

Comprehensive Pharmacognostic Evaluation and Quality Standardization of *Pterocarpus Santalinus* L.F. Heartwood

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Abstract

Pterocarpus santalinus L.f. (Red Sandalwood) is an endangered medicinal plant widely used in traditional Indian medicine for the management of inflammation, diabetes, skin disorders, and wound healing. Owing to its high therapeutic value and commercial demand, the species is frequently adulterated or substituted, emphasizing the need for scientifically validated quality control parameters. The present study aimed to establish comprehensive pharmacognostic, physicochemical, phytochemical, and chromatographic standards for the authentication and quality assessment of *P. santalinus* heartwood.

Pieces of sandal heartwood were examined for macroscopic and microscopic techniques, following the standard pharmacognostic ways. In addition, various physical parameters like moisture content, residue upon drying, ash values, and extractive values were also studied following the methods specified in pharmacopoeia. A screening of phytochemical characteristics was done to recognize and classify the most important groups of bioactive compound constituents in the heartwood. The thin-layer chromatography was run on an ethanol extract of Crude drug and compared with Santalin marker.

The presence of a few of these characteristic diagnostic features - lignified fibers with a thick wall, bordered-pit xylem vessels of the heartwood, multiseriate medullary rays, a very large number of prismatic calcium oxalate crystals, and starch, grains was detected microscopically. Physicochemical analyses showed a compliance with the pharmacopoeial standards, and that means good purity and suitable quality of the heartwood specimen. Phytochemical tests corroborated the presence of plant-derived phenolic compounds flavonoids tannins, glycosides, and so on - all of the other bioactive substances too. Thin-layer chromatography (TLC) has yielded a chromatographic fingerprint of the substance which has shown the presence of its main constituents, like santalin A and santalin B. There are also weak bands due to a presence of the minor constituents - santalin C and santalin D. TLC fingerprint perfectly matches the reference profile which is suitable for authentication and setting up quality control.

This current investigation brings authentic, trustworthy, and reproducible pharmacognostic physicochemical phytochemical, and chromatographic benchmarks, which may authenticate and standardize the heartwood part of *Pterocarpus santalinus*. Besides, these results offer a set of helpful reference data for detecting the authenticity of the drug, the quality test, and for preventing adulteration

Keywords: *Pterocarpus santalinus*, Red Sandalwood, Pharmacognostic standardization, TLC.

How to cite this article: Swapnil S. Dounde^{*1} Kanchan Chauhan². Comprehensive Pharmacognostic Evaluation and Quality Standardization of *Pterocarpus Santalinus* L.F. Heartwood. *Int J Drug Deliv Technol.* 198;16(81s):1648-1656. DOI: 10.25258/ijddt.16.81s.198.

Introduction

Medicinal plants are still a part of healthcare systems all around the world and be natural sources of bioactive compounds for the prevention and treatment of many diseases. WHO estimates that many the population of the world turns to traditional herbal medicines for primary healthcare. There is no doubt that the interest in herbal products is on the rise, and this has raised the necessity for scientific quality control measures that can help ensure the purity safety therapeutic value, efficacy, and consistency of the products. Pharmacognostic standardization technique is one method, which can

reliably be used to identify crude plant materials and at the same time reduce the potential risk of misuse, adulteration, and fraudulent substitution. The tree with scientific name of *Pterocarpus santalinus* L.f. comes from the family of Fabaceae (legumes) and is known locally as Red Sandy Wood and scientifically as Red Sandalwood or Red Sanders. It is a rare and protected flora of the Southern Eastern Ghats of India which means people have to rely on cultivation of this tree in plantations. The heartwood of these valuable trees is deep red colored due to its red pigment and can be of medicinal use and a source of income also. Herbal

medicine practitioners and medical scholars have, since years been using this valuable plant for the treatment of inflammation, skin ailments wounds haemorrhage, diabetes fevers dysentery, and a few other minor illnesses too. It is the phytochemicals of this plant that are mainly responsible for its use in herbal medicines. The major classes of molecules that characterize this plant are phenolic compounds flavonoids tannins, lignans and the characteristic red pigments, santalins are some. Studies of *P. santalinus* in a pharmacologic manner suggest that this tree has got anti-inflammatory antioxidant antimicrobial, liver-protective antidiabetic anticancer, wound-healing, and immunomodulatory activities. These biological activities explain why it has been one of the most sought after ingredients by researchers and producers of herbal medicines so that their products could be herbal remedies, and thereby increase the demand of authentic Red Sandalwood material on the market. Still, the authenticity of the herbal material is tools for the quality assessment and standardization of herbal raw materials and are also recommended by the regulatory guidance document of the international regulatory body for herborized medicinal products. Although *P. santalinus* has been widely studied for the pharmacological profile and plant constituents, full pharmacognostic reference standards for its heartwood are very scarce yet. In fact, a detailed description of the characters in microscopic diagnosis together with the standard physicochemical factors is necessary for enabling a correct identification as well as the quality control of the commercial crude drug samples. So, the setting forth of reference standards shall facilitate the

Materials and Methods

Collection and preparation of Plant

The dark, heartwood part of *Pterocarpus santalinus* L.f. Heartwood of the tree was obtained from a supplier specializing in supplying crude drug material at local herbal in Pune, Maharashtra. Authentication of the plant was carried out by Ayurveda Scientific Research Centre, Regional Cum Facilitation Centre, Western Region, Regional Centre National Medicinal Plants Board (NMPB), Ministry of AYUSH and Government of India Pune Maharashtra. The authenticated plant species was confirmed as *Pterocarpus santalinus* L.f. and an authentication certificate was issued (Specimen No. ESN/240247). Heart wood was washed to remove extraneous matter, shade-dried when required and ground to a coarse powder using a laboratory grinder. Finely powdered sample, which passed through sieve

a problem. It is because the geographical range of this species is limited, the growth is slow, the price is high in the market, and the protection status is restricted. So genuine material has been in short supply regularly. Besides that, due to a lack of knowledge among the users and traders, and high profit margin, substitution and adulteration with other wood species are common nowadays in some regions which lead to low quality of the herbal products, loss of safety and effectiveness also.

Authentication of crude herbal drugs is that means the most important aspect before use in pharmaceutical drug formulations. Pharmacognostic evaluation offers the criteria that are scientifically accepted for the identification of medicinal plants based upon macroscopic features; anatomical characters; powder microscopy; physicochemical constants; fluorescence characteristics; and phytochemical analysis. Such aspects are generally considered to be the basic regulations adherence, quality assurance, and the development of standardized herbal formulations. In this view, this study aims at determining pharmacognostic and physicochemical standards for the heartwood of *Pterocarpus santalinus* L.f. via macroscopic inspection, anatomical characterization, powder morphology, physicochemical determination, fluorescence reaction, and primary phytochemical investigations. The information obtained through this study is anticipated to yield valid reference standards for identification, evaluation for quality, and standardization of this medically very important plant and to also facilitate its use in herbal medicines and pharmaceutical research.

no 80, was transferred to an airtight container and kept in the absence of light, moisture and dry place cool and protected from light and moisture. Guidelines of Indian Pharmacopeia and the World Health Organization were followed to conduct pharmacognostic, physicochemical, phytochemical and chromatographic study.

Result

The plant is known by different vernacular names across various Indian languages, including Lal Chandan and Rakta Chandan in Hindi; Agaru etc.

Organoleptic Characteristics

The organoleptic characteristics of the authenticated *Pterocarpus santalinus* L.f. heartwood, including its characteristic deep reddish-brown colour, compact texture, and smooth cut surface, are shown in **Figure 1**.



Figure 1. Organoleptic authenticated heartwood blocks of *Pterocarpus santalinus* L.f.

The following table 1 gives details about Organoleptic characteristics of *Pterocarpus santalinus* L.f. heartwood

Table 1. Organoleptic characteristics of *Pterocarpus santalinus* L.f. heartwood

Features	Observation
Colour	Deep reddish-brown to dark red
Odour	Characteristic, faint, non-aromatic
Taste	Slightly astringent and bitter
Shape	Rectangular woody blocks with straight grain
Size	Variable (approximately 5–10 cm in length and 3–5 cm in width in the collected samples)
Surface	Smooth, finely grained with distinct longitudinal striations
Texture	Hard, compact, dense and moderately heavy
Fracture	Splintery with a fibrous appearance

The following table 2 gives details about Powdered Characteristics of *Pterocarpus santalinus* L.f. heartwood with different reagents

Table 2. Powdered Characteristics of *Pterocarpus santalinus* L.f. heartwood with different reagents

Treatm ent	Visib le light	UV 254 nm	UV 365 nm	Observatio n
Powder as such	Red	Dark red	Red	Characteris tic colour of

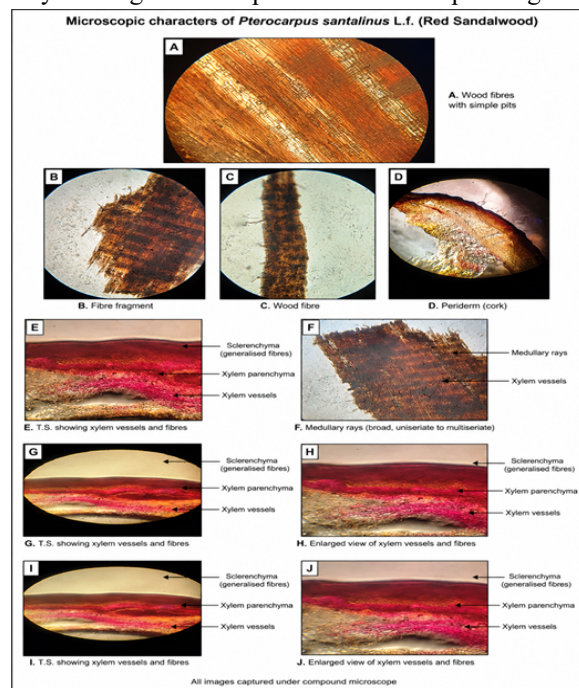
Microscopic Characteristics of *Pterocarpus santalinus* L.f.

The microscopic characteristics of *Pterocarpus santalinus* L.f. heartwood are presented in Figure 2,

				heartwood powder
Powder + 1 N HCl	Light yello w	Red	Red	Acid- induced colour change
Powder + 1 N NaOH	Pale red	Red	Red	Mild alkaline colour change
Powder + 50% HCl	Light yello w	Fluoresc ent red	Fluoresc ent red	Intense fluorescen ce under UV light
Powder + 50% H ₂ SO ₄	Dark red	Dark red	Dark red	Uniform dark red appearance
Powder + 50% HNO ₃	Dark brow n	Brown	Reddish brown	Oxidative colour transforma tion
Powder + Methan ol	Red	Fluoresc ent red	Light red	Enhanced fluorescen ce in methanol
Powder + Methan ol + 1 N NaOH	Red	Brown	Red	Alkaline methanolic response

Microscopic study of *Pterocarpus santalinus* heartwood confirmed the presence of secondary xylem features typical of such tissue. Transverse and longitudinal sections showed a compact mass of hard

lignified thick-walled wood fibres interspersed with clearly marked xylem vessel elements having wide lumina. The vessel elements were mainly solitary but occasionally they formed radial groupings, and were surrounded by lignified xylary tissues. The medullary rays penetrating the xylary tissue appeared like very thin parallel lines. The surface of the heartwood showed only a very thin periderm (cork) layer. The lignified tissues in the stained preparations showed very strong reddish-pink coloration pointing to



Powder Characteristics of Red Sandal Wood

The coarse powdered heartwood of *Pterocarpus santalinus* L.f. prepared for powder microscopic analysis is shown in Figure 3. To determine the identity of the herbal material, a microscopic study on the heartwood of *Pterocarpus santalinus* L.f. was carried out. It was found that the main constituents of the powder were lignified fiber fragments, xylem vessel elements, and different sized fragments of reddish-brown heartwood tissues with the color typical of such heartwood being imparted by the numerous reddish to orange tissue fragments evenly distributed throughout the powder. Longitudinally elongated cells with walls of considerable thickness

extensive secondary wall thickening. The dense aggregation of xylem fibres, the striking xylem vessel elements with radial groups of vessel elements and the good condition of secondary xylem are among the main reasons for the anatomical identification of *Pterocarpus santalinus* heartwood and its authenticating. These microscopic details can act as the basic pharmacognostic data for distinguishing the genuine crude drug from other drugs or potential adulterants related to its source.

Figure 2. Transverse section of *Pterocarpus santalinus* L.f

and with ends of these cells pointed, together with vascular elements characterized by lignified walls were abundantly found. The heartwood cells were represented through the tiny red and orange tissue fragments. Also identified at the micro level were the cells of fibrous tissue and xylem parenchyma further reinforcing that the herb was derived from a woody plant. So, the presence of these micro characters work as useful parameters to confirm the identity of this herbal drug, for its authentication, and help it to be distinguished from the counterfeit drugs. The diagnostic powder microscopic characteristics of *Pterocarpus santalinus* L.f. heartwood are presented in Figure 4.



Figure 3. Coarse powdered heartwood of *Pterocarpus santalinus* L.f.

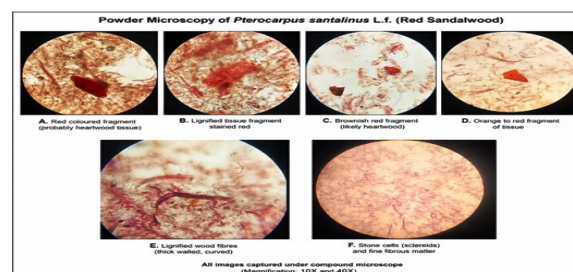


Figure 4. Powder Characteristics of *Pterocarpus santalinus* L.f.

Physiochemical Parameter

Figure 5 illustrates the representative samples used for physicochemical evaluation of *Pterocarpus santalinus* L.f. heartwood, showing (a) the total ash obtained after incineration and (b) the powdered



Figure 5 (a) Total Ash Value (b) Loss on Drying of Red Sandal Wood heart wood

The following table 3 gives details about Physiochemical Parameter

Table 3 Result of Physiochemical Parameter

Extractive values

The determination of extractive values in different solvents was carried out using standard extraction techniques, as illustrated in Figure 6. The test for extractive value measures how much active compounds are taken out of a herbal medicine, if there was a specific solvent used. This is an essential part of quality control for checking the purity and

heartwood sample used for loss on drying determination.

Physiochemical Parameters were performed and presented in table no. 3

Sr. No.	Physicochemical Parameter	Result (% w/w)
1	Total Ash Value	1%
2	Acid Insoluble Ash Value	0.1%
3	Loss on Drying	1%

reliability of the herbal product. The technique uses the principle of taking away the water-soluble compounds of a pre-determined quantity of powdered medicinal plant by using solvent that is properly selected, per standard requirements. When the solvent is dried off, the remaining dry extract is weighed, which gives the extractive value.



Figure 6 Extractive values in different solvents of Red Sandal heart wood

The following table 4 gives details about Extractive value of Red Sandal heart wood

By using different solvents

Table 4 Result of Extractive value

Sr no	Solvent	Extractive value in %
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Qualitative Analysis Test

To determine if the different phytochemical components are present in a plant extract, a qualitative analysis is done. This type of experiment gives a first-hand understanding of what are the major bioactive constituents of the plant that play an important role in its therapeutic effects. The nature of

Phytochemical Test	Water	Chloroform	Butanol	Petroleum ether	Dichloromethane	Acetone	Ethanol	Ethyl acetate
Alkaloids (Wagner's test)	+	-	+	-	-	-	+	+
Flavonoids (NaOH)	+	-	+	-	-	+	+	+

1	Water	10
2	Ethanol	20
3	Acetone	14
4	Ethyl acetate	9
5	Chloroform	7
6	Dichloromethane	8
7	Butanol	3
8	Pet Ether	1

the test relies on very specific chemical reactions that occur between phytochemical components and suitable reagents that lead to characteristic color changes or precipitates revealing the presence of a given group of compounds.

The following table 5 gives details about Qualitative analysis of Red Sandalwood *Pterocarpus santalinus*

test)								
Phenols & Tanins (FeCl ₃ test)	+	-	+	-	-	+	+	+
Saponins (Foam test)	+	-	+	-	-	-	+	+
Glycosides (Keller-Kiliani test)	+	-	+	-	-	+	+	+

Terpenoids (Salkowski test)	+	++	+	++	++	+	+	+
Steroids (Liebermann-Burchard test)	-	++	+	++	++	+	+	+
Carbohydrates (Molisch's test)	+	-	+	-	-	-	+	+
Prot	+	-	+	-	-	-	+	+

Thin Layer Chromatography (TLC)

chromatography analysis was carried out using a mixture of 7 parts toluene and 3 parts ethyl acetate (v/v) as the solvent to find out if santalin was present in the ethanolic extract of Pterocarpus santalinus. On a silica gel G-coated TLC plate, the extract and santalin standard were spot-applied, developed in a saturated atmosphere chamber. The plate was dried



Figure 7 Thin Layer chromatography of Red Sandalwood extract

The following table 6 gives details about R_f value of Red Sandalwood extract

eins (Biu ret test)							+	
Resins (Acetone-Water test)	+	++	+	++	++	+	+	+
Quinones (NaOH test)	+	++	+	++	++	+	+	+
Anthraquinones (Borntraeger's test)	+	++	+	-	++	+	+	+

and examined under UV light at 254 nm and also in daylight after development. Under both UV light and daylight, the plate could show four different spots which correspond to the four isomers namely Santalin A, Santalin B, Santalin C, and Santalin D. which shown in figure no 7.

Table 6 R_f value of Red Sandalwood extract

Santalin Component	Typical R _f Value Range
Santalin A	0.50 – 0.65
Santalin B	0.40 – 0.55
Santalin C	0.20 – 0.35
Santalin D	0.10 – 0.20

Discussion

The quality, safety, and efficacy of herbal treatments heavily rely on the verification, as well as the uniform preparation of the natural botanical matter. A good example is *Pterocarpus santalinus* L.f. commonly referred to as the Red Sandalwood. Such herbs with high medicinal and commercial value are also more likely to be falsified or mixed due to their restricted geographical occurrences and high demand. Because of this, creating accurate pharmacological and physical and chemical references is highly desirable for both species identification and ensuring that such herbs meet the standard requirement. The findings of organoleptic characteristics such as color as deep red-brown, smooth surface, compact, and splintery fracture. These gross features make it a quick and easy screening method for the identification of raw drug material before subjecting sample materials for a more detailed laboratory analysis. In the microscopic analysis the secondary xylem, which is well developed, consisted of a dense lignified cell wall material from fibers, vessels, and rays - which are the key structural features for verifying species at the pharmacognosy level. In powder microscopy, the findings include lignified fiber fragments, vessel elements, and reddish-brown heartwood tissue that are very reliable characters of an authentic powdered drug sample in which the external features have been totally lost or destroyed by grinding. The physicochemical features in this research were found to be below the limits defined by the major pharmacy reference publications showing that the drug sample was of good quality and purity. The lower percentages of total ash show that there is negligible contamination and higher percentage of organic matter while low content of moisture means that the sample was not very damp and Because of this less likely to suffer deterioration or microbial activity during storage periods. In reality the alcohol soluble extractive content is higher than that of water-solubility is an indication that ethanol is a very efficient solvent for extracting the main bioactive constituents of *P. santalinus*.

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Preliminary phyto-chemical screening has revealed the presence of flavonoids phenolics tanning components glycosides Terpenoids, saponins, and resins. These constituents are known for their various health-benefitting properties ranging from antioxidant, anti-inflammatory to antimicrobial, healing of sores, etc. and also hepatoprotective, which in many ways, justify its traditional medicinal application of Red Sandalwood. Among the identified phytochemicals, phenolic compounds and a few flavonoid compounds together accounted for most the biological activity linked to *P. santalinus*. Thin-layer chromatography yielded a reproducible chromatographic fingerprint showing spots that represent various coloured constituents. So, this fingerprint can be used for routine quality control and detection of adulteration in commercial samples. The combination of pharmacognostic study, physicochemical analysis, fluorescence properties, phytochemical tests, and TLC fingerprinting together offer a broad-spectrum quality control strategy for the assessment of *P. santalinus* heartwood. This research's standards will likely help authenticating of botanicals, standardization work pharmacopoeias, and the development of herbal products.

Conclusion

The present study was done to describe the pharmacognostic, phytochemical, physicochemical parameters of *Pterocarpus santalinus* L.f. The evaluated parameters represented the key diagnostic characteristics of the plant and facilitate its accurate identification, even in powdered form. These characteristics are valuable for detecting adulterants and substitutes, thereby ensuring the authenticity, quality, and therapeutic efficacy of the plant material. Furthermore, the findings of this study provide essential reference data that can be used in the development and standardization of the plant's pharmacognostic monograph. Also this study would be a valuable resource to pharmacognosist, traditional herbal medicine producers, regulatory laboratories, and research working in phytopharmaceutical formulations.

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