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PHARMACOGNOSTIC STUDY AND PHYTOCHEMICAL SCREENING ON STEM BARK OF OROXYLUM INDICUM (L.) VENT

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ABSTRACT

Medicinal plants are an important component of natural resources and they are used as a source of food, medicine, fibres, tannin, gum and allied uses. *Oroxylum indicum* (L.) Vent. (*Bignoniaceae*) also known as 'Sonapatha' is an important endangered medicinal plants of indigenous medical system for over hundreds of years. It has been used as a single drug and as a component for preparation of ayurvedic compound preparations viz. Dashmularistha and Chyavanprash etc. Plants are used in treating in various human ailments like jaundice, dysentery, asthma, allergic disease, urticaria, sore throat, measles, laryngitis, hoarseness and diarrhea. The present paper provides a detailed account of the phytochemical evaluation and pharmacognostic studies of Sonapatha stem bark. The study includes macroscopy, microscopy, and powder microscopy studies, fluorescence study, physicochemical parameters, preliminary phytochemical screening High Performance Thin Layer Chromatography fingerprint profile. Established parameters can be used as standards for identification and quality control of the plants in compound formulations and also preparation of a monograph of the plant.

KEYWORDS: Oroxylum indicum, Phytochemical screening, Physico-chemical studies, HPTLC fingerprints profile.

INTRODUCTION

Medicinal plants play an important role in the prevention and treatment of various human ailments since time immemorial (Samanta et al., 1999). In Indian system of medicines a large

number of herbal and herbo-mineral drugs have been used to cure various types of diseases. Ayurveda is one of the traditional systems of medicine practiced in India back to 6000 BC. (Gupta et al., 2006). *Oroxylum indicum* (L.) Vent. (*Bignoniaceae*) also known as 'Sonapatha' is an important endangered medicinal plants. It is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern China, in Indo-China and the Malaysia ecozone. It is diversely available in the forest of National Park in Assam, India, reported from Sri Lanka (Ceylon) (Kirtikar and Basu, 2001).It is large medium size tree 12-15 meter (40 feet) height. Stem bark colour is off brown. Leaves are 2.5 to 5 inch long, broad, leaflets are 4.5 inch long and 3.5 to 4.5 inch broad having sharp edges. Flowers colour is purple and stalk is 0.5-1.5 feet long. Fruits long curve downward and resemble the wings of a large bird or dangling sickles or swords in the night and 1 to 3.5 feet long, 2.5 to 4.5 inch broad. Seeds are flat 2.5-3.5 inch long and 1.5-2-5 inch in wide. The flowers are born in rainy season and fruiting session in December to March (Dalal and Rai 2004). *Oroxylum indicum* used to various Ayurvedic preparations viz. Chyavanaprasa Awaleha, Amartarista, Dasamula, Dhanawantara Ghrita, Dantyadyarista, Brahma Rasayana, Narayana Taila and mentat (mental drug) etc. (Balkrishna, 2005; Kumar *et al*., 2009; Anonymous, 1998). Sonapatha different parts are used as tannins, dyestuffs and wood (Laupattarakasem *et al*., 2003; Gupta *et al*., 2008). *Oroxylum indicum* stem bark decoction is used to cure cancer (Mao, 2002). In various tribes of India, bark and seeds of the plant are used in fever, stomach disorders, pneumonia and respiratory troubles (Panghal *et al*., 2010; Patil *et al*., 2008, Raut *et al*., 2009). The purpose of this study there are no reports of systematic pharmacognostic investigation of stem bark of this plant. So present study was designed to scientific evaluation of endangered medicinal plant Sonapatha stem bark. The study includes macro and microscopic characters, powder microscopic characteristics, High Performance Thin Layer Chromatography fingerprints, preliminary phytochemical screening and physicochemical parameters. The information generated by this particulars study provides relevant harmacognostical and phytochemical data needed. for proper identification and authentication of Sonapatha stem bark.

MATERIAL AND METHODS

Collection and processing of plant material

The fresh plant stem bark of *Oroxylum indicum* was collected from the, Sati-uiyaforest of Chitrakoot, Satna (M.P.) in the month of March. Samples were authenticated (Jain 1991, Kirtikar and Basu 1935, Verma *et al*.1993) by Botanist Dr. Manoj Tripathi of Ayurveda

Sadan (Research Laboratory), Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/109/2016) prepared as per standard procedure (Jain and Rao 1977) and maintained in the herbarium of faculty of Science and Environment, Mahatma Gandhi Chitrakoot Gramodaya University, Chitrakoot, Satna (M.P.) for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of High Performance Thin Layer Chromatography fingerprint profile.

Macroscopic study

Macroscopic or organoleptic characters like appearance, colour, odour and taste were evaluated.

Powder microscopic study

The dried stem bark were subjected to powdered and completely passes through 355 µm IS Sieve (old sieve number 44) and not less than 50% pass on through 180 µm IS Sieve (old sieve number 85). About 2 g of powder washed thoroughly with potable water, pour out the water without loss of material. Mounted a small portion in glycerine, warmed a few mg with chloral hydrate solution, wash and mounted in glycerin. Treat a few mg with iodine solution and mount in glycerine, about 1 g of powder warmed over water bath with Chloral hydrate solution till brown fumes appear, cool and wash with water thoroughly and mount a small portion in glycerin and seen under microscope at 40X x 10X magnification of the Trinocular Research Microscope (Evans 2003and Kokate 2006).

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid insoluble ash value and water soluble ash were calculated (Anonymous 2000 and Anonymous 2007).

Preliminary phytochemical studies

Preliminary phytochemical tests were carried out on ethanolic and water extract for the presence\absence of phyto-constituents like alkaloids, flavanoids, steroids, terpenoids, tannins, resins, carbohydrates, proteins and saponins (Tripathi and Sikarwar 2015, Tripathi *et al*. 2015).

High Performance Thin Layer Chromatography (HPTLC)

For High performance thin layer chromatography, the powdered stamens 5 gm of sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60. thick walled long fibres, prismatic crystals of calcium oxalate, longitudinally cut medullary rays and transversely cut medullary rays with parenchymatous cells (Figures 1 to 8).

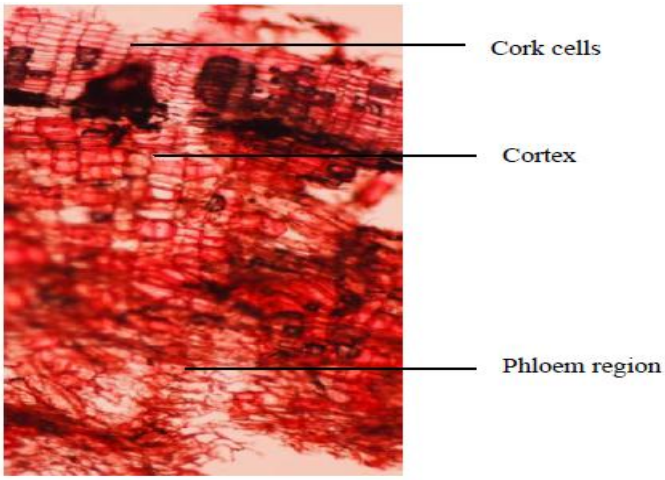

Figure 1: Detailed TS of Sonapatha stem bark.

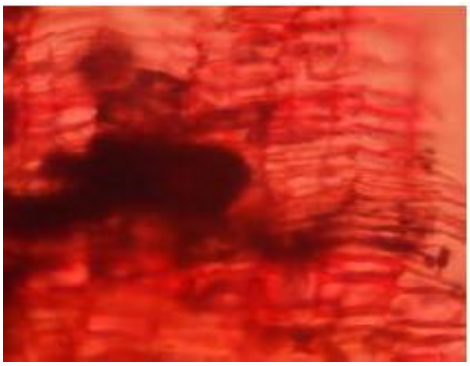

Figure 2: Cork cells in sectional view.

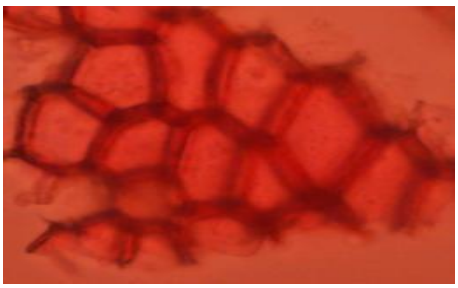

Figure 3: Cork cells in surface view.

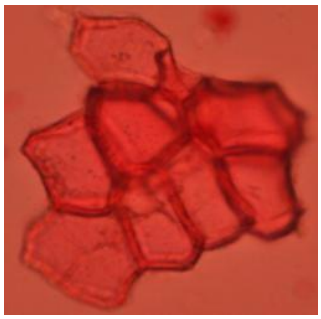

Figure 4: Groups of stone cells.

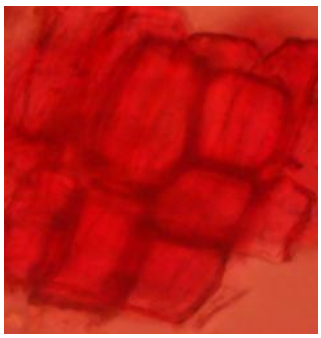

Figure 5: Parenchymatous cells.

Figure 6: Thick walled fibres.

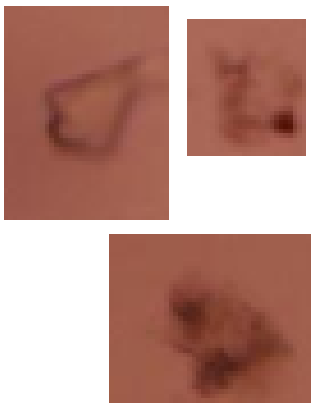

Figure 7: Prismatic crystals of calcium oxalate.

Figure 8: Longitudinally cut medullary rays.

F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of *Toluene: Ethyl acetate (7:3* v\v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, Thin Layer Chromatography plate was dried with the help Figure-1 Detailed TS of Sonapatha stem bark of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at 254nm, 366nm and day light with Win cat software and R_f values noted (Ansari 2006, Choudhary et al. 2014).

RESULTS AND DISCUSSION

Macroscopic, microscopic and powder microscopic study

Sonapatha stem bark colour is off brown, taste acrid, bitter, odour astringent. Detailed Transverse Section of Sonapatha stem bark shows mu8-12 layered cork cells, cortex wide consisting thich walled rectangular parenchymatous cells some are embeded with prismatic crystals of calcium oxalate, wide lumen, round to hexagonal stone cells present in the cortex. medullary rays present. Stem bark powder examined under microscope shows cork cells in sectional view, thick walled cork cells in surface view, parenchymatous cells, thick walled with wide lumen tetragonal to hexagonal group of stone cells.

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in (Table 1).

Table 1: Physico-chemical analysis of the Sonapatha stem bark.

S. No.	Parameters	Results
1	Foreign matter	1.9%
2	Loss on drying at 105^0C	5.24%
3	Ethanol-soluble extractive	14.50%
4	Water- soluble extractive	21.15%
5	Total ash value	11.4%
6	Acid-insoluble ash value	2. 4%

Preliminary phyto-chemical investigation

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. Phyto- chemical results of the drug are given in (Table 2).

Table 2: Phytochemical analysis of Sonapatha stem bark

S. No.	Name of phyto-constituents	Result
1	Alkaloids	Present
2	Carbohydrates	Present
3	Protein	Present
4	Saponin	Present
5	Steroids	Present
6	Resin	Absent
7	Tannin	Present
8	Flavonoid	Absent

of this drug. High performance thin layer chromatography finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The High performance thin layer chromatography profile also helps to identify and isolate's important phyto-constituents. These finding could be helpful in identification and authentication.

Table 3: Rf values of HPTLC finger prints profile of test solution of Sonapatha stem bark.

R_f values	Test solution of Sonapatha stem bark		
	At 366 nm(before derivatization)	At 366nm (after derivatization)	UV light (after derivatization)
R_f1	0.04 (light pink)	0. 06 (light pink)	0.32 (light black)
R_f2	0.08 (dark pink)	0.18 (light brown)	0.54 (brown)
R_f3	0.32 (brown)	0.32(yellow)	0.60 (brown)
R_f4	0.34 (reddish brown)	0. 34(sky blue)	0.70 (green)
R_f5	0.60 (dark red)	0.60(yellow)	0.80 (dark brown)

High performance thin layer chromatography

High performance thin layer chromatography

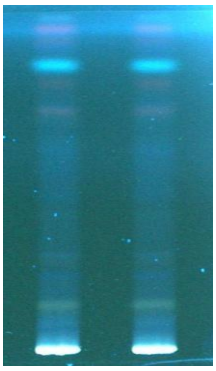

Figure 9: HPTLC fingerprint profile at 366nm before derivatization.

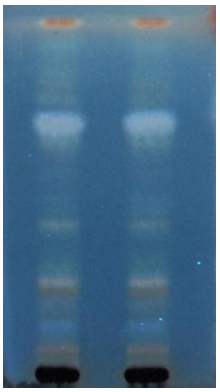

Figure 10: HPTLC fingerprint profile at 366nm after derivatization.

Figure 11: HPTLC fingerprint profile at UV After derivatization.

(HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the Thin Layer Chromatography plate. Major spots R_f values with colour were recorded under, 366nm, after derivatization 366nm and UV light. Chromatogram profile and R_f values are given (Figure.9 -11 and Table -3).

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Sonapatha stem bark. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity.

CONCLUSION

Oroxylum indicum has numerous uses in traditional medicine to treat several ailment *viz.* in treating cancer, fever, stomach disorders, pneumonia and respiratory troubles etc. It is also used to various Ayurvedic preparations *viz.* Chyavanaprasa Awaleha, Amartarista, Dasamula, Dhanawantara Ghrita, Dantyadyarista, Brahma Rasayana, Narayana Taila and mentat (mental drug) etc. Due to its wide therapeutic importance it is worthwhile to standardize it for use as drug. The present study reveals. phytochemical screening and pharmacognostic study of drug *Sonapatha*, which would be of immense value in botanical identification and authentication of plant drug may help us in preventing its adulteration.

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