AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems
Research Article

PHYTOCHEMICAL SCREENING OF *SAPTAPARNA* (*ALSTONIA SCHOLARIS* R. Br.) BARK

Kamlesh Bhogayata[1], B. R. Patel[2], Bhakti Chhaya[3], Krunal Doshi[4*], Joban Modha[5]

[1]Assistant Professor, Dept. of Dravyaguna, Sheth J. P. Govt. Ayurved College, Bhavnagar, Gujarat, India.

[2]Assistant Professor, Dept. of Dravyaguna, IPGT & RA, Gujarat Ayurved University, Jamnagar, India.

[3]Assistant Professor, Dept. of Shalakya, Sheth J. P. Govt. Ayurved College, Bhavnagar, Gujarat, India.

[*4]Lecturer, Dept. of Dravyaguna, [5]Principal, Indian Institute of Ayurvedic Pharmaceutical Sciences, Gujarat Ayurved University, Jamnagar, Gujarat, India.

Article info

Article History:

Received: 25-03-2015

Accepted: 23-05- 2015

KEYWORDS: *Saptaparna, Alstonia scholaris* R. Br., Phytochemical, Physicochemical, Thin layer chromatography.

***Corresponding Author**
Dr. Krunal Doshi
Lecturer
Dept. of Dravyaguna
Indian Institute of Ayurvedic Pharmaceutical Sciences
Gujarat Ayurved University
Jamnagar, Gujarat, India.
Mob: +919898907572
Email: krunaldoshi760@gmail.com

ABSTRACT

The Ayurvedic Pharmaceutical Industries use barks of many herbal drugs like *Neema, Vata, Udumbara, Parisha, Saptaparna, Plaksha, Baboola* etc. for the manufacturing of different herbal medicines. The stem bark of *Alstonia scholaris* R. Br. commonly known as *Saptaparna* in *Ayurved*, is in demand, due to its antipyretic, galactogogue, cardiotonic, anticancer, anti-helminthic etc. activities. But, the identification and separation of these barks drugs from each other is very difficult. Hence, a preliminary study has been done to ensure the basic phytochemical profile of *A. scholaris* R. Br. stem bark for identification of herbal drug. Physicochemical parameters, preliminary phytochemical screening, quantitative estimation of alkaloid and Thin Layer Chromatography (TLC) were carried out in the present study. Physicochemical data revealed, there is more amount of water soluble extractive value (27.10% w/w) than alcohol soluble extractive value (7.40% w/w). Facts of phytochemical screening showed presents of alkaloid, carbohydrate, tannin, saponin, flavanoids and cardiotonic glycosides in the sample. Result of TLC identification showed 7, 8, and 6 spots under short UV, long UV and after spray reagent respectively. The present study on phytochemical investigation of *A. scholaris* R. Br. bark will be helpful in developing standards for quality, purity and sample identification of this plant.

INTRODUCTION

Phytochemistry is a branch of science that deals with the chemicals obtained from plants with desirable bio-logical activities.[1] Several conventional chemical based drugs, herbal and plant based medicinal drugs, also known as herbal medicine, traditional medicine or complementary medicines are quite popular in developing countries like India. Plants have been a major source of therapeutic agents since time immemorial and traditional herbal systems of medicine, like *Ayurveda*, resulted in the revival of ancient traditions of medicine.[2] Therefore indispensable scientific authentication of these medicinal values of plants will pave the way for future herbal drugs.[3] Herbal medicine has become an integral part of standard healthcare, based on a combination of time-honored traditional usage and ongoing scientific research. Rising interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety.[4]

The Apocynaceae family consists of about 250 genera and 2000 species of tropical trees, shrubs and vines. This family is known for plants that have a very high biological activity and medicinal properties.[5] Some of the well-known plant of this family such as *Rauwolfia serpentina*, *Alstonia scholaris* and *Alstonia venenata* are known for the ample amount of medicinal potential.[6] *Saptaparna-Alstonia scholaris* R. Br. is an important medicinal plant in folklore medicine. It grows throughout India, in deciduous and evergreen forests and in plains. Juice of leaves and tincture of the bark acts as a powerful galactogogue and also used in cases of snake bite.[7] Milky juice of the plant is applied on wounds and ulcers. The bark is bitter, acrid, astringent, digestive, laxative, thermo genic, antipyretic, galactogogue, cardiotonic and tonic. It is

useful in abdominal disorders, fevers, leprosy, skin diseases, chronic and foul ulcers, asthma, bronchitis and helminthiasis.[8] The bark extract induces the cellular immune response at low doses and inhibited the delayed type of hypersensitivity reaction at high doses.[9] The alkaloid fraction of *A. scholaris* were founded to have a potential anticancer agent.[10] It's bark extract showed chemo preventive potential against skin tumor genesis in Swiss albino mice.[11]

Barks of different herbal drugs are greatly used by the Ayurvedic Pharmaceutical Industries. Stem bark of *Alstonia scholaris* R. Br. is also used as one of the ingredients in many of the formulations. But the different barks of herbal medicines seem are very similar in size, shape, color and other morphological characters. Now the question arises related to identity and genuineness of the stem bark of *Saptaparna* (*A. scholaris* R. Br.). However, phytochemical screening performs the major parts to overcome this problem and assists us greatly for the correct identity. Hence, an attempt has been made to ensure properties of *Saptaparna* (*Alstonia scholaris* R. Br.) stem-bark through analytical study by following materials and methods.

MATERIALS AND METHODS

Procurement of sample

Bark of *Alstonia scholaris* R. Br. was collected from the periphery of Gujarat state by Pharmacy department of IPGT & RA, GAU, Jamnagar. Further the authenticity of the sample was confirmed by the experts of Gujarat Ayurved University and by comparing their characters mentioned in various floras. (Fig. 1)

Preservation of sample

The drug was thoroughly washed with running water and cut into small pieces and some pieces were preserved in the solution containing Formalin: Glacial Acetic Acid: Ethanol 70 % in 5: 5: 90, while some pieces were dried in sunlight and powdered for studying the different activities. (Fig. 2)

Physicochemical study

Moisture content, ash values (total ash, acid insoluble ash), and extractive values (alcohol soluble extractive, water soluble extractive), pH value, particle size were determined by following standard analytical procedures.[12,13]

Preliminary phytochemical screening

Five grams coarse powder of the stem bark was subjected for extraction with methanol (100 ml), keeping it for overnight with initial occasional shaking up to 6 hrs. and then set aside. After 24 hrs, it was filtered and alcoholic extract was collected. Similarly, water extract was prepared. Both the extracts were evaporated to dryness. The dried extracts were weighed, and percentage yield was calculated. The extracts were used for preliminary phytochemical screening with a set of various chemical tests viz., Dragendorff's Mayer's, Hager's, and Wagner's tests for alkaloids; ferric chloride, lead acetate, potassium dichromate, and dilute iodine tests for tannins and phenolics; and foam test for saponin glycosides.[14]

Quantitative estimation of alkaloid

As Alkaloids were found to be present in the sample by qualitative analysis, it was determined quantitatively following two different methods.

A. Method – 1 accurately weighed 5 gm sample was taken in volumetric flask & was wetted with dilute HCl. After 24 hrs it was made basic by adding dilute Ammonium hydroxide. Now it was transferred to 250 ml separating funnel and extracted with chloroform. It was repeatedly washed 2 to 3 times. The chloroform extract were mixed and evaporated to dryness in an empty evaporating dish which was previously weighed. Thus, the amount of alkaloid in the sample was obtained with respect to air dried sample.[15]

B. Method – 2 after taking 5 gm test sample in dry petridish, it was moistened with 0.5 N NH_4OH and was allowed to dry. By adding 100 ml ethyl acetate to both the sample, it was transferred to a conical flask. Shake thoroughly. Filter the same and concentrate it in a previously weighed evaporating dish. The weight of alkaloid obtained was calculated with respect to air dried sample.[16]

Thin Layer Chromatographic identification (TLC Study)

TLC is widely used for separation of an individual compound from a mixture. Observing the intensity one can make identification of the compound and Rf value of separated spots. TLC has become important tool for both qualitative and quantitative analysis.

Chromatographic Condition

- **Preparation of sample**: Sample was extracted for obtaining the alkaloid fraction by following the method as described earlier and those alkaloid fractions was used for T.L.C. study.
- **Stationary phase:** Silica gel GF_{254} Pre-coated plates (Merck).
- **Plate activation:** Plate was activated in oven at 110^0 C for 60 min.
- **Mobile phase:** Chloroform: 5%MeOH NAOH (11:1)
- **Spray reagent:** Dragendroff's reagent
- **Application of sample:** Extract of the sample was spotted on TLC plate and TLC plate placed vertically in the saturated chamber as per standard procedure. The solvent is allowed to run. Rf values of all the resolved spots were noted down.
- **Visualization:** 1.Under short UV (254 nm), 2. Under long UV (366 nm), 3. After spraying with Dragendroff's reagent.

RESULTS AND DISCUSSION

Physicochemical study

Table-1 shows the values obtained of the different parameters studied. It indicates 1.72 % of moisture present in the sample as well as the nature of sample whether the drug is hygroscopic or not by which drug can protect longer period of time. Stem bark of *A. scholaris* R. Br. having more amount of water soluble extractive value (27.10% w/w) than alcohol soluble extractive value (7.40% w/w). It also represent the quality of the sample by indicating the amount of present total ash in sample i.e. 7.50% w/w. Value of silica and silicate (Acid insoluble ash) is very low in the sample (1.92%), hence the particles of sand are very less and the sample is of high quality. Stem bark is also very near to neutral pH value i.e. 6.97. The Particle consistency of powdered is carried out to determine the different type of particles size present in that drug sample. The Particle size of test samples is not much variable except the particles below mesh no.120. Particle size measuring parameter indicates the nature of powder, in which 91.80 % powder is coarse powder from the taken sample.

Table 1: Physicochemical data of *Saptaparna* (*Alstonia scholaris* R. Br.) stem bark

S.No.	Parameters	Stem Bark
1.	Loss on drying	1.72 % w/w
2.	Water soluble extractive	27.10 % w/w
3.	Methanol soluble extractive	7.40 % w/w
4.	Ash value	7.50 % w/w
5.	Acid insoluble Ash	1.92 % w/w
6.	pH value	6.97
7.	Particle size	
	A. above 60 mesh	91.80 % w/w
	B. between 60-85 mesh	4.60 % w/w
	C. between 85-120 mesh	2.43 % w/w
	D. below 120 mesh	1.13 % w/w

Preliminary Phytochemical screening

The qualitative test conducted for alkaloids by using Mayer's reagent and Dragendroff's reagent was positive in the sample. Some other test like carbohydrate, tannin, saponin, flavanoids and cardiotonic glycosides were also positive in the sample of *A. scholaris* R. Br. Stem bark. The test conducted to detect the phenols, steroid and anthroquinone glycosides were negative in the sample. (Table 2)

Table 2: Phytochemical profiles of *Saptaparna (Alstonia scholaris* R. Br.) stem bark

S. No.	Tests	Name of reagents	Stem bark
1.	Alkaloids	Mayer's reagent	Positive
		Dragendroff's reagent	Positive
2.	Carbohydrate	Molisch's test	Positive
3.	Tannin	Ferric chloride reagent	Positive
		5% lead acetate & KOH	Positive
4.	Phenols	Neutral $FeCl_3$	Negative
5.	Saponin	Lead acetate test	Positive
6.	Flavanoid	Shinoda's test	Positive
7.	Steroid	Salkowski reaction	Negative
8.	Cardiotonic glycosides	Keller – Kiliani test	Positive
9.	Antheraquinone glycosides	Borntrager's test	Negative

Quantitative estimation of alkaloid

Quantitative estimation of alkaloid was done by two different methods in which the method adopted secondly proved to get more amount of alkaloid from the stem bark of *saptaparna - A. scholaris* R. Br. The amount of alkaloid by the first method is 0.04% and 0.46% by second method which clearly indicate the difference. (Table 3)

Table 3: Quantitative estimation of alkaloid from the sample

S. No.	Sample	Alkaloid (% w/w)	
		Method-1	Method - 2
1.	*Saptaparna* (*A. scholaris* R. Br.) stem bark	0.04 %	0.46 %

Thin Layer Chromatographic identification (TLC Study)

Table 4 represents the different R_f values observed under the different visualization. Among them highest number (8) of sports were observed under long UV (366 nm) and lowest (6) number of spots were observed after spraying of Dragendroff's reagent. The R_f values 0.16 and 0.24 are observed under all three visualization, revealing the chemical separate out at these R_f values are similar. This also denotes the separation of chemical present in the stem bark and more clearly detected under 366 nm wave length. By this method the drug genuineness can be easily identified and it will be helpful for the Ayurvedic Pharmaceutical Industrial as basic tool for identification of stem bark of *Saptaparna* (Fig. 3).

Table 4: R_f values of *Saptaparna* (*A. scholaris* R. Br.) stem bark under different visualization

S. No.	Visualization	No. of spots	R_f values
1.	Short UV (254 nm)	7	0.11, 0.16, 0.24, 0.28, 0.41, 0.61, 0.92
2.	Long UV (366 nm)	8	0.16, 0.24, 0.31, 0.47, 0.54, 0.64, 0.74, 0.85
3.	After spraying of Dragendroff's Reagent	6	0.07, 0.16, 0.24, 0.35, 0.43, 0.46

CONCLUSION

Herbal bark drugs are derived from varied resources with similarity in external features, leads to standardizations of herbal drugs for appropriate identification of drugs. The stem bark of *Saptaparna* (*A. scholaris* R. Br.), is an herbal drug with great medicinal values. Thus, the present phytohemical screening will be helpful as referential information for identification and standardization of this plant material as basic tool for identification.

REFERENCES

1. Karthishwaran K, Mirunalini S, Dhamodharanand G. and Arulmozhi V., Phytochemical investigation of Metha-nolic Extract of the Leaves of Pergulariadaemia, Jr of biological sciences, 10 (3), 2010, 242-246.
2. Misraet al., A comparative study on phytochemical screening and antibacterial activity of roots of Alstonia scholaris with the roots, leaves and stem bark, Int. J. Res. Phytochem. Pharmacol., 1 (2), 2011, 77-82.
3. Trivedi PC, Medicinal Plants: Traditional Knowledge, (New Delhi: I.K. international Pvt. Limited, 2006) 216-217.
4. Atal CK, Sharma ML, Kaul A and Khajuria A., Immunomodulating agents of plant origin: Preliminary screening, J Ethnopharmacol, 41, 1986, 185-192.
5. Kalaria Pankti, Gheewala Payal, Chakraborty Manodeep and Kamath Jagadish, A phytopharmacological review of Alstonia scholaris: A panoramic herbal medicine, International journal of Research in Ayurveda & Pharmacy, 3(3), May-June, 2012, 367-371.
6. Vaidyanatha IT, Joel J, Arunkumar TV and Lipin Dev MS, Phytochemical screening and antimicrobial activity of Alstonia scholaris flowers (L) R. Br., Int J Pharm Res Dev, 3(4), 2011, 172-178.
7. Krunal et al., Pharmacognostical standardization of Saptaparna (Alstonia scholaris R. Br.) Stem bark, Pharma Science Monitor, 5(3), Jul-Sep, 2014, 248-255.
8. Kirtikar KR and Basu BD, Indian Medicinal Plants, Vol. I, (Allahabad: Lalit Mohan Basu Publishers, 2002) 111-114.
9. Iwo MI, Soemardji AA, Retnoningrum DS and Sukrasno UUM, Immunostimulating effect of pule (Alstonia scholaris L. R. Br., Apocynaceae) bark extracts, Clinical emorheology and Microcirculation, 23, 2000, 177-183.
10. Jagetia GC and Baliga MS, Evaluation of anticancer activity of the alkaloid fraction of Alstonia scholaris (Sapthaparna) in vitro and in vivo, Phytotherapy Research, 20 (2), 2006, 103-109.
11. Baliga MS: Alstonia scholaris Linn R Br in the Treatment and Prevention of Cancer: Past, Present, and Future. Integrative Cancer Therapies, 9 (3), 2010, 261-269.
12. Anonymous. Ayurvedic Pharmacopoeia of India. 1st ed., Part 2, Vol. 2, (New Delhi: Govt. of India, Ministry of Health of Family Welfare, 2008) 156.
13. Harborne JB, Phytochemical methods. A Guide to Modern Techniques of Plant Analysis. (Berlin: Springer -Verlag, 2005).
14. Shukla VJ and Bhatt UB, Methods of Qualitative Testing of Some Ayurvedic Formulations, (Jamnagar: Gujarat Ayurvedic University, 2001), 5-12.
15. Stephen KS and Sim, Medicinal Plant Alkaloids: An Introduction for Pharmacy Students, (Canada: University of Torronto Press, 1969) 44.
16. Kamalesh Bhogayata, An evaluation of ethnomedicinal properties of Saptaparna (Alstonia scholaris R. Br.) w. s. r. to Essential Hypertension, doctoral diss., Gujarat Ayurved University, Jamnagar, MD, 2008.

Cite this article as:
Kamlesh Bhogayata, B. R. Patel, Bhakti Chhaya, Krunal Doshi, Joban Modha. Phytochemical Screening of Saptaparna (Alstonia Scholaris R. Br.) Bark. AYUSHDHARA, 2015;2(2):71-76.
Source of support: Nil, Conflict of interest: None Declared

Fig. 1: Different parts of *Saptaparna (A. scholaris* R. Br.)

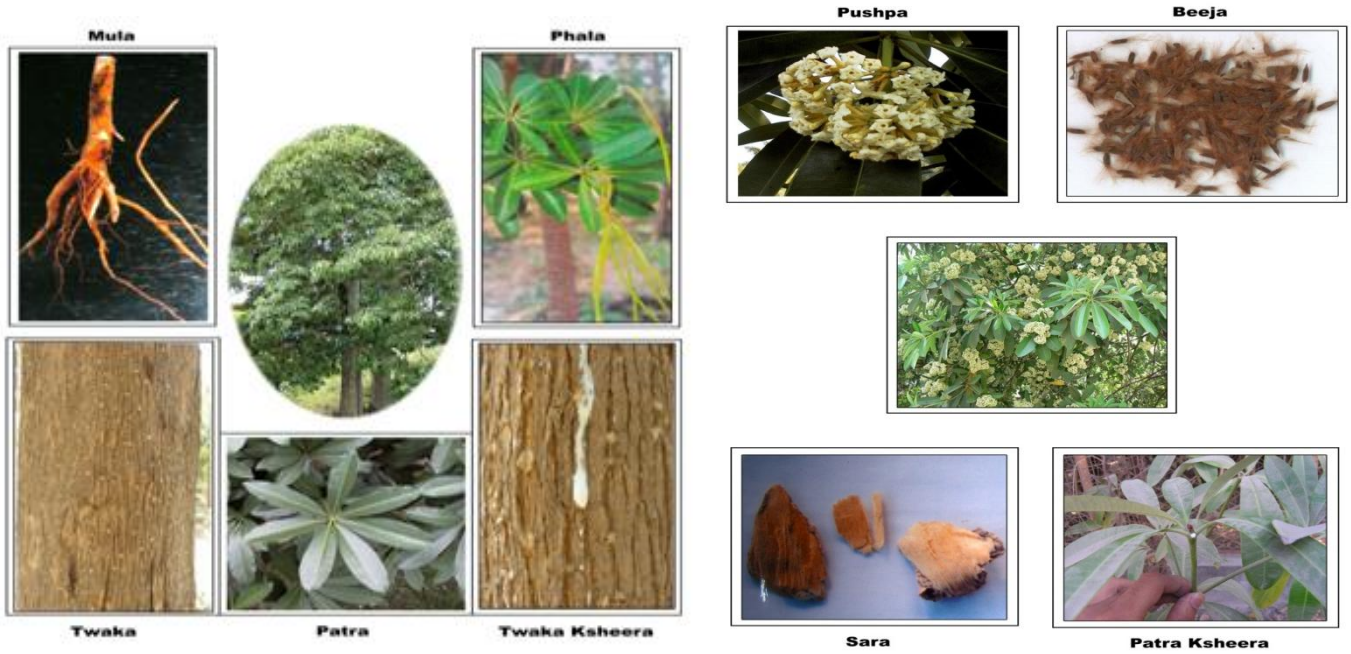

Fig. 2: Bark and Stem bark powder of *Saptaparna (A. scholaris* R. Br.)

Stem of *Saptaparna*

External surface of fresh stem bark

Internal surface of fresh stem bark

External surface of dry stem bark

Internal surface of dry stem bark

Powder of stem bark

Fig. 3: Images of UV chamber & TLC plate under different visualization of *Saptaparna* (*A. scholaris* R. Br.) stem bark

UV Chamber

Under Short UV

Under Long UV

After spraying dragendroff's reagent