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PHYTOCHEMICAL ANALYSIS OF BARK SKIN OF SARACA INDICA (ASHOKA) AND SHOREA ROBUSTA (SHAL)

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ABSTRACT

Shorea robusta (Shal) and *Saraca indica* (Ashoka) are important traditional Indian medicinal plants used in various ailments and rituals. The use of different parts of these plants like leaves and resins as a medicament for treatment of various inflammatory conditions is well documented in literature. However, the studies on phytochemical constituents and the medicinal properties in the bark skin of these plants are scanty. Initially the bark samples were subjected to macroscopy and microscopy under which organoleptic characters like appearance, size, shape, colour, taste, odour etc. were noted. The aqueous and alcoholic extracts of bark skin of *Saraca indica* and *Shorea robusta* were prepared and evaluated for their phytochemical analysis using different methods. The extractability of aqueous and alcoholic extract of bark skin of *Saraca indica* and *Shorea robusta* were found to be 20.26%, 18.33% and 30.17%, 24.57% respectively. Phytochemical analysis of both the extracts of Ashoka revealed the presence of tannin, essential oil, terpenoid and steroid whereas both extracts of Shal revealed the presence of tannin, resins, terpenoid, and essential oil.The analgesic properties of bark skin of Ashoka and Shal mentioned in the Ayurveda literature can be attributed to the presence of different bioactive compounds such as alkaloids and steroids as revealed by phytochemical analysis of the present study.

KEY WORDS: *Saraca indica*, *Shorea robusta*, phytochemical analysis.

INTRODUCTION

Ashoka is the most ancient tree of India, generally known as "shok briksh", having botanical name *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging family *Caesalpinaceae*. *Saraca indica* is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational and having antiestrogenic activity against menorrhagia[1]. The phytochemical analysis and analgesic property of leaf extract of *Saraca indica* has been evaluated in previous studies,[2,3] but the phytochemical analysis and analgesic property of its bark (Ashok Chhal) which is mentioned in Ayurveda literature (Charak Samhita) has not been studied so far.

Shal (*Shorea robusta* Roxb. ex Gaertn. f) belongs to the family *Dipterocarpaceae* (two-winged fruit) which is a large sub-deciduous tree, found in deciduous forests throughout the India, covering part of North, East and Central India up to an altitude 900 – 1700 m. The various parts of the plant are traditionally used in India for the treatment of diverse ailments. The oleoresin exuded from the cut bark has astringent and detergent properties[4]. In Ayurveda, the leaves are used as anthelmintic and alexiteric. *S. robusta* leaf extract was found to possess significant analgesic activity[5,6] and its resin along with some other constituents has also shown potential in wound healing[4,7]. However, limited studies are available on the phytochemical analysis and analgesic property of its bark skin which is mentioned in Ayurveda literature (Charak Samhita).

In view of the above medicinal importance the present study was undertaken to know the phytochemical constituents in the bark skin of Ashoka and Shal.

MATERIAL AND METHODS

The dried stem bark of Ashoka and Shal received from Government Ayurvedic College, Nagpur was used for the study. Initially all the samples were subjected to bark macroscopy and microscopy under which organoleptic characters like appearance, size, shape, colour, taste, odour etc. were noted.

Preparation of Extract

The alcoholic and aqueous extracts were prepared by the method described by Rosenthaler, 1930[8].

Preparation of Aqueous Extract

100 gm of powdered materials (*Saraca indica* and *Shorea robusta*) were taken in flasks in which 1000 ml of distilled water was added. The flasks were kept on heating mantle for boiling. Heating was allowed till the content was reduced to half. The contents were cooled and filtered and then poured in petri-dishes and placed on hot plate for complete evaporation. The extract was cooled at room temperature and weighed to calculate the extractability percentage and stored in a dessicator. The colour of extracts of *Saraca indica* was blackish dark brown and *Shorea robusta* was dusty brown.

Preparation of Alcoholic Extract

50 gm of powdered material was subjected to extraction in a Soxhlet's extraction apparatus using absolute ethanol. These were heated on heating mantle till the colourless solvent started returning back in the reservoir. Then the contents were transferred to dry petri-dishes and kept at room temperature till the complete evaporation of the solvent. The petri-dishes were weighed to calculate the extractability percentage and finally stored in a dessicator. The colour of extracts of *Saraca indica* was reddish brown and *Shorea robusta* was dark brown.

Phytochemical Analysis

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug using different methods (Table I). General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them[9].

Table I: Methods Used For Phyochemical Analysis of Shal And Ashoka.

S.No.	Chemical compound	Method used
1.	Alkaloids	Dragendorff's test, Hager's test, Wagner's test, Mayer's test
2.	Carbohydrates	Anthrone test, Benedict's test, Fehling's test, Molisch's test
3.	Flavonoids	Shinoda's test
4.	Triterpenoids	Liebermann-Burchard's test
5.	Proteins	Biuret's test, Millon's test
6.	Resins	The extract dissolved in acetone and the solution was poured into distilled water. Turbidity indicated the presence of resins.
7.	Saponins	In a test tube containing about 5 ml of an aqueous extract of the drug add a drop of sodium bicarbonate solution, shake the mixture vigorously and leave for 3 mints. Honeycomb like forth is formed.
8	Steroids	Liebermann-Burchard's test, Salkowski Reaction

9	Tannins	To 1 ml of plant extract, a few drops of 5% FeCl3 solution was added. Green colour indicated the presence of gallotannins while brown colour tannins.
10	Starch	0.015g of Iodine and 0.075g of Potassium Iodide was dissolved in 5 ml of distilled water and 2 – 3 ml of aqueous extract of drug was added. Blue colour indicated the presence of starch.

Thin Layer Chromatography (TLC)

TLC was performed on TLC plates prepared with silica gel containing binder. A known quantity of sample was dissolved in a known volume of solvent and the sample applied on pre-coated TLC plates. Development of the chromatogram is effected after the solvent of the applied sample was completely evaporated. Examination of developed plates done under UV254nm and UV366nm, followed by derivatisation by using Liebermann reagent (LB) and anisaldehyde and other spray reagent.

RESULTS

Test Report of Shal

1. Macroscopic Examination

Macroscopially the outer surface was rough, greyish brown in colour, had thick, protuberated, transverse cracks and fine longitudinal striation. The inner surface was brown in colour, fibrous, the fibres were longitudinal and oblique and oblique cracks were prominent.

2. Microscopy of Powder

Powder was greyish brown in colour and microscopial examination showed fragments of cork cells, stone cells, thick walled fibre patches of adjacent cells of fibres with prismatic crystals of calcium oxalate, medullary rays with interlocking arrangements.

3. Organoleptic Test

The odour was not characteristic but the taste was slightly bitter (astringent).

Table II: Different Chemical Properties of Shal (*Shorea Robusta*)

1.	**Chemical reaction with different reagents**			
	S. No.	**Treatmemt**	**Colour in ordinary light**	**Colour in UV lamp Long wave- 366 nm**
	1.	Drug as such	Brown	Brown
	2.	Drug + Nitrocellulose	Dark brown	Brown
	3.	Drug + Picric acid	Light brown	Brown
	4.	Drug + HCl $_{conc.}$	Dirty brown	Brown

	5.	Drug + H_2SO_4 conc.	Black	Brown
	6.	Drug + HNO_3 (50%)	Orange	Brown
	7.	Drug + 1 N NaOH in MeOH	Black	Brown
	8.	Drug + 1 N NaOH in MeOH + Nitrocellulose	Black	Brown
	9.	Drug + NH_4OH	Blackish brown	Brown
	10.	Drug + $FeCl_3$	Green	Brown
	11.	Drug + Acetic acid Glacial	Brown	Brown
	12.	Drug + Sudan-III	Reddish brown	Brown
2.	**Loss on drying (w/w %)**		**6.5 %**	
3.	**Ash Values (w/w %)**			
	a) Total ash		3.36 %	
	b) Acid insoluble ash		1.15 %	
	c) Sulphated ash		5.15 %	
4.	**Extractive Values (% w/w)**			
	a) Water soluble extractives		30.17 %	
	b) Alcohol soluble extractives		24.57 %	
5.	**Successive extraction**			
	a) Petroleum ether 60 - 80^0 C		3.73 %	
	b) Methanol		21.65 %	
6.	Chemical test qualitative		Tannin + ve Resins + ve Essential oil + ve Terpenoid + ve	
7.	Swelling Index		0.5 ml/gm	
8.	Foaming index		100	
9.	Volatile Content		2.5 %	

Test Report of Ashoka (*Saraca indica*)

1. Macroscopic Examination

Macroscopically the bark was channeled, externally it was dark grey in colour, smooth with circular lenticels and transversely ridged, sometimes cracked. Internally it was reddish-brown with fine longitudinal strands and fibres; fracture splintery exposing striated surface; a thin whitish continuous layer was seen below the cork layer.

2. Microscopic Characters of Powder

Powder showed periderm – phellem, phellogen and phelloderm, stone cells with many patches of sclereids; parenchyma cells containing yellowish mass and prismatic crystals of calcium oxalate; phloem parenchyma, sieve tube with companion cells and phloem fibres and crystal fibres.

3. Organoleptic Test

The odour was not characteristic and the taste was bitter.

Table III. Different Chemical Properties of Ashoka (*Saraca Indica*)

1.	**Chemical reaction with different reagents**			
	S. No.	**Treatment**	**Colour in ordinary light**	**Colour in UV lamp Long wave- 366 nm**
	1.	Drug as such	Brown	Yellow
	2.	Drug + Nitrocellulose	Brown	Brown
	3.	Drug + Picric acid	Yellowish brown	Brown
	4.	Drug + HCl $_{conc.}$	Dark brown	Brown
	5.	Drug + H_2SO_4 $_{conc.}$	Black	Brown
	6.	Drug + HNO_3 $_{(50\%)}$	Brown	Brown
	7.	Drug + 1 N NaOH in MeOH	Greenish brown	Brown
	8.	Drug + 1 N NaOH in MeOH + Nitrocellulose	Brown	Brown
	9.	Drug + NH_4OH	Grey	Brown
	10.	Drug + $FeCl_3$	Green	Brown
	11.	Drug + Acetic acid $_{Glacial}$	Grey	Brown
	12.	Drug + Sudan-III	Cherry brown	Brown
2.	**Loss on drying (w/w %)**		13.5 %	
3.	**Ash Values (w/w %)**			
	a) Total Ash		10.66 %	
	b) Acid Insoluble Ash		1.33 %	
	c) Sulphated Ash		12.45 %	
4.	**Extractive Values (% w/w)**			
	a) Water Soluble Extractives		20.26 %	
	b) Alcohol Soluble Extractives		18.33 %	
5.	**Successive extraction**			
	a) Petroleum ether 60 - 80^0 C		2.54%	
	b) Methanol		20.65%	
6.	Chemical test qualitative		Tannin + ve Essential oil + ve Terpenoid + ve Steroid + ve	
7.	Swelling Index		1.0 ml/gm	
8.	Foaming index		Less than100	

The percent extractability of aqueous and alcoholic extracts of *Saraca indica* was found to be 20.26% and 18.33% respectively. The percent extractability of aqueous and alcoholic extracts of *Shorea robusta* was found to be 30.17% and 24.57% respectively. The colour of aqueous extract of Ashoka and Shal were blackish dark brown and dusty brown respectively and semisolid in consistency. The alcoholic extract of Ashoka and Shal were reddish brown and dark brown respectively and solid consistency.

Thin Layer Chromatographic Study of Ashoka And Shal

The petroleum ether extract (Soxhlet) of Ashoka exhibited several brownish spots on chromatoplate indicating the presence of different fatty acids in the extract at Rf - 0.25, 0.31, 0.40, 0.50 & 0.64. Similarly petroleum ether extract of Shal also exhibited several brownish spots on chromatoplate indicating the presence of different fatty acids in the extract at Rf - 0.38, 0.43, 0.50 & 0.58.

The methanol extract (Soxhlet) of Ashoka exhibited several spots on chromatoplate at Rf - 0.14, 0.20, 0.24, 0.28, 0.37, 0.52 and 0.59. Similarly methanol extract of Shal also exhibited several spots on chromatoplate at Rf - 0.02, 0.17, 0.20, 0.24, 0.31, 0.40, 0.52, 0.61, 0.71 & 0.77.

UV Exposure Studies

Under UV (254 nm), 8 florescent zones were visible at Rf - 0.10, 0.15, 0.18, 0.25, 0.28, 0.30, 0.35 and 0.48 in case of Shal whereas Ashoka displayed 4 distinct florescent zones at Rf – 0.10, 0.20, 0.40 & 0.45. On exposure under UV (366 nm) the same chromatoplate displayed 3 florescent zones at Rf - 0.20, 0.26 and 0.32 in case of Shal and 3 florescent zones at Rf – 0.36, 0.40 and 0.70 in case of Ashoka.

DISCUSSION

The macroscopic description of bark of *Saraca indica* was in agreement with previous literature [11,12,13]. The different phytochemical analysis of bark skin of Ashoka revealed the presence of tannin, essential oil, terpenoid and steroid. In previous studies the presence of procyanidin, epicatechin, 11'- deoxyprocyanidin B, catechin, leucopelargonidin and leucocyanidin were reported from bark skin[1]. In addition to these compounds the dried bark of Ashoka also reported to contain glycoside, flavanoids and saponins[10]. In another study the phytochemical analysis of methanolic extract of leaves of *Saraca indica* revealed the presence of tannins, triterpenoids, saponin, flavonoids and glycosides[2]. The use of *Saraca indica* as anti-inflammatory, analgesic, spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour, anti-progestational, antiestrogenic against menorrhagia and anti-cancer as mentioned in Ayurveda literature may be due the presence of above phytochemical constituents. It was previously studied that the oleoresin of the *Shorea robusta* Gaertn has the chemical constituents such as nor -triterpene, dammarenolic acid, asiatic acid, dipterocarpol, triterpenic acid, tannic acid and phenolic content and possesses antibacterial, analgesic and wound healing effect.[14] Another study showed the presence of alkaloids,

carboxylic acids, fatty acids, phenols, saponins and steroids in the extracts of *Shorea robusta*, wheras catechols, coumarins, proteins, tannins, and volatile oils were found in low concentrations [15]. In other studies the preliminary phytochemical screening of the methanol extract of *S. robusta* leaves revealed the presence of alkaloids, triterpenes, phenols, anthraquinones and cardiac glycosides[5] whereas the ethanolic extract of resin powder revealed the presence of triterpenoids, sterols and resin[4]. However, no phytochemical study on bark skin of Shal was found in literature but the above studies on leaves corroborates with the finding of the present study in which tannin, essential oil, resins, terpenoid were reported to be present.

CONCLUSION

The percent extractability of aqueous and alcoholic extracts of *Saraca indica* was found to be 20.26% and 18.33% respectively and the percent extractability of aqueous and alcoholic extracts of *Shorea robusta* was found to be 30.17% and 24.57% respectively. Phytochemical analysis of both the extracts of Ashoka revealed the presence of tannin, essential oil terpenoid and steroid. In Shal tannin, essential oil, resins and terpenoid were found to be present. The different medicinal properties of Ashoka and Shal mentioned in the Ayurveda literature can be attributed to the presence of these bioactive compounds as revealed by phytochemical analysis. However further clinical studies are necessary to explore the therapeutic efficacy of bark skin of Ashoka and Shal.

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