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Pharmacognostical, Physicochemical and Phytochemical screening of leaves of *Shorea robusta* Gaertn.F

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ABSTRACT

Aim: The value of traditional medicinal plants increasing day by day because of their advantages over synthetic products. The present study deals with the determination of Pharmacognostical, Physicochemical and Phytochemical evaluations of leaves of *Shorea robusta* gaertn.

Methods: Macroscopic and microscopical studies of leaves were included under Pharmacognostical evaluation whereas ash value, extractive value, moisture content and fluorescence behavior was the parameters determined for Physicochemical evaluation. Phytochemical screening was performed on extracts (ethanol and water) for the confirmation of presence of plant metabolites.

Results: According to findings, the leaves show cork cells, trichomes and vessels. Physicochemical analysis of leaves revealed 1.53% w/w total ash value, 4.97% w/w alcoholic extractive value, 1.08% moisture content. *S. robusta* leaves comprise a number of bioactive compounds such as flavonoids, tannins, and alkaloids.

Conclusion: This information will be used for further pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity and sample identification.

Keywords: Phytochemical screening, Pharmacognostic, Shorea robusta, Ash value, Sal, Extractive value

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INTRODUCTION

Plants are the most ancient and vital source of medicines. After the synthesis of aspirin at the end of the nineteenth century, research on herbal products ceased and researchers began concentrating on synthetic and semi-synthetic drugs. However, there has been a rise in the use and interest in drugs of natural origin in recent years, particularly botanical drugs. Approximately 25% of prescribed medicines are derived from higher plants, and this figure rises to 50% when animal and microbial products are included [1].

The natural biological diversity is a very useful resource for improving health and treating many diseases in many different human societies. In various regions on the earth, including Asia, South America, and Africa, a variety of plant species are still used as remedies for various diseases. Because of the numerous environmental factors, like conditions of the soil and biological zones, India is well-known for its botanical richness & for diversity of aromatic & medicinal plants [2]. Herbal medicine, as defined by the WHO, refers to labeled products containing plant parts or mixtures of them, including crude juices, gums, fatty oils, or other forms [3]**.** The value of these plants is diminishing with the passing of time, and combined efforts are required to preserve it [2]**.** The current study emphasizes on its botany, traditional uses, pharmacognostical, physicochemical, and phytochemical studies, providing preliminary data to explore its therapeutic potential and other future research possibilities [4]. Pharmacognosy has grown in popularity in recent years because it is an effective tool for the authentication and identification of plant raw materials. As a result, evaluating pharmacognostical parameters is an essential stage while working with herbal drugs [5]**.** The aim of the current research is to determine the various evaluation parameters of leaves of the plant *S. robusta*, which is native to Himachal Pradesh, Orissa's Eastern districts, and the Eastern Ghats of Andhra Pradesh [2]**.**

S. robusta, also known as sal or shala tree, is a type of tree from the Dipterocarpaceae family as shown in Table 1[6]. Sal trees can be found all over India; They account for around 13.3 percent of the entire forest regions in the country, which encompasses parts of north region, east region and Central Indian regions from the lowlands up to altitudes of 900 to 1700 metres. It covers the Jaintia, Garo & Khasi hills in the northern states of Punjab, Himachal Pradesh, and Haryana, as well as the outer Himalayas and sub-Himalayan regions of Assam and Tripura. It spreads eastward from western Bengal. In the south, from Orissa to Vishakhapatnam, and in the east, from a large portion of south-eastern Madhya Pradesh to the western regions of Chindawara and Hoshangabad. Dispersed in Nepal and Bhutan as well [7].

TABLE -1: TAXONOMICAL CLASSIFICATION OF S. ROBUSTA

Taxonomical classification	
Kingdom	Plantae
Subkingdom	Viridiplantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Malvales
Family	Dipterocarpaceae
Genus	Shorea
Species	*S. robusta*

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S. robusta is an evergreen tree that may grow to a maximum height of 50 meters. The *S. robusta* tree reaches a height of approximately eighteen to thirty-two meters and a girth of 1.5 - 2 meters. The crown has an outward-facing, spherical shape. Bark is 2.5 cm thick, dark brown in color, and has deep vertical grooves [4]. In old trees, the trunk becomes shallow. Simple, shiny, and glabrous leaves measure between 10 and 25 cm in length, are in general oval at the base, and taper sharply towards the apex like in Figure 1. Young leaves emerge reddish, then turn light green before turning darkish green as in figure 2 [8]. Flowers are velvety pubescent, pale yellow or cream in color, and borne in lax, terminal or axillary panicles. Fruit is ovoid, 1-1.5 cm long, slightly fleshy, slightly yellowish green to reddish, with persistent sepals that resemble wings and are 5-7 cm long, linear, and 10-nerved. It consists of single ovoid seed. Young foliage and flowers are produced by the plant in March through April, and fruiting starts in the summer. The booming period of flowers and fruits are march & June respectively [7]. Limonene, alpha pinene, beta- pinene, limonene, kaempferol, myricetin, quercetin, hydrolysable tannins, ellagic acid, catechins, resins, esters, salicin and its derivatives are the chemical constituents present in this plant [9].

Figure1: Leaves of S.robusta

Figure 2: Leaves of S.robusta

In Ayurveda and Unani medicine, *S. robusta* (Dipterocarpaceae), is frequently used to treat a variety of illnesses. According to Ayurveda, the drug Shala contains Kashaya rasa, rukshaguna, sheetavirya, katuvipaka, and it calms pitta & kapha, thus preventing krimis creation and development [4]. This tree is extensively utilized in Unani medicine as well as in the Ayurvedic medical system for a variety of ailments [6]. The plant gum is used in traditional remedies for stomach issues, infections, and skin problems, and as a cleanser and an ingredient in ointments with bee wax to treat foot cracks, cuts, burns, and ear and eye issues. Seeds are used on pus-filled wounds; a mix of oleoresin and cow ghee helps treat hemorrhoids, and reduces pain and swelling [10]. *Shorea robusta* leaves have anti-inflammatory and antioxidant properties, which can aid in the reduction of inflammation and oxidative stress. They also have antibacterial qualities, preventing the development of some bacteria and fungus. Furthermore, the leaves have showed promise in wound healing and possible anti-diabetic activities. However, further study is required to completely comprehend and confirm these pharmacological actions [4].

The current research compiles fragmented information on this plant's botany, phytochemistry and physiochemistry. We anticipate that this research will emphasizes the significance of *S. robusta* & offer fresh approach for future research [6].

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MATERIALS AND METHOD

Plant material collection

S. robusta leaves were collected from Vindhya Herbals Bhopal, Madhya Pradesh & the leaves were authenticated by Botany department of Career College, Bhopal.

Macroscopic studies

S. robusta dried leaves were cleaned, allowed to air dry in the shade for 10 to 15 days, and then characteristics such as color, odor, shape and size were assessed [11].

Fluorescence analysis

A fluorescence analysis of powdered dried leaf was carried out. On a clean, grease free microscopic slide 1-2 drops of freshly made solution of the reagent was mixed with the small quantity of plant's powder. The slide was then gently tilted to mix the additions, and the procedure was allowed to sit for some time. After that, in UV chamber the slide was kept to get examined in both long (at 365 nm) & short (at 254 nm) UV radiations. Different reagents were applied in various radiations, and the colors that were seen were noted [12].

Microscopy study/ powder microscopy

After removal of color from the coarse powder by boiling it in 5% KOH, a small quantity of decolored powder applied with glycerin was put on a glass slide. A cover slip was applied after the powder had been strained with phloroglucinol, and it was then examined under a microscope [13].

Physiochemical study

Ash value

a) Determination of total ash: Total ash is made up of phosphates, carbonates, silica, and silicates, as well as physiological and non-physiological ash, such as sand and soil [14]. In a tarred silica dish, 3.00 grams of air-dried powdered drug was precisely weighed and then it was incinerated until it was carbon-free at a temperature no higher than 450 ℃ and weighed again after cooling. The total ash percent was computed using air dried drug as a reference [13].

b) Determination of water-soluble ash: Water soluble ash is the part of the total ash which is soluble in water. The total ash was boiled in 25 ml of water for five minutes, then the undissolved solid was removed using ash-free filter paper, and the mixture was then burned at 450°C. The water-soluble ash percent was then computed using air-dried drug as a reference [14].

c) Determination of Acid-insoluble ash: By treating total ash with dilute HCl and evaluating the residue, acid-insoluble ash is determined. By comparing the limit of this to the total ash value for the same sample, it is possible to distinguish between the contaminating material and the drug's natural ash. This limit shows the contamination of siliceous materials such as sand. The total ash was produced by boiling about 2N solution of HCl (25ml) for about five minutes, the indissoluble substance was gathered using ash-free filter paper, cleaned with hot water, cooled in a desiccator before ignition and weighed again. The percent of acid insoluble ash in the air-dried drug was determined [14].

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d) Determination of Sulphated ash value: Before being ignited in a tared silica crucible to a constant weight, 1 gm of air-dried powder drug underwent treatment with diluted sulphuric acid. The obtained ash was weighed. The sulphated ash percent was computed using dried drug [14].

Extractive Value

a) Alcohol soluble extractive value: 5 grams of the dried powder drug should be macerated with 100 ml of the specified strength of ethanol for 24 hours in a sealed flask, stirring frequently for initial six hrs of maceration and left still for the remaining 18 hrs. In order to prevent ethanol loss, the filtrate as filtered rapidly. Next, evaporated 25 milliliters of filtrate to dryness in a flat-bottomed, tar-covered dish. Then, dried it at 105°C and weighed the outcome. Computed the ethanol-soluble extract percentage based on the air-dried substance [13].

b) Water-soluble extractive value: 5 grams of the dried powder sample should be macerated with 100 milliliters of the specified strength of chloroform water for 24 hours in a sealed flask, stirring frequently for initial six hours of maceration and left still for the remaining 18 hrs. In order to prevent solvent loss, the filtrate was filtered rapidly. Then, evaporate away a total of 25 milliliters of the filtrates to dryness in a flat-bottomed dish that has been covered in tar. Then, dried it at 105°C and weighed the outcome. Computed the water-soluble extract percentage based on the air-dried substance [13].

Loss on drying: The mass reduction from drying is shown as a percentage weight by weight. In a petri dish, a small amount of drug powder, about 5–6g was precisely weighed and kept for four hours at 110°C in a hot air oven. In each case, the weight reduction was measured after cooling in a desiccator. This process was repeated until a stable weight was achieved.

$$\text{Loss on drying (\%)} = \frac{\text{Loss in wt.}}{W} \times 100$$

W = sample wt. in gram [13].

Phytochemical study

Test for Alkaloids

In 5 ml of distilled water, about 50 mg of the extract were dissolved. until an acid reaction took place. The filtrate was checked for alkaloids as mentioned below (Roopalatha & Mala Nair, 2013).

a) Dragendroff's Test: 1-2 milliliter of Dragendroff's reagent were mixed with few milliliters of filtrates. The appearance of a bright yellow precipitate indicated a positive test result [16]**.**

b) Mayer's Test: Added one milliliter of Mayer's reagent with one milliliter of filtrate. A white-yellow or cream-colored precipitate showed the presence of alkaloids. [13].

c) Wagner's Test: A brownish-red precipitate that forms after adding two milliliters of Wagner's reagent (Iodo-potassium iodide) to 1 ml of the filtrate implied the presence of alkaloids [13].

Test for Saponins

Foam Test: In the test tube, 5 milliliters of the test's solution were mixed vigorously for five minutes. The test was confirmed by the formation of stable foam [15].

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Test for Glycosides

a) Legal Test - The extract should be dissolved in pyridine before being made alkaline with sodium nitroprusside solution. The development of deep red colour indicated the positive test result.

b) Baljet Test - Add one milliliter of sodium picrate's solution to one milliliter of the test extract, & the yellowish orange colour showed the positive test result.

c) Borntrager's Test - To 1 milliliter of filtrate, added a few milliliters of dil. Sulphuric acid. Boiled the filtrate, then filtered it and extracted it with chloroform. 1 mL of ammonia was added to the chloroform layer. Anthraquinone glycosides are present as shown by the ammoniacal layer's formation of red colour [13].

Test for Phenolic Compounds and Tannins

a) Added a small amount of the test solution to a solution of basic lead acetate. The existence of tannins was indicated by the appearance of white precipitate.

b) To check for tannins, added $FeCl_3$ solution to one milliliter of the filtrate and observed for a deep blue or dark green-black colour [13].

Test for Flavonoids

Shinoda's Test: The existence of flavones or flavanols is confirmed by the intense cherry red or orange red colour that is produced by the alc. and aq. extract of powder that has been mixed with magnesium foil and highly concentrated hydrogen chloride.

a) The extract undergoes treatment with NaOH; the existence of flavones is confirmed by the development of yellow color.

b) When the extract undergoes treatment with conc. H_2SO_4, the appearance of an orange or yellow colour shows the presence of flavone.

c) After receiving treatment with 10% sodium chloride, the alcohol and aqueous extract develops a yellow color, which denotes the existence of coumarins [13].

Test for Triterpenoids

Noller's Test: In two milliliters of sulfoxide chloride solution, mixed two or three tin metal granules. The appearance of pink color in test tube after adding one milliliter of the extract & warming it up confirms the triterpenoid's presence [13].

Fixed Oils & Fats Spot Test: Using the filter paper, press a small amount of the extracts. Paper with oil stains on it likely has fixed oils in it [13].

Test for carbohydrates

Molisch's test: Along the side of test tube, two to three milliliters of strong sulfuric acid & some drops of 1% alpha-naphthol were mixed with one milliliter of the filtrate. The test was confirmed through a purple-colored ring that formed where two liquids converged [15].

Protein test

Biuret test: Add 5 drops of one percent $CuSO_4$ solution and two milliliters of 10% NaOH to two milliliters of the filtrate. Mix well. Violet or purple color confirms the positive test result [15].

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Test for amino acids

Millon's test: In one milliliter of test solution was mixed with 5 drops of Millon's reagent. It was heated in boiling water bath for 10 minutes, cooled, and mixed with 1 percent sodium nitrite. Red color confirmed the test [15].

Test for Phytosterols

a) Liebermann-Burchard's Test: After mixing the 2 mg extract with two milliliters of acetic anhydride and heating it, one milliliter of strong sulfuric acid was added to the test tube. The brown ring at the layer's junction and dark green top layer confirmed the presence of phytosterols [15].

RESULT AND DISCUSSION

Pharmacognostical study

An appropriate standard for identification and authentication of crude drug is provided by pharmacogenetic studies such as macroscopic and microscopic characteristics.

Macroscopic evaluation

The macroscopic parameter was helpful in identifying plant matter easily & also acts as an important standardization factor.

Organoleptic studies showed that the *S. robusta* leaves are simple, shiny, glabrous, oval shaped leaf with dark green in color. Length varies from 10 - 25cm with apex tapering into a long point and width varies from 5-12 cm as shown in Figure 3. The odor is aromatic and taste is bitter. *S. robusta* powde**r**: The powdered form was examined for appearance as well. The leaf powder is dark green in color, having aromatic odor & bitter taste as mentioned in Table 2.

TABLE 2: MACROSCOPIC EVALUATION OF LEAF OF *S. ROBUSTA*.

S. NO	Parameters	Result
1.	**Leaf**	
	Color	dark green upper surface, whitish green lower surface
	Shape	Oval at base with long pointing tapering apex
	Size	Length 10 -25 cm, width 5-12 cm
	Taste	Bitter
	Odor	Aromatic, characteristic
2.	**Powdered drug**	
	Color	Light green
	Odor	aromatic
	Taste	bitter

Figure 3: Macroscopic study of leaves

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Microscopic study

Stomata, resin crystals, calcium oxalate druse crystals, simple starch grains, and oil globules were all visible in the leaves of S. robusta as in Figure 4.

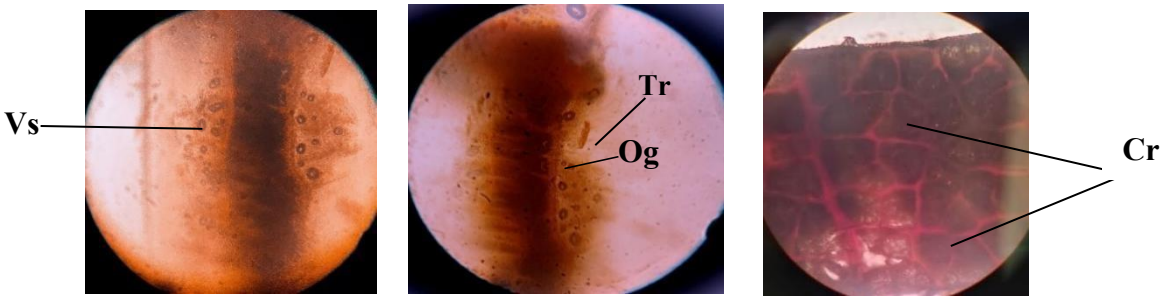

Figure 4: Microscopic findings of leaves of *S. robusta*, where Vs-vessels, Tr – trichomes, Og- oil globules, Cr – Cork cells

Physiochemical study

Several physicochemical parameters, including ash, extractive values, and loss on drying, were determined. The findings are summarized in Table 3. These data were useful in identifying and determining the purity of the crude drug collected.

TABLE 3: PHYSIOCHEMICAL STUDIES OF LEAF POWDER OF *S. ROBUSTA*

Physicochemical Parameters	% w/w
Total ash	1.53
Acid insoluble ash	0.41
Water soluble ash	0.84
Alcohol soluble extractive value	4.97
Water soluble extractive value	3.55
Loss on drying	1.08

Fluorescence analysis

The fluorescence analysis of *S. robusta* leaves powder were performed and were given in Table 4.

TABLE 4: LEAF POWDER EXTRACT'S FLUORESCENCE CHARACTER UNDER UV LIGHT

Treatment	Visible light	UV light (254nm)
Alc. extract	Greenish black	Brick red
Aq. extract	Yellowish brown	Pale yellow

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Phytochemical study

The powdered *S. robusta* extracts contained the tannins, compounds called flavonoids, alkaloids, and terpenoids, according to primary phytochemical screening of the extract solution as shown in Table 5.

TABLE 5: QUALITATIVE PHYTOCHEMICAL SCREENING TEST RESULTS

Experiment	Alc. Extract	Aq. Extract
Alkaloids	+	-
Saponins	+	-
Anthral glycoside	-	-
Cardiac glycoside	+	+
Anthraquinone	-	-
Phenol	+	+
Tannins	+	+
Flavonoids	+	+
Terpenoids	-	+
Fixed oils	-	-
Carbohydrates	+	+
Starch	-	-
Protein	-	-
Amino acid	-	-
Steroids	+	+

Present (+) and absent (-)

Traditional/herbal remedies play an important role in health care systems today because they are inexpensive, safe, and people believe in them. As the use of these herbal remedies has grown, concerns about their effectiveness, safety, and quality have emerged. Undoubtedly, the goal of standardizing medicinal plants is to guarantee their effectiveness as a form of treatment. Identification of the crude drug will be possible through morphological along with anatomical studies of parts of the plant. It will be possible to determine whether the drug is authentic by using the data through the preliminary phytochemical screening. Adulteration can be detected using ash values, extractive values, and moisture contents. The combination of pharmacognostical, physicochemical, and phytochemical studies provide a comprehensive understanding of the medicinal properties and quality parameters of *Shorea robusta* leaves. These studies form the basis for further research, including pharmacological investigations, formulation development, and the development of herbal medicines derived from this plant species. It is important to note that the specific findings of these studies may vary depending on the methodology and geographical origin of the plant material, so it is necessary to refer to specific research articles or publications for detailed information.

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Abbreviations

Alc. - Alcoholic

Aq. - Aqueous

$CuSO_4$ – Copper sulphate

$FeCl_3$ – Ferric chloride

HCl – Hydrochloric acid

KOH- Potassium hydroxide

H_2SO_4 – Sulfuric acid

NaOH – Sodium hydroxide

UV - Ultra-violet

WHO – World Health Organization

Declarations

Conflict of Interest

There is no conflict of interest, The authors alone are responsible for the content and writing of the paper.

Ethical approval

Not applicable

Consent to participate

Not applicable

Availability of data and materials

Not applicable

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