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Seasonal Variation Studies and Pharmacognostic Evaluation of *Alstonia scholaris* R.Br. Bark

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Abstract – *Alstonia scholaris* is known as 'Saptaparna' in Ayurvedic System of Medicines and the bark is used for the treatment of various diseases. It has various ethnomedicinal values as different traditional communities find diverse medicinal properties. The present communication deals with the seasonal variation studies of the stem bark of this plant. The bark was collected in the month of January, July and November. There is no macro-microscopical changes in all the three seasons but the ash values showed significant differences. Crude fibre content showed sharp decline from January to July to November. Total tannin percentage was found maximum in January and minimum in June. TLC finger print profile showed more concentration of constituents in January as compared to June and November. Hence, the bark may show more efficacy when collected in January.
Keywords – *Alstonia scholaris*, pharmacognosy and seasonal variation

Introduction

The bark of *Alstonia scholaris* R. Br. (Apocynaceae) 'Saptaparna' is considered as astringent, bitter tonic, laxative, anthelmentic, febrifuge, antiasthmatic, antiliprotic, antimalarial and also useful in diseases of heart, leucoderma, tumors, dental caries, stomachache and has hypotensive and anticancer activities (Chopra *et al.*, 1958). The fresh bark juice with milk is reported to be administered in leprosy and dyspepsia. The milky juice or latex is applied to ulcers, sores, tumours and rheumatic pain and is used for curing toothache (Nadkarni, 1954; Kirtikar and Basu, 1984). Ethnobotanically the bark is used for tuberculosis (Tiwari *et al.*, 1979); in stomachache (Ghosh and Sensarma, 1997); as an antidiarrhoeal and antidysenteric (Ahluwalia, 1968; Sharma *et al.*, 1979; Ahmad and Siddiqui, 1985; Das and Kant, 1988; Jha *et al.*, 1989; Tewari *et al.*, 1990; Siddique and Husain, 1991; Kumar, 1992); galactagogue (Borthakur, 1993; Girach and Aminuddin, 1995); as anti-inflammatory and for rheumatic pains (Hemadri and Rao, 1983; Mohapatra, 1991); astringent, anthelmintic, antiperiodic in leprosy, dyspepsia (Choudhury *et al.*, 1993); post delivery complaints (Chandra and Pandey, 1985; Chandra *et al.*, 1985); as an antispasmodic (Chandra *et al.*, 1987); in asthma (John, 1984; Tiwari and Majumdar, 1996); for both pneumonial and malarial fever, leucoderma (Sharma *et al.*, 1979; Maheshwari *et al.*, 1980; Ahmad and Siddiqui, 1985; Singh and Prakash, 1994). It is applied for massage (Singh and Dhar, 1993) and also used for wounds (Sen and Pradhan, 1999). Both stem and root bark is used for leucorrhoea (Bhandary *et al.*, 1995). The latex is reported to be used in spermatorrhoea (Sexena *et al.*, 1988) and post partum abdominal pains (Singh and Krishna, 1983). *A. scholaris* is reported to be rich in echitamine type alkaloids. The alkaloids echitamine chloride, echitamine and 17-*O*-acetylechitamine were isolated from the bark (Gupta and Tandon, 2004).

The bark is collected by pharmacies and have been used extensively. The studies on its documentation of medicinal utility are well known. The bark has utility in Ayurvedic and Unani systems of medicine and also used by local medico-practitioners for various purposes.

Though the crude drug is worked out with various aspects, there is no criteria for exact period of collection of bark. Hence, the detailed pharmacognostic work with modern tools was initiated. The bark is collected in three different seasons and studied with conventional as well as modern pharmacognostical tools. The bark shows significant variation in three different seasons. The studies on seasonal variation highlight the period of collection of the

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bark. The observations on these investigations are presented in the communication.

Experimental

The bark of *Alstonia scholaris* R.Br. was collected from Banthara (Lucknow, Uttar Pradesh, India) in three seasons viz. January, July, and November. The bark samples were dried and every sample was processed for detailed pharmacognostic investigations. Microscopic studies were carried out on transverse and tangential longitudinal sections (TS and TLS) by using saffranin as the staining agent (Johansen, 1940). Fluorescence powdered drug analysis was carried out according to the methods described by Chase and Pratt (1949) and Kokoski *et al.* (1958). The Physico-chemical parameters viz. successive Soxhlet extractives, total ash, acid insoluble ash, alcohol and water soluble extractives, and crude fibre were determined by Indian Pharmacopoeial methods (1966). The total tannin was estimated by AOAC method (1984).

For comparative TLC 10% solution of chloroform soluble extract of the bark materials, collected in three seasons, were prepared separately. 15 μl of each test solutions were applied on precoated silica gel F254 TLC plate (E. Merck) with the help of CAMAG Linomat IV Applicator. The plate was developed to a distance of 8.0 cm at room temperature 30 °C. The TLC plate was scanned with CAMAG TLC Scanner 3 with the help of the software Cats 4.

Results

The trees of *Alstonia scholaris* R.Br. (Family : Apocynaceae) are found in high rainfall tract. Leaves are in whorls of 5 - 7, coriaceous; flowers in umbellate cymes, greenish-white; follicles long, cylindrical; seeds with coma (Plate 1 A). It is distributed throughout the moist regions of India in West Bengal and West coast forests of South India, upto 600 m.

Organoleptic characters – The *Alstonia scholaris* tree shows oozing of milky latex immediately when bark was cut. In July the latex was thinner while in January the latex was much more thicker. Dried bark channeled or occasionally quilled, 3 to 7 mm thick, light in weight, quite brittle. Outer surface is rough; dark grey in colour, with lenticels. Whole bark readily separated into two longitudinal parts. Both the parts show characteristic lock and key system. Inner surface is rough and buff coloured and showing protuberated longitudinal ridges and oblique, fine, wavy striations. The texture of bark is spongy and fracture short and coarse (Plate 1 B & C).

Microscopic characters – TS of outer bark shows outer most crushed cells of rhytidoma followed by a broad zone of cork about 80-140 layers. The secondary growth is anomalous, the cork cambium arises at 3 or 4 distinct places and develops rectangular cork cells outer side and angular parenchymatous cells towards inner side. All the cells of this portion are suberized except the cambium region.

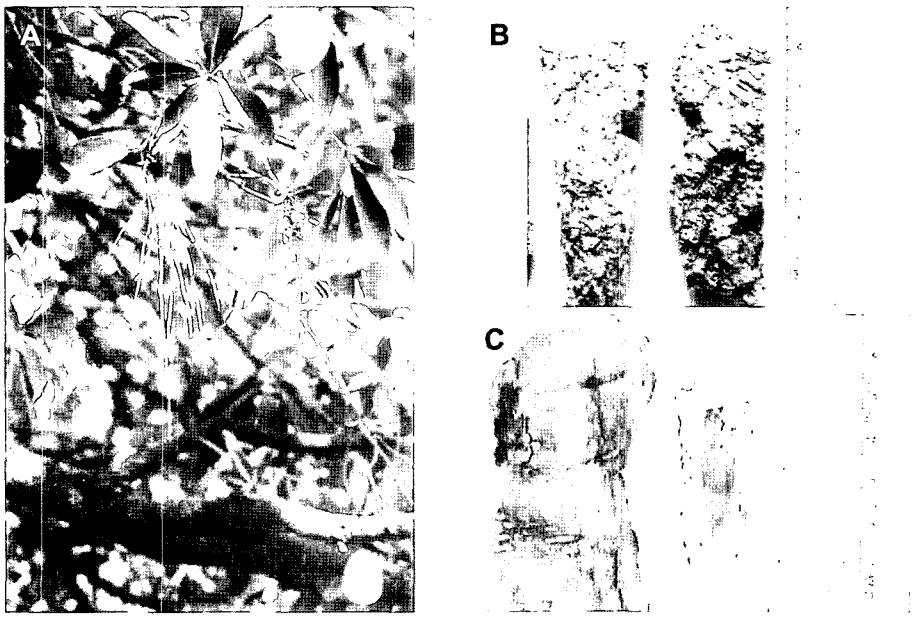

Plate 1. Macroscopy of *Alstonia scholaris* R. Br.
A : A twig, B : stem bark (outer surface), C : stem bark (inner surface)

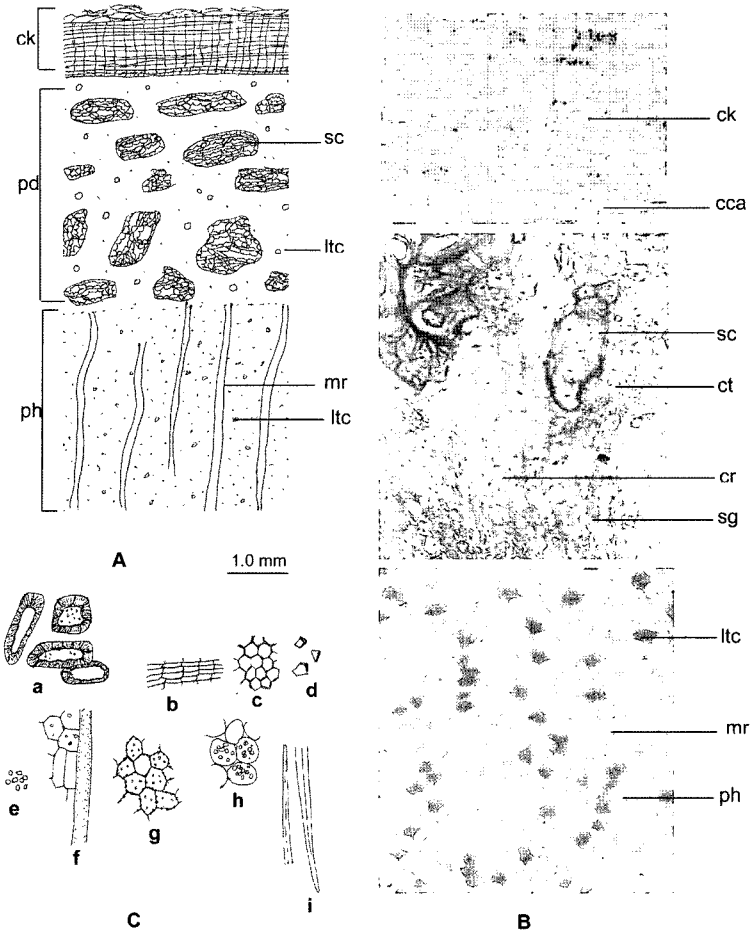

Fig. 1. Microscopy of dried bark of *Alstonia scholaris*.

A : diagrammatic TS of stem bark, B : TS of stem bark, C : powder.

cca, cork cambium; ck, cork; cr, crystals of calcium oxalate; ct, cortex; ltc, latex canals; mr, medullary rays; pd, phelloderm; ph, phloem; stc, stone cells; sg, starch grain; **powder: a,** stone cells; **b,** transversely cut cork cells; **c,** cork cells in surface view; **d,** prismatic crystals of calcium oxalate; **e,** starch grains; **f,** latex canal with adjacent cells; **g,** pitted parenchyma; **h,** parenchyma with starch grain; **i,** fibre.

Table 1. Behaviour of the powdered bark with different chemical reagents

solvent	visible light	254 nm	366 nm
powder	yellow	brown	greenish-grey
powder + nitro-cellulose in ethyl acetate	golden- yellow	brown	yellow
powder + 1N NaOH in methanol	brown	yellowish-brown	yellowish green
powder + nitro-cellulose in ethyl acetate + 1N NaOH in methanol	yellow	brown	yellow
powder + 1N NaOH (aqueous)	yellow	brown	golden-yelllow
powder + 1N HCl	white	coffee	lemon-yellow
powder + dil. HNO_3	yellowish-red	brownish black	brown
powder + dil. H_2SO_4	yellowish-brown	chocolate-brown	coffee

TS inner bark shows outer most layers of crushed cells followed by secondary cortex. The cortical cells are parenchymatous containing abundant simple starch grains. Stone cells are present in the cortical region. Phloem is made up of phloem parenchyma which is somewhat wavy in outline, sieve tubes and companion cells. Laticeferous canals are abundant in the inner bark. Phloem and phelloderm regions consist of many rhomboidal calcium

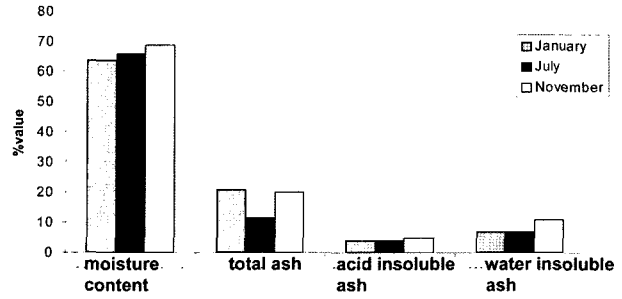

Fig. 2. Comparative moisture content and ash values of *Alstonia scholaris* bark collected in different seasons.

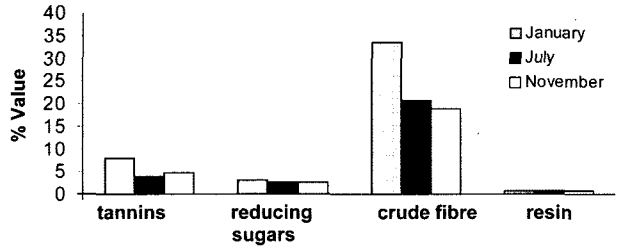

Fig. 3. Comparative physico-chemical constituents of *Alstonia scholaris* bark collected in different seasons.

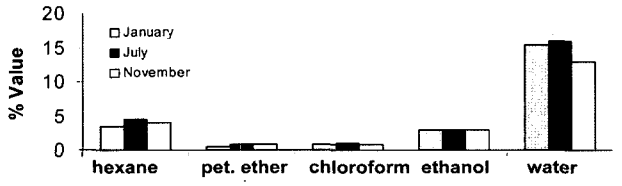

Fig. 4. Comparative successive Soxhlet extractives of *Alstonia scholaris* bark collected in different seasons.

Table 2. Color of the successive Soxhlet extractives of the bark

solvent	visible light	254 nm	366 nm
hexane	yellow	brown	greenish-yellow
chloroform	pale yellow	brown	olive green
acetone	golden-yellow	brown	greenish-yellow
ethanol	glossy brick red	brown	green

oxalate crystals and pitted cells. These pitted cells are filled with alkaloids. Some cells with tannin content are also observed. In the TLS the medullary rays are heterogeneous 2 - 3 cells broad and 5 - 15 cells long (Fig. 1 A & B).

Powder – It shows cork cells in surface and transverse view stone cells of various shapes and sizes, forming a group of 2 to 20, pitted parenchyma filled with starch grains, thin walled parenchyma, fibers 400 - 470 μm long, with blunt ends, latex canal, prismatic crystals of calcium oxalate and starch grains (Fig. 1 C). The behavior of

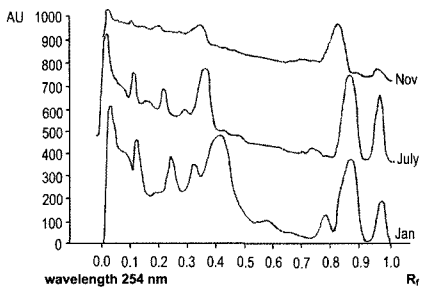

Fig. 5. TLC finger print profile of chloroform extract under UV 254 nm. Jan, January; Nov, November.

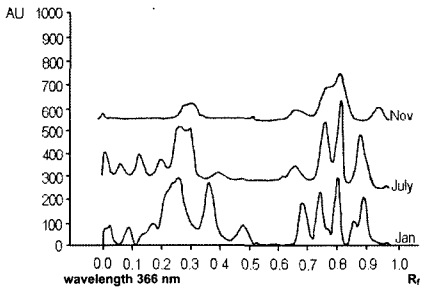

Fig. 6. TLC finger print profile of chloroform extract under UV 366 nm. Jan, January; Nov, November.

Table 3. Comparative account of HPTLC fingerprints of chloroform extract at UV 254 nm and 366 nm

R_f Value	Jan.	July	Nov.	R_f Value	Jan.	July	Nov.
0.07	+	–	–	0.16	–	+	–
0.13	+	+	–	0.24	+	+	–
0.16	+	+	–	0.29	+	+	–
0.24	+	+	–	0.36	+	+	–
0.29	+	+	–	0.42	–	+	+
0.36	+	+	–	0.46	+	+	–
0.42	–	+	+	0.61	+	+	–
0.46	+	–	–	0.74	–	+	+
0.61	+	–	–	0.81	+	+	–
0.72	–	–	+	0.86	+	+	–
0.81	+	+	–	0.92	+	+	+
0.89	+	+	+	0.98	+	+	+
0.98	+	+	+				

powdered bark with different chemical reagents under visible and UV lights is presented in Table 1.

Physico-chemical studies – The comparative physico-chemical values of the bark samples collected in three seasons are depicted in the histograms (Fig. 2 & 3). The percentage of successive Soxhlet extractive are presented in Fig. 4 and the colours of these extractives under visible

and UV light are presented in (Table 2).

The preliminary phytochemical screening of ethanolic extract of the powdered bark shows presence of resins, tannins, alkaloids, carbohydrates, sugars, starch, flavonoids and saponins.

Moisture content and acid insoluble ash shows gradual increase from January to November. Ash value shows significant difference in three seasons. Total tannin was found maximum in January and minimum in June while the resin content and reducing sugars are constant in three seasons. Crude fibre content shows sharp decline from January to July to November.

Comparative thin layer chromatography – The chloroform extract was chromatographed in a solvent system cyclohexane : chloroform: formic acid (25 : 75 : 0.01) and scanning was done under UV 254 and 366 nm (Fig. 5 and 6). The R_f values of all the components observed are given in Table 3.

Conclusion

Collection of bark as a drug should be done carefully in proper season which will definitely increase drug value and positively give good clinical results. The results indicate that the *A. scholaris* bark may show more efficacy when collected in January.

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