Organoleptically, fine powder of root bark is yellowish brown in colour (Fig.4) and bitter in taste. Fig.5 and Fig.6 show the characteristic features, *i.e.* storified multi-layered, thick and thin-walled cork like layered bricks; angular compact thick walled cork cells almost square in transverse and longitudinal sections and polygonal in surface view; few medullary ray cells; thick walled aseptate fibres with tapering ends; group of fibres traversed by medullary ray cells; few group of lignified schlerenchyma; elongated lignified short fibre like stone cells; thin walled angular to polygonal parenchyma of secondary cortex with rhomboid crystals inside cells; starch grains single to compound 5 µm to 26 µm in diameter of different shapes; prismatic or rhomboid crystals of calcium oxalate; secondary phloem tissue with sieves and few starch grains inside phloem parenchyma (Fig.5 and Fig.6).

Fig 4: Root Bark powder of *Alstonia scholaris*

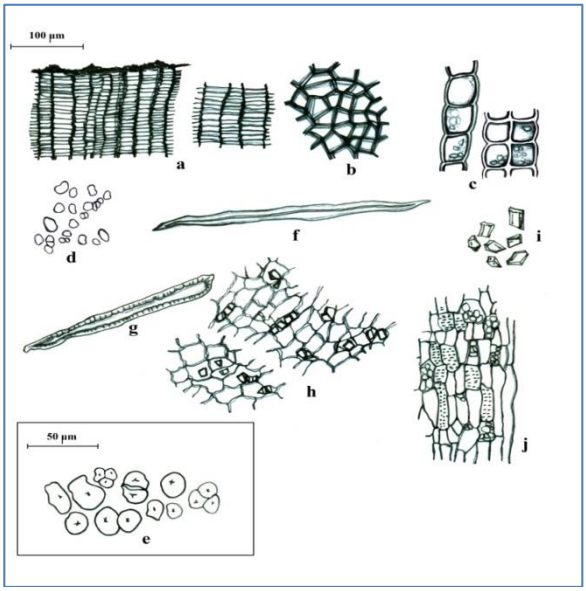
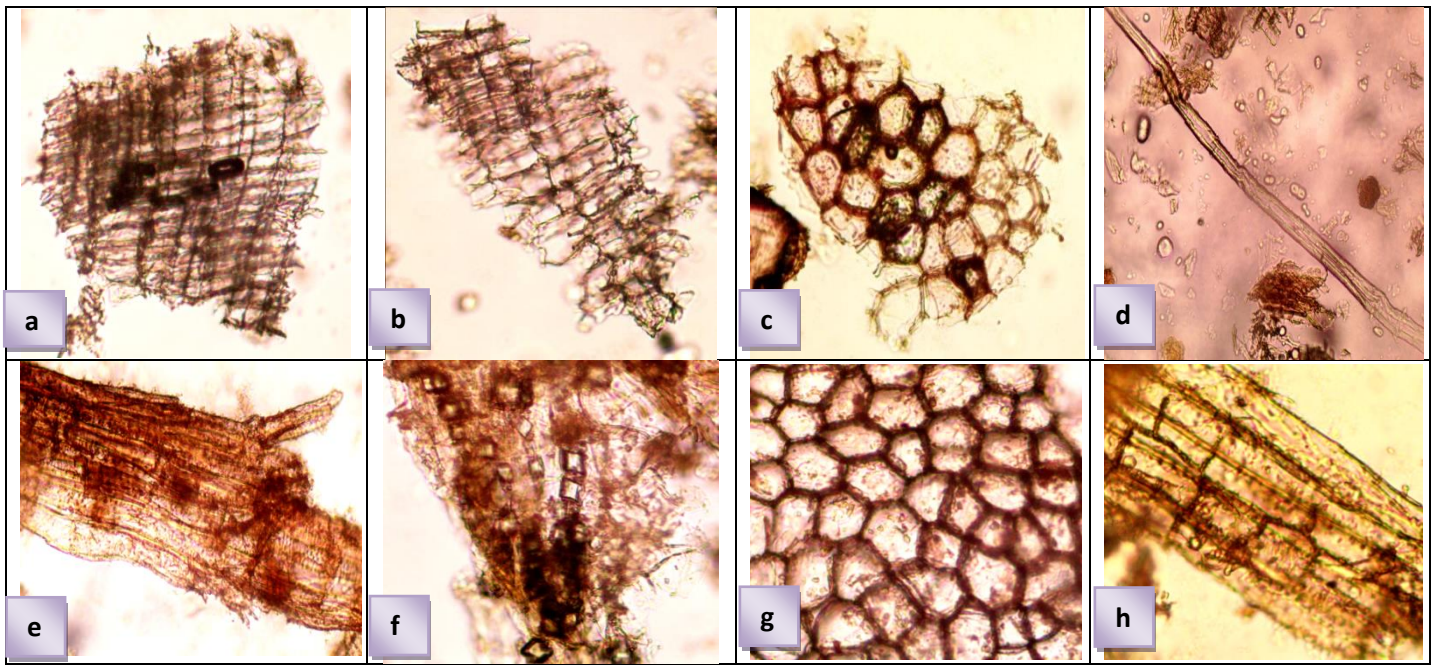


Fig 5: Camera Lucida drawing of powder microscopy of root bark of *Alstonia scholaris*
a: cork cell layers; **b:** group of cork cells in surface view; c: medullary ray cells; **d, e:** starch grains in different dimension; **f:** aseptate fibre; **g**: elongated stone cell; **h:** parenchyma with crystals; **i:** prismatic crystals of calcium oxalate pitted xylem vessels; **j:** secondary phloem tissue.



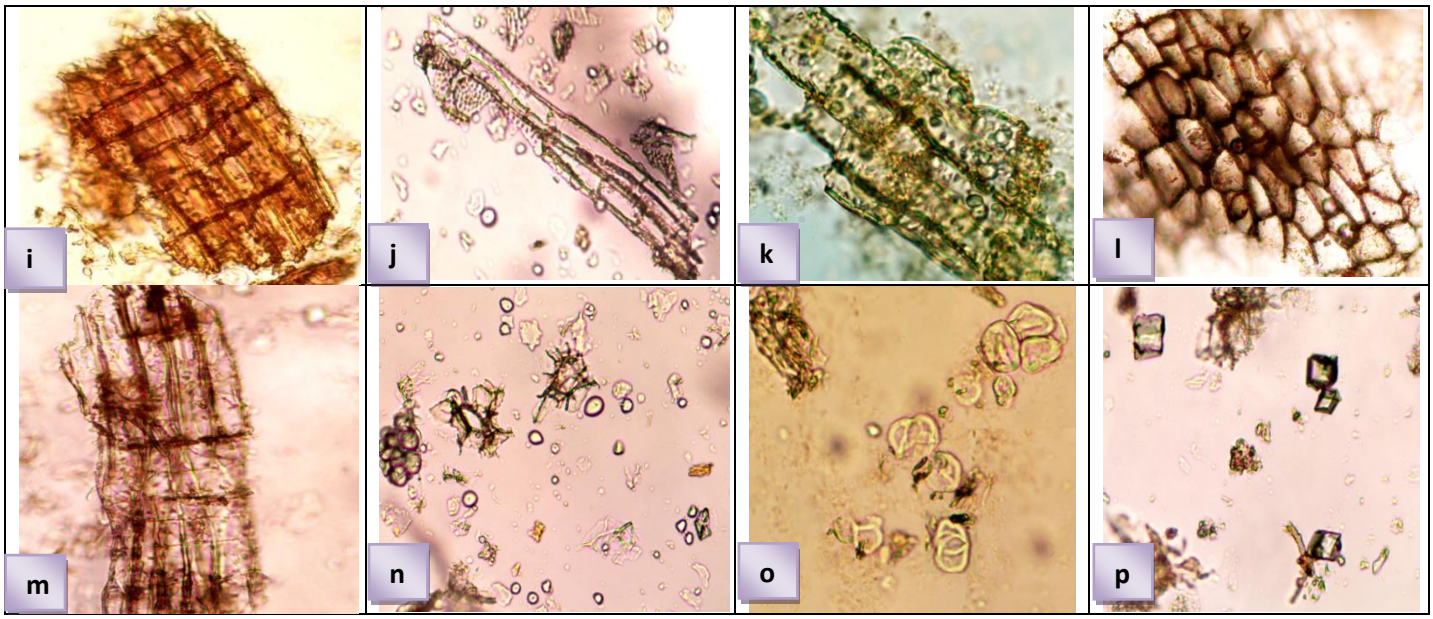


Fig 6: Photomicrographs of powder microscopy of root bark of *Alstonia scholaris*
a, b: cork cell layers; **c:** group of cork cells in surface view; **d:** fibre; **e:** group of fibres; **f:** prismatic crystals embedded in cortical tissue; **g:** thick walled cells of schlerenchyma; **h, i:** group of fibres traversed by medullary ray cells; **j:** fragmented secondary phloem tissue; **k:** parenchyma with starch grains; **l:** angular cells of secondary cortex; **m:** secondary phloem with medullary rays; **n, o:** starch grains in different dimension; **p:** prismatic crystals of calcium oxalate.

ii) Root core (root wood): Organoleptically, fine powder of root is bright yellowish buff in colour (Fig.7) and bitter in taste showing the characteristic features, *i.e.* multi-layered, thick and thin-walled cork like layered bricks; prismatic crystals of calcium oxalate; single starch grains 5 µm to 28 µm in diameter along with compound (2 to 3) ones; angular compact thick walled cortical parenchyma; rectangular to polygonal pitted parenchyma; aseptate fibre single or in group with tapering end, few with crystals embedded in them; group of thick walled oval to rectangular stone cells, few are specially shoe shaped in appearance; phloem tissue with sieves and phloem parenchyma; xylem vessels with spiral and pitted thickenings on wall (Fig.8 and Fig.9).

Fig 7: Root core (Root wood) powder of *Alstonia scholaris*

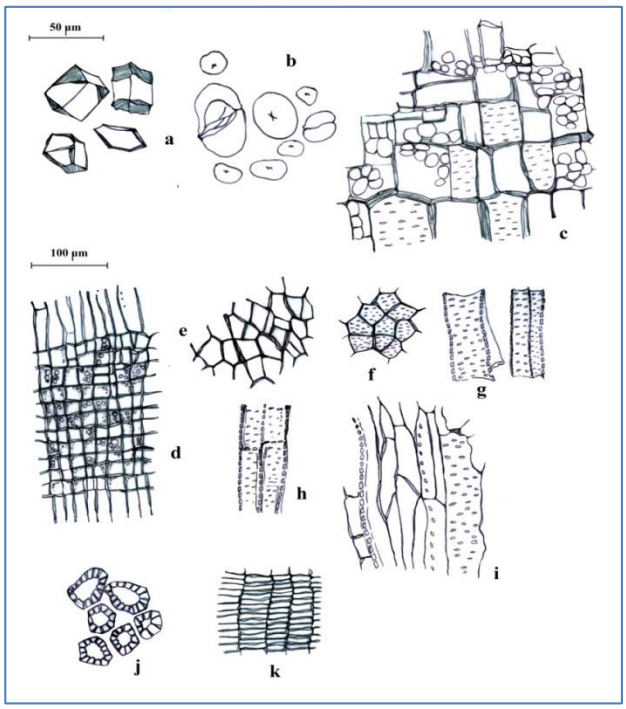


Fig 8: Camera Lucida drawing of powder microscopy of root bark of *Alstonia scholaris*:
a: prismatic crystals of calcium oxalate; **b:** starch grains; **c:** phloem tissue with starch grains; **d:** group of fibres traversed by medullary ray cells; **e:** cortical parenchyma; **f:** pitted polygonal parenchyma; **g:** pitted xylem vessels; **h:** pitted xylem parenchyma; **i:** portion of phloem tissue with sieves; **j:** stone cells of different shape; **k:** cork cell layers.

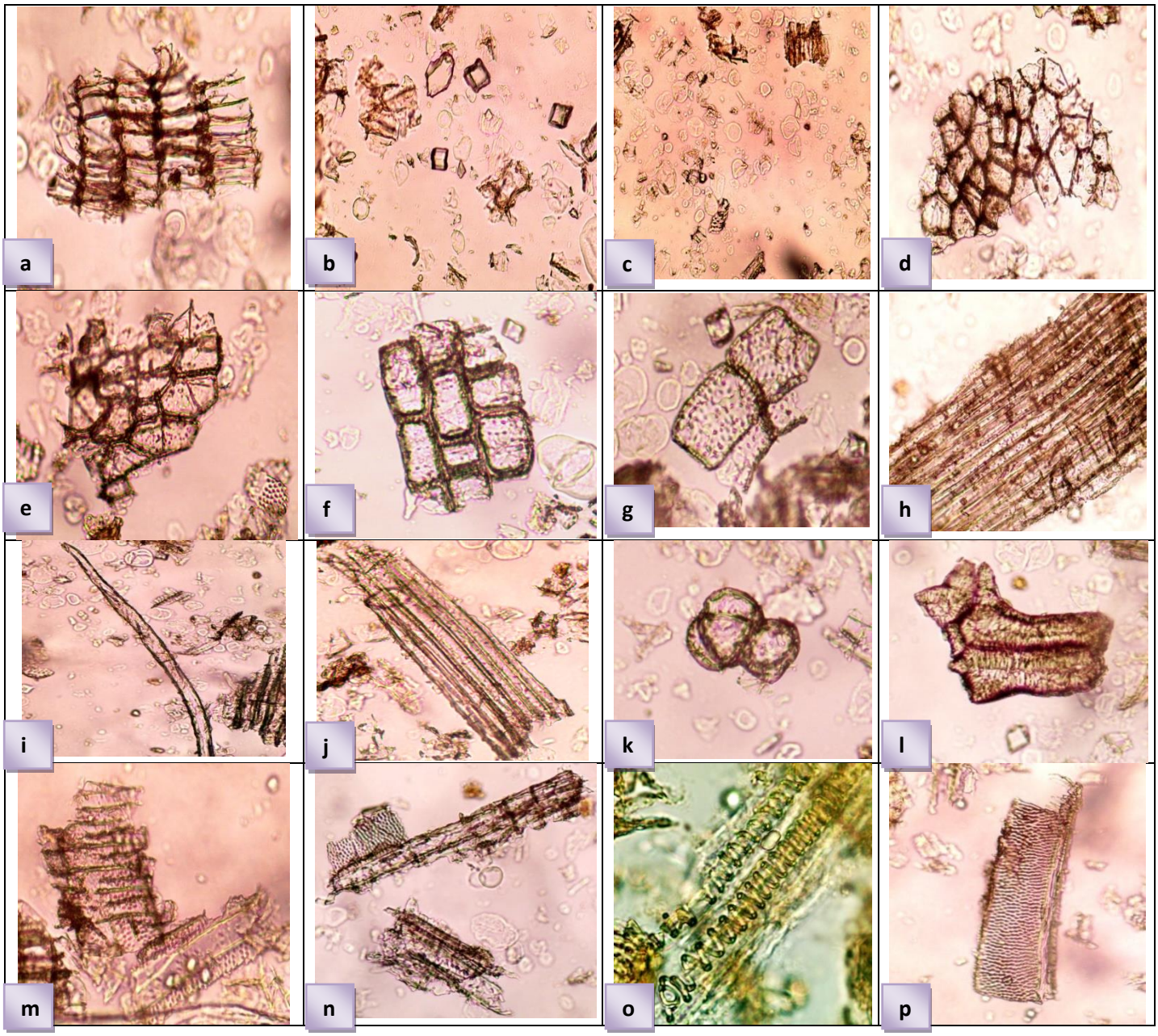


Fig 9: Photomicrographs of powder microscopy of root of *Alstonia scholaris*
a: cork cell layers; **b:** prismatic crystals of calcium oxalate; **c:** starch grains; **d:** angular parenchyma; **e:** polygonal pitted parenchyma; **f, g:** rectangular pitted parenchyma; **h:** group of fibres with crystals; **i:** single fibre; **j:** group of fibres;; **k:** oval stone cells; **l:** elongated stone cells (shoe-shaped); **m, n:** fragmented phloem tissue; **o:** spiral xylem vessel; **p:** pitted xylem vessel.

4. Conclusion

This communication furnishes pharmacognostic parameters of the root and root bark of *Alstonia scholaris* (Saptaparṇa). These data can be used for the authentication and identification of these parts of this important plant.

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