

# A Comparative Pharmacognostic Evaluation of Different Extracts of *Shorea robusta* Gaertn. f. Resin

Sushma Vashisht\*, Manish Pal Singh, Viney Chawla

Rajiv Academy for Pharmacy, N.H. #2, Delhi Mathura Byepass, P.O. Chattikara, Mathura-281001, Uttar Pradesh, India

#### ABSTRACT

*Shorea robusta* (Shal) is an important traditional Indian medicinal plant used in various ailments and rituals. The use of different parts of this plant like leaves and resin as a medicament for treatment of various conditions is well documented in literature. However, the studies on phytochemical constituents and medicinal properties in the resin of this plant are scanty. All three samples carried out microscopic characters, ash values, extractive values, T.L.C., and chemical tests. The extractability of methanol, ethanol and chloroform extracts of *Shorea robusta* were found to be 44.85%, 48.57% and 4.48% respectively. Phytochemical analysis of the extracts of *Shorea robusta* revealed the presence of alkaloids, flavonoids, triterpenoids and amino acids. The presence of alkaloids and triterpenoids were confirmed by qualitative tests followed by TLC.

**Key Words:** *Shorea robusta,* phytochemical analysis, triterpenoids, flavonoids. **DOI:** 10.24896/eijppr.2017711

# eIJPPR 2017; 7(1):1-5

1

#### **INTRODUCTION**

Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used popular folks medicine. It has been shown that in-vitro screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigation [1].

The chosen medicinal plant namely *Shorea robusta* resin belongs to the Dipterocarpaceae family. *Shorea robusra* Gaertn.f. is widely distributed in India, Nepal and Bhutan. In India, the species are distributed from Himachal Pradesh to Assam, Tripura, West Bengal, Bihar and Orissa, eastern districts of Madhya Pradesh extending further to the eastern ghats of Andhra Pradesh [2]. Different parts of the plant are traditionally used for the treatment of diverse purposes. The leaves are used to treat wounds, ulcers, itching, leprosy, gonorrhea, cough, earache and headache [3, 4]. The oleoresin exuded from the cut

bark has astringent and detergent properties [5]. The bark is also used to treat diarrhea [6], dysentery [7], wounds [8], ulcers and itching [9]. In Unani system of medicine, the resin is used for treating menorrhagia, enlargement of spleen and for relieving eye irritations [10]. In Ayurveda the leaves are used as anthelmintic and alexiteric. *Shorea robusta* leaf extract has been found to possess significant anti-inflammatory activity [11]. The present study has been undertaken the 3extracts of resin evaluated its pharmacognostic parameters by comparison of standard data.

### **MATERIALS AND METHODS**

Sample source: The resin of *Shorea robusta* samples was purchased from local market of Delhi. Microscopic characters

*Colour:* - The untreated part of the drug was taken and colour of the drug was examined under sunlight.

*Odor and Taste:* - A small portion of the drug was taken, slowly and repeatedly inhaled the air over the material and examined the odor. And taste, a small portion of drug was taken on the tongue and find out the taste of drug.

Phone: +91-9996002936

Corresponding author: Ms. Sushma Vashisht

Address: Research Scholar, Department of Pharmacology, Rajiv Academy for Pharmacy, N.H. #2, Delhi Mathura Byepass, P.O. Chattikara, Mathura-281001, Uttar Pradesh, India

e-mail 🖂 vashishtsush@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Received: 02 November 2016; Revised: 22 February 2017; Accepted: 25 February 2017

*Size and Shape:* - Width and length of fruit was measured with the help of scale. Shape of fruit was confirmed by comparing with literature.

*Surface characteristic*: - Longitudinally wrinkled and ridges were confirmed by comparing with literature.

# Ash values of resin powder

*Total Ash:* - 3 gm of drug was weighed and incinerated in a China dish at a temperature not exceeding 450° until free from carbon, cooled and weighed, until a constant weight was obtained for three successive readings. Percentage of ash was calculated with reference to air dried drug.

Total Ash = 
$$\frac{Wt. \text{ of ash X 100}}{Wt. \text{ of drug}}$$

*Acid-Insoluble Ash:-* Boil the total ash was obtained for 5 mins with 25 ml of dilute hydrochloric acid, collect the insoluble matter in a gooch crucible, it insoluble matter was wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

*Water soluble ash:-* The ash obtained as described in the determination of total ash was boiled for 5 min. with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited at a temperature not exceeding 450 °C. The procedure was repeated until a constant weight was observed. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug.

### Extractive values of resin powder

*Alcohol-soluble extractive:*- 5 gm of accurately weighed powdered drug was taken in a stopper conical flask and add 100 ml of 90% alcohol, and shake constantly for 6 hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:-

Alcohol-Soluble Extractive = <u>Wt. of extractive X 100</u> Wt. of drug

*Water Soluble extractive:* -5 gm of accurately weighed powdered drug was taken in a stopper conical flask and add 100 ml of chloroform water, and shake constantly for 6 hr in an electrical shaker and kept overnight for maceration and then filter carefully and filter was evaporated to dryness and weight of extractive was taken and percentage was calculated by:-

> Water-Soluble Extractive= <u>Wt. of extractive X 100</u> Wt. of drug

### Foreign matter

Weighed accurately 250 g of the crude sample was spread out in a thin layer. The sample was inspected with the unaided eye or with the use of a magnifying lens (10 X) and the foreign matter was separated manually as completely as possible and weighed. The percentage of organic matter was weighed and determined with reference to the weight of the drug taken.

# Loss on drying

About 2-5g of the prepared air dried material was accurately weighed in a previously dried and tarred flat weighing bottle. The sample was distributed evenly and was placed in the drying chamber (Oven). Drying was carried out by heating to 100-150 °C, the bottle was removed from the oven and the bottle was closed promptly and allowed to cool to room temperature and then weighed. The experiment was repeated until two consecutive weighing did not differ by more than 5 mg, unless otherwise stated in the test procedure. The loss in weight on drying was then calculated [12].

# Chemical test [13]

## Test for alkaloids:-

Small portion of solvent free all the extract were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents, such as Mayer's reagent, and Hager's reagent.

*Mayer's reagent:* Few drops of Mayer's reagent were added to the methanol, ethanol and chloroform extracts, a cream color precipitate was observed.

*Hager's reagent:* Few drops of Hager's reagent were added to the methanol, ethanol and chloroform extracts, a yellow precipitate was observed.

## Test for amino acids:-

*Millon's test:* Few drops of Millon's reagent were added to the methanol, ethanol and chloroform extracts, a white precipitate was observed.

### Test for flavonoids:-

*Shinoda test:* 5 ml of ethanol and few drops of concentrated hydrochloric acid were added to the methanol, ethanol and chloroform extracts, brown colour appeared.

### Test for steroids and triterpenoids:-

Small portion of (2 mg) of extract was hydrolyzed with dilute hydrochloric acid for few hours in water bath and was subjected to Salkowaski test.

#### Salkowaski test:-

2 ml chloroform and 2 ml concentrated sulphuric acid was added to the 1 ml of extract, stirred, red colour was observed in chloroform layer and in acid layer greenish yellow fluorescence was observed.

### Test for tannins:-

*Ferric chloride test:* Treat the extracts with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

### Chromatographic studies (T.L.C.) [14]

Thin layer chromatography was performed on silica gel G coated glass plates. The adsorbent silica gel G was coated to a thickness of about 0.3 mm on previously cleaned TLC plates of 7x3 cm using conventional spreader. The plates were placed in hot air oven at 105 °C for 30 min. for activation. The compounds were applied as a spot on the activated plate about 2 cm above from the bottom.

**Preparation of plates-** Silica gel with a mean pore width of preferably 6 to 10 nm is used as a base material. As smaller the particles better the separation efficiency. Silica gel plates of 0.2 mm thickness were prepared by spreading method and final spot taken on a silica gel coated plate of uniform thickness (0.2 mm) and develop it in the solvent system to a distance of 0.8 cm.

**Activation plates-** Plates were activated at 105 °C for 45 min. in an electric oven.

**Test solution-** The methanol, ethanol and chloroform extracts of *Shorea robusta* resin were used for the chromatograph.

**Solvent system-** Toluene: Ethyl acetate: Methanol (7:2:1)

**Visualization of spots-** Spray the plate with anisaldehyde sulphuric acid reagent solution (prepare 0.5 ml p-anisaldehyde in 50 ml glacial acetic acid and 1 ml 97% sulphuric acid).

**Chamber preparation-** A clean and dry chamber was taken. The chamber was lined with the filter paper. The strips of filter paper should be cut in such a way that a window remains allowing observation of the development process. Solvent was introduced to a height of 0.5 to 1 cm in the chamber which was carefully tilted in order to moisten the filter paper and to equilibrate the chamber with solvent vapor. The closed chamber was allowed to saturate with solvent vapors. The TLC was then introduced in the chamber in such a way that the system the system just wet the lower edge of the plate sorbet. The solvent system should not wet the part of the plate where the spots were applied, any contact between the side of the plate and the filter paper should avoid.

**Development of chromatogram-** The solvent migrates up the plate through the sorbet by capillary action. The substance was separated as a result of interaction between the samples, mobile and stationary phase into individual component. Migration behavior of the separated substance is given in the form of RF value (relative to front).

RF = <u>Distance traveled by solute (solute front)</u>

Distance traveled by solvent (solvent front)

Ascending development of chromatogram was done. The plates were removed from the chamber, when the solvent front had reached the predetermined height and the solvent front was marked precisely with pencil. Then the plate was dried and observed under UV light.

### **RESULTS AND DISCUSSION**

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there has been an emphasis standardization of medicinal plants of therapeutic Despite potential. the modern techniques, by identifications evaluation of plant drugs pharmacognostic studies is still more reliable, accurate and in expensive means [15]. According to World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

Shorea robusta is an important ayurvedic drug which has also been studied extensively by different investigators. Shorea robusta not only destroy pathogenic bacteria but it also used in wound healing, anti-inflammatory, analgesic, and antioxidant. Different parts of Shorea robusta such as leaves, stem bark; floral parts were used for the various pharmacological activities.

Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The organoleptic or macroscopic studies yielded important characteristics, such as the fractured surfaces of fresh and dried resin, typical tongue sensitizing tasteless and odourless of the resin, which are useful diagnostic characters (Table 1).

Table 1.	Macrosco	pically stud	ly of <i>Shorea</i>	robusta	resir
			.,		

Tuble II Macroscopically study of bhorea robusta reshi				
S. No.	Parameter	Standard values of <i>Shorea robusta</i>	Test values of Shorea robusta	
1.	Odour	Odourless	Odourless	
2.	Colour	Dark brown to pale amber or yellow red	Dark brown and yellow	
3.	Shape	Cylindrical shape	Cylindrical	
4.	Taste	Tasteless	Tasteless	
5.	Surface characteristics	Brittle pieces, irregular, cylindrical	Cylindrical pieces	
6.	Size	1.5-5 cm	2-5 cm	

3

The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash, and watersoluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash', which is derived from the plant tissue itself, and 'nonphysiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acidinsoluble ash is a part of total as hand measures the amount of silica present, especially as sand and siliceous earth. Water-soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards (Table 2).

The plant material was subjected to preliminary phytochemical screening involving successive solvent extraction by different solvents in order of increasing polarity to obtain diverse polar and non-polarphy to constituents possessing different solubility pattern, followed by various chemical tests for qualitative detection of various chemical constituents. As per photochemical screening, the resin of *Shorea robusta* contains mainly alkaloid and triterpenoids. Alkaloid and triterpenoids was found to be appreciable as compared with other constituents. The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. The colour of the extract sometimes may roughly indicate the physical and chemical features of constituents present (Table 3).

Table 2. Standardization of resin powe	ler
--	-----

S. No.	Parameters	Standard values of Shorea robusta	Test values of Shorea robusta
	Total ash value	NMT 1%	0.66%
1.	Water soluble	NMT 1%	0.33%
	Acid insoluble	NMT 0.7%	Negligible
2.	Methanol soluble extractive value (alcohol soluble extractive)	NMT 60%	44.85%
3.	Ethanol soluble extractive value	NMT 60%	48.57%
3.	Water soluble extractive value (Chloroform extract)	NLT 1.5%	4.48%
4.	Loss in weight on drying at 105°C	NMT 12%	4.4%
5.	Foreign organic matter	NMT 2%	1.25%

NMT-Not more than, NLT-Not less than

**Table 3.** Results of phytochemical screenings of successive extracts of fruits of *Shorea robusta*

Chemical test	Methanol extract	Ethanol extract	Chloroform extract
Test for alkaloids			
Hager's test	+ ve	+ ve	+ ve
Mayer's test	+ ve	+ ve	—Ve
Test for amino acid			
Millon's test	+ ve	+ ve	—Ve
Test for flavonoids			
Shinoda test	+ ve	+ ve	—Ve
Test for steroids			
and triterpenoids			
Salkowaski test	+ ve	+ ve	—Ve
Test for tannins			
Ferric chloride test	+ ve	+ ve	—Ve

#### Table 4. T.L.C. Identification

S. No.	Shorea robusta extracts	R <sub>f</sub> Values of spot of standard	R <sub>f</sub> Values of spot of test
1.	Methanol extract	0.85	0.83
2.	Ethanol extract	0.80	0.76
3.	Chloroform extract	0.96	0.87

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. Experimental conditions of TLC and hence, the obtained  $R_f$  value differed to some extent from that of literature. The chromatographic profile may serve as a characteristic finger print for qualitative of resin (Table 4).

After present investigation it can be concluded that the pharmacognostical study of resin of Shorea robusta yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. As previously mentioned, resin of Shorea robusta being morphologically variable species, these information will also be helpful to differentiate resin of Shorea robusta from the closely related other species and varieties of resin of Shorea robusta. The all three extracts were compared with standard data, the methanol extract was more same pharmacognostical parameters as standard data.

#### REFERENCES

[1] Adlkha, M., Bhargava, A., Kapoor, R., Sharma, L.N. and Singh, C., 2014. Ayurvedic medicinal plant *Shorea robusta* (*shala*). *Innovare Journal of Ayurvedic sciences*, 2(4), pp. 18-21.

[2] Chattopadhayay, D., 2012. Inhibition of  $TNF-\alpha$  and Inos expression by shorea robusta L: an ethnomedicine used for anti-inflammatory and analgesic activity. *Evidence Based Complement Alternal. Med.* 1-14.

[3] Duddukuri, G.R., Rao, D.E., Kaladhar, D.S.V.G.K., Sastry, N., Rao, K.K., Chaitanya, K.K. and Sireesha, C., 2011. Preliminary studies on in vitro antibacterial activity and phytochemical analysis of aqueous crude extract of Shorea robusta floral parts. *Int J Curr Res*, *3*(8), pp.21-23.

4

[4] Jyothi, G., Carey, W.M., Kumar, R.B. and Mohan, K.G., 2008. Antinociceptive and antiinflammatory activity of methanolic extract of leaves of Shorea robusta. *Pharmacology online*, *1*, pp.9-19.

[5] Kalaiselvan, A., Gokulakrishnan, K., Anand, T., Akhilesh, U. and Velavan, S., 2013. Preventive effect of Shorea robusta bark extract against diethylnitrosamine-induced hepatocellular carcinoma in rats. *Int Res J Medical Sci*, *1*(1), pp.2-9.

[6] Murthy, K.S.R., Lakshmi, N., Raghu, D., 2011. Biological activity and phytochemical screening of oleoresin of Shorea robusta gaernt. *Tropical subtropical agroeco*, 14(3), pp.787-791.

[7] Kalaiselvan, A. and Gokulakrishnan, K., 2012. Bark extract of Shorea robusta on modulation of immune response in rats. *Int J Recent Scienti Res*, *3*(8), pp.693-697.

[8] Mathavi, P. and Nethaji S., 2014. In-vitro antioxidant activity of Shorea robusta leaf extract. International *Journal of research in biochemistry & biophysics*, 4(3), pp. 22-26.

[9] Mukharjee Hemanta, and Durbadal Ojha. Wound healing activity of *Shorea robusta* leaf extract. *Journal of Ethanopharmacology*, 2013: 149, pp. 665-669.

[10] Santhoshkumar, M., Anusuya, N. and Bhuvaneswari, P., 2013. Antiulcerogenic effect of resin from Shorea robusta Gaertn. f. on experimentally induced ulcer models. *International Journal Pharmacy and Pharmaceutical Science*, *5*(1), pp.269-72.

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR) | February 2017 | Volume 7 | Issue 1 | Page 1-5 Vashisht S., A Comparative Pharmacognostic Evaluation of Different Extracts of *Shorea robusta* Gaertn. f. Resin

[11] Nainwal, P., Bhatt, R., Nanda, D. and Saini, P., 2013. Screening of in vitro anti-inflammatory activity of aqueous extract of leaves of Shorea robusta. *Int J Pharmacol Screen Method*, *3*(2), pp.43-45.

[12] Naikodi, M.A.R., Nagaiah, K. and Waheed, M.A., 2012. Phytochemical Standardization of Oleo Resin of Shorea robusta Gaertn (Dipterocarpaceae) with modern analytical technique. *International Journal of Phytomedicine*, *4*(4), p.503.

[13] Quality Standards of Indian Medicinal Plants. Gupta, A.K., 2003. *Quality standards of Indian medicinal plants. Volume 1.* Indian Council of Medical Research, New Delhi, pp. 207-209.

[14] Trease, G.E., 1996. Phenols and phenolic glycosides in Trease and Evans pharmacognosy and Biliere Tindall London, pp: 832.

[15] Srikanthmurthy, K.R., 2000. Bhavaprakasha of Bhavamishr (pp. 160). *Krishnanda Academy Varanasi*.

HOW TO CITE THIS ARTICLE: Vashisht, S., Singh, M.P. and Chawla, V., 2017. A Comparative Pharmacognostic Evaluation of Different Extracts of *Shorea robusta* Gaertn. f. Resin. *International Journal of Pharmaceutical and Phytopharmacological Research*, 7(1), pp.1-5, DOI: 10.24896/eijppr.2017711