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Development of quality control parameters for the standardization of *Pterocarpus santalinus* Linn. F. leaf and stem

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Abstract
Traditional therapeutic systems remain significant resources of healthcare worldwide that are reported to be safe and generate minimum side effects compared to synthetic medicines. However the main problem with herbal drugs is they are prone to adulteration and substitution. So, it is of utmost importance to lay down quality control parameters for plant under study. In the present work, *Pterocarpus santalinus* Linn. f. Red sanders an endemic, endangered species of India, an important traditional medicinal plant, was evaluated for its pharmacognostic, phytochemical, physicochemical and fluorescence analysis of leaf, stem and bark. Preliminary qualitative phytochemical screening revealed the presence of maximum amount of saponins in leaf, saponins and triterpenes in stem, flavonoids and steroids in bark. Physicochemical evaluation included loss on drying, total ash, water soluble ash, acid insoluble ash, sulphated ash and extractive values. These analysis will be valuable towards establishing pharmacognostic standards for identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

Keywords: *Pterocarpus santalinus*, macroscopical, microscopical, pharmacognostic standardization, physicochemical, phytochemical screening, fluorescence analysis

1. Introduction
Pterocarpus santalinus Linn. F. is commonly known as Red sandal and belongs to the family Fabaceae. It is a genus of trees and woody climbers distributed in the tropics throughout the world and is an endangered plant species. *P. santalinus* is a small to medium sized deciduous tree having a dense, round crown reaching a height of 10 to 15 m with a girth of about 90 to 160 cm. The bark is typically dark brown in colour with rectangular plates and deeply fissured when matured. When blazed, it exudes a red colour gum with numerous pink streaks. The red wood yields a natural dye santalin, which is used in coloring pharmaceutical preparations and foodstuffs. Traditionally it has many medicinal uses [1]. There are many reported activities for *P. santalinus*. For eg. Wound healing potential [2]; antibacterial activity and hepatoprotective activity [3, 4]; ant helicobacter pylori effect [5]; anti-cancer activity [6]; antidiabetic activity [7]; anti-inflammatory, analgesic, and antioxidant activities [8] anti-inflammatory and cytotoxic activity [9]; acute and sub-chronic oral toxicity [10].
Plants have been considered as one of the best reservoir of many medicinally valuable compounds. There are number of drugs like Khellin, Glargine, Palavering, Quinine, Reserpine, Ephedrin, Vinblastine and Vincristine, etc [11] already in use which have originated from plants. Thus there has been an increasing demand for green medicine. The therapeutic ability of plants lies in their chemical constituents. Medicinal plants are rich in a variety of secondary metabolites. Different metabolites are present in different plants and their concentration vary even in different parts of the same plant. Secondary metabolites are structurally and chemically diverse group of compounds and consists of phenols, flavonoids, alkaloids, cardiac glycosides, tannins, saponins, terpenoids, essential oils, etc. The secondary metabolites are known to show a number of biological activities like anticancer and anti-inflammatory, antioxidant [12, 13], anticancer, cardiovascular and anti-inflammatory [14], antiulcer [15], anti-inflammatory [16], anthelmintic activities [17], antimicrobial [18] etc.
Pharmacognostical standardization is an efficient tool to establish quality control parameters of plants. It helps to assure the authentication of plants and prevention of adulteration. These studies also ensure reproducible quality of plant material and herbal products in trade. Standardization and quality control of plants are also essential for the worldwide acceptance of herbal products in modern system of medicines. It is quite well known that the phytochemicals (secondary metabolites) present in the plants are responsible for the pharmacological activity

They show and it is very important to screen and lay down standardization parameters so that plants can be successfully identified and free from adulteration and substitution. Hence, in the present work, pharmacognostic, physicochemical and preliminary phytochemical analysis has been done in leaf, stem and bark of *Pterocarpus santalinus*.

***Pterocarpus santalinus* Linn. F.**
Scientific name:-
***Pterocarpus santalinus* Linn. F.**
Family:- Fabaceae
Vernacular Name:- Rakta-chandan
Part used – Leaf, stem and bark

2. Materials and methods

2.1. Plant collection

The leaves, stem and bark of *P. santalinus* were collected from Surat in August, 2016 Gujarat, India. All the three parts were separated, washed thoroughly was tap water, shade dried and homogenized to fine powder and stored in closed container for further studies. For physicochemical studies, 10 g of dried powder of leaf was extracted by using solvents of different polarities. The solvent was evaporated to dryness and dried crude extract was stored in air tight bottles at 4 ºC. Macroscopic and microscopical characters were studied as described by Khandelwal [19]. Photographs at different magnifications were taken by using digital camera.

2.2 Pharmacognostic studies

Macroscopical studies

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of leaves and stem of plants. Were observed [20].

Microscopic studies

Microscopic studies were carried out by preparing thin sections of leaf and petiole. The thin sections were further washed with water, stained with safranin and mounted in glycerine for observation and confirm its lignification's. The powder microscopic studies were also carried out and the specific diagnostic characteristic features were recorded [19].

2.3. Qualitative Phytochemical Analysis

The detection of alkaloids, flavonoids, tannins, phlobatanins, saponins, steroids, cardiac glycosides, triterpenes and anthocyanins were carried out following the procedure of Harborne, [21] and Ram *et al*. [22]. The presences of the specific phytochemicals were designated with (+) sign whereas the absence of the group was indicated with (-) sign.

2.4. Physicochemical analysis

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash and extractive value were determined as per WHO guidelines [23]. The procedure followed is as described earlier [24].

2.5. Fluorescence analysis

Fluorescence study of different plants powder was performed as per Kokashi *et al* [25] and Moteriya *et al*., [26]. A small quantity of the plants powder was placed on a grease free clean microscopic slid and 1-2 drops of freshly prepared reagent solution were added, mixed by gentle tilting of the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colours observed by application of different reagents in different radiations were recorded.

3. Results and discussion

Medicinal plants are increasingly being used as natural cure in the treatment and or prevention of many diseases. Now-a-days their demand is increasing tremendously. Thus to ensure reproducibility of its therapeutic efficacy, it is very important to lay down standardization and quality control parameters [27]. In order to establish the identity and purity of the medicinal plant under study, the first step is the macroscopic and microscopic studies. Organoleptic evaluation is a simple qualitative technique based on the study of morphological and sensory profile of plants which are useful as diagnostic characters of leaf, seed, fruit or root. Organoleptic evaluation has been done for *Eucalyptus globules* leaves [28], *Terminalia bellerica* leaf and stem [29]; *Caesalpinia pulcherrima* flower [30].

3.1. Organoleptic and macroscopic characteristics of *P. santalinus*

Organoleptic and macroscopic characteristics of *P. santalinus* leaves and stem are given in Table 1 and Fig. 1. The macroscopic study showed that the leaf was alternate, simple leaf with entire margin, long petiole (5.5 cm), apex sub-acute and base symmetrical, with surface smooth and texture semi smooth.

Leaves

The leaf was simple, trifoliate and alternate; shape was lanceolate to ovate, margin entire. Apex sub-acute, venation reticulate, odour was fragrant scent and taste was fragrant taste. The average leaf size was 15.5 cm in length, 13.7 cm in width, single middle leaf was 10.5cm in length and 8.9cm in width (Fig.1 and Table 1).

Stem

The stem was redish brown, woody, erect, cylindrical, up to 50 to 51cm diameter in trunk and 8 to 9 m (26 feet) tall, odour was fragrant scent and taste was aesthetics (Rasa). Outer surface was rough and hard (Table 1). The organoleptic and macroscopic characteristics laid down can serve as diagnostic parameters for identifying the plant and serve as a tool for preventing accidental or intentional substitution or adulteration.

Microscopic characteristics

T.S of petiole

The transverse section of *P. santalinus* leaf is shown in Fig.2 the T.S. of petiole is shown in (Fig. 2A) the epidermis was single layered. Cortex consisted of 8-9 layers with white latex secretory gland. The vascular bundle" bean" or "pea" shape varied from center to margin. The vascular bundle were conjoint collateral open type (Fig. 2 A).

Leaves

The T.S. of leaf epidermis with palisade tissue is shown in Fig.2 B. The single layer epidermis, consisted of palisade tissue on lower surface (Fig. 2B). Vascular bundles were conjoint collateral open type placed on ventral surface (Fig. 2C). The cortex consisted of 7-8 layers of parenchymatous tissue, cluster crystal of calcium oxalate were present (Fig. 2D). The reticulate venation was seen on the lower surface of the leaf (Fig. 2E); anomocytic stomata were present on the lower epidermis (Fig. 2F).

Stem

The transverse section of *P. santalinus* stem is shown in Fig.3. The epidermis was single layered, thick cuticle layer was present, Many unicellular trichomes were present (Fig.3.A). The cork cambium cells were 2-3 layered consisted of chloroplast cells, the pericycle was surrounded by vascular bundles (Fig.3 B). The vascular bundles were concentric type of arrangement; secondary xylem consisted of Meta xylem, xylem vessels, trachieds, fibro trachide. Secondary phloem consisted of sieve tubes, companion cells, medullary rays and it was radially elongated (Fig.3 C_1, C_2). The cortex consisted of 7-8 layers with white latex secretary glands with polygonal lignified parenchymatous cells were also found (Fig.3D). The pith was small and consisted of parenchymatous. cells cluster crystals of calcium oxalate present in it. (Fig. 3 E).

Powder microscopy of leaves

The crude powder of *P. santalinus* leaves was dark green in colour, fine, odour was characteristic and taste was slight bitter. The powder microscopy characteristics are shown in Fig.4. The specific characteristics determined from the powder study under microscopic investigation showed border pitted vessels, anomocytic stomata, phloem, spiral and reticulate vessels, etc

Powder microscopy of stem

The crude powder of *P. santalinus* stem was reddish brown in colour, fine, odour was fragrant scent and taste was aesthetics (Rasa). The powder microscopy characteristics are shown in Fig.5. The specific characteristics determined from the powder study under microscopic investigation showed annular vessels, border pitted vessels, pitted vessels, scalar form vessels, etc.

The microscopic characteristics laid down are characteristics of the plant under study. These parameters will be helpful in identifying the genuine drug especially is powder form when the plant loses its morphological characteristics. This will help in preventing adulteration and substitution [31]. Such parameters were evaluated for other plants by other researchers [32, 33, 34].

3.2. Phytochemical analysis

The results of qualitative phytochemical analysis of the crude powder of *P. santalinus* leaf, stem and bark are shown in Table 2. The leaf had maximum amount of saponins and stem had saponins and triterpenes while bark had high amount of flavonoids and steroids. Some amount of tannins, cardiac glycosides and anthocyanin were present and phlobatannis were completely absent in all the three parts (leaf, stem and bark). In the present work, the preliminary qualitative phytochemical analysis revealed different phytoconstituents in different parts of the same plant *P. santalinus*. The therapeutic efficacy of a plant depends on the nature of phytoconstituents present in them. Hence *santalinus* leaf, stem and bark can be effectively used as a natural source of antimicrobial, anticancer, antiulcer and other biological activities which can be worked out. Such preliminary phytochemical screening is important and useful and has been attempted for other plants and parts [35, 36].

3.3. Physicochemical study

The physiochemical classification of *P. santalinus* leaf, stem and bark are shown in Table 3. The moisture content of leaf, stem and bark was 8.05%, 11.00% and 5.75% respectively. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash in leaf was 8.91%, while water soluble ash and acid insoluble ash was 5.08 and 1.00 respectively. The total ash in stem was 9.25% while water soluble ash and acid insoluble ash was 1.33 and 0.83 respectively %. While the total ash in bark was 3.33% while water soluble ash and acid insoluble ash was 1.33 and 0.50 respectively % and the sulphated ash in leaf, stem and bark was 10.16, 5.83 and 3 respectively %. The extractive values of leaf, stem and bark are shown in Table 6. The maximum extractive value was found in leaf and stem in methanol solvent and bark in acetone solvent while minimum was in petroleum ether solvent in all the three parts leaf, stem and bark of *P. santalinus*.

The determination of various parameters of physicochemical study is important and it useful in detecting the purity of drug. Presence of low grade drugs, exhausted drugs and presence of silica or sand can be easily detected and thus genuine drugs and adulterated or substituted drugs can be easily identified especially in powder form. Extractive values also varies in different solvents and it is also indicative of purity of the crude drug [37, 38].

3.4. Fluorescence analysis

The fluorescence character of powdered drug plays a very important task in the purpose of quality and transparency of the drug material. The fluorescence characteristics of leaf, stem and bark powder of *P. santalinus* are summarized in Tables 4-6. Some constituents show fluorescence in the visible range in daylight. The UV light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Similar fluorescence analysis is reported for other plants for eg. *Terminali arjuna* [39]; *Solanum virginianum* [40], *Buchanania lanzan, Artocarpus hirsutus* and *Terminalia coriacea* [41].

Pharmacognostic studies have been done on different parts by different researchers. For eg. Lakshmi *et al.*, [42] worked on *T. travancorensis* leaves and bark; Rakholiya *et al.*, [43] worked on different parts of *M. indica;* Kumar *et al.*, [44] worked on different parts of *P. pinnata* stem and bark; Anitha *et al.*, [45] worked on different parts of *A. sessilis* stem; Thomas *et al.*, [46] worked on *A. carambola* fruit; Qadir *et al.*, [47] worked on different parts of *A. cocculus* seed; Murugan *et al.*, [48] worked

on different parts of *O. parvifolia* whole plant; Kumar *et al.*, [49] worked on different parts of *H. integrifolia* leaf and root bark; Leela *et al.*, [50] worked on different parts of *A. nilotica* flowers; Apraj *et al.*, [51] worked on different parts of *C. aurantifolia* peel. Bapodara *et al.*, [52] worked on different parts of *P. granatum* leaf; Nagani *et al.*, [37] worked on different parts of *C. quadrangular is* stem; Moteriya *et al.* [26] worked on different parts of *M. indica* leaf and stem.

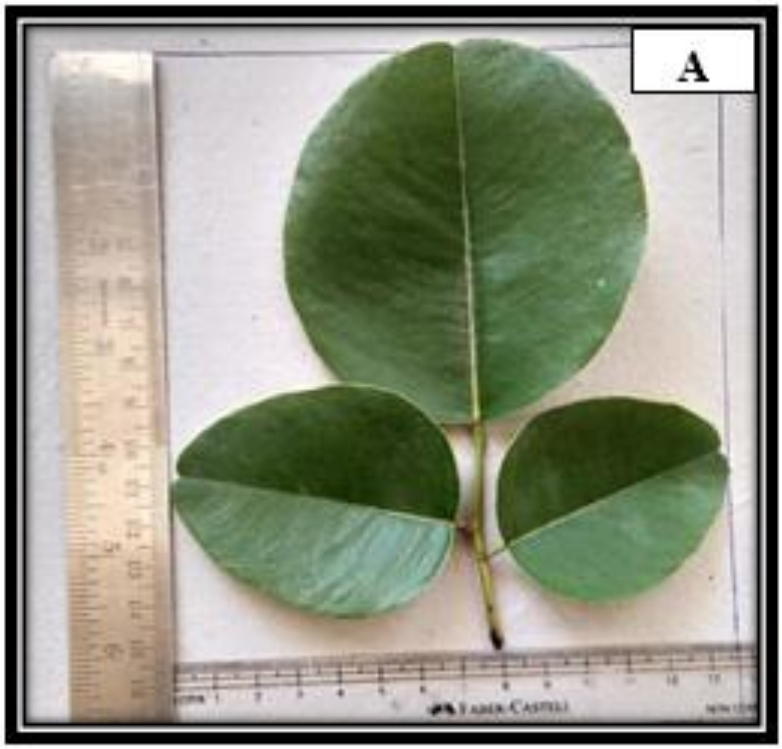


Fig 1: Macroscopic characteristics of *Pterocarpus santalinus* Linn. F.

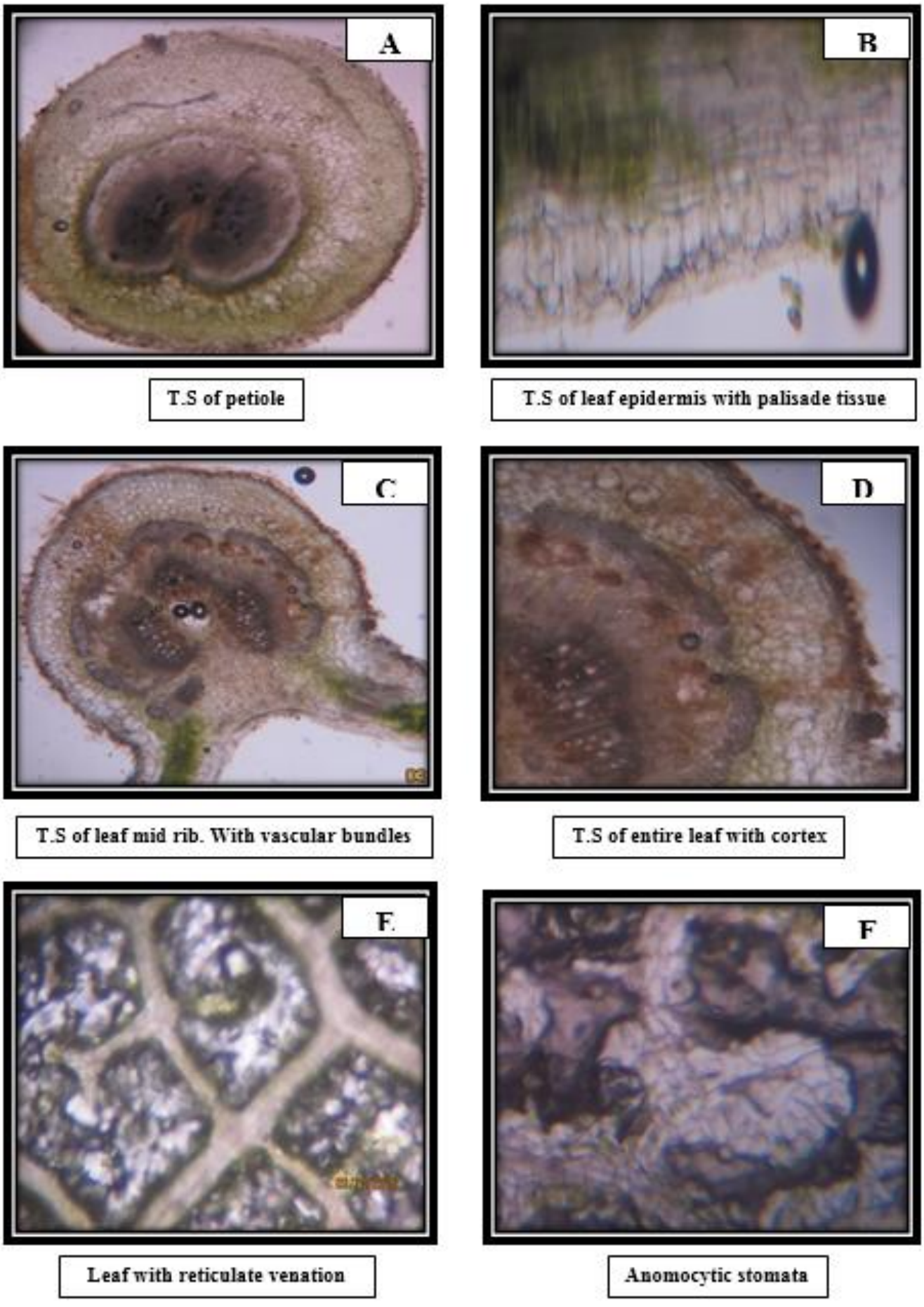


Fig 2: Microscopic characteristics of *Pterocarpus santalinus* leaf

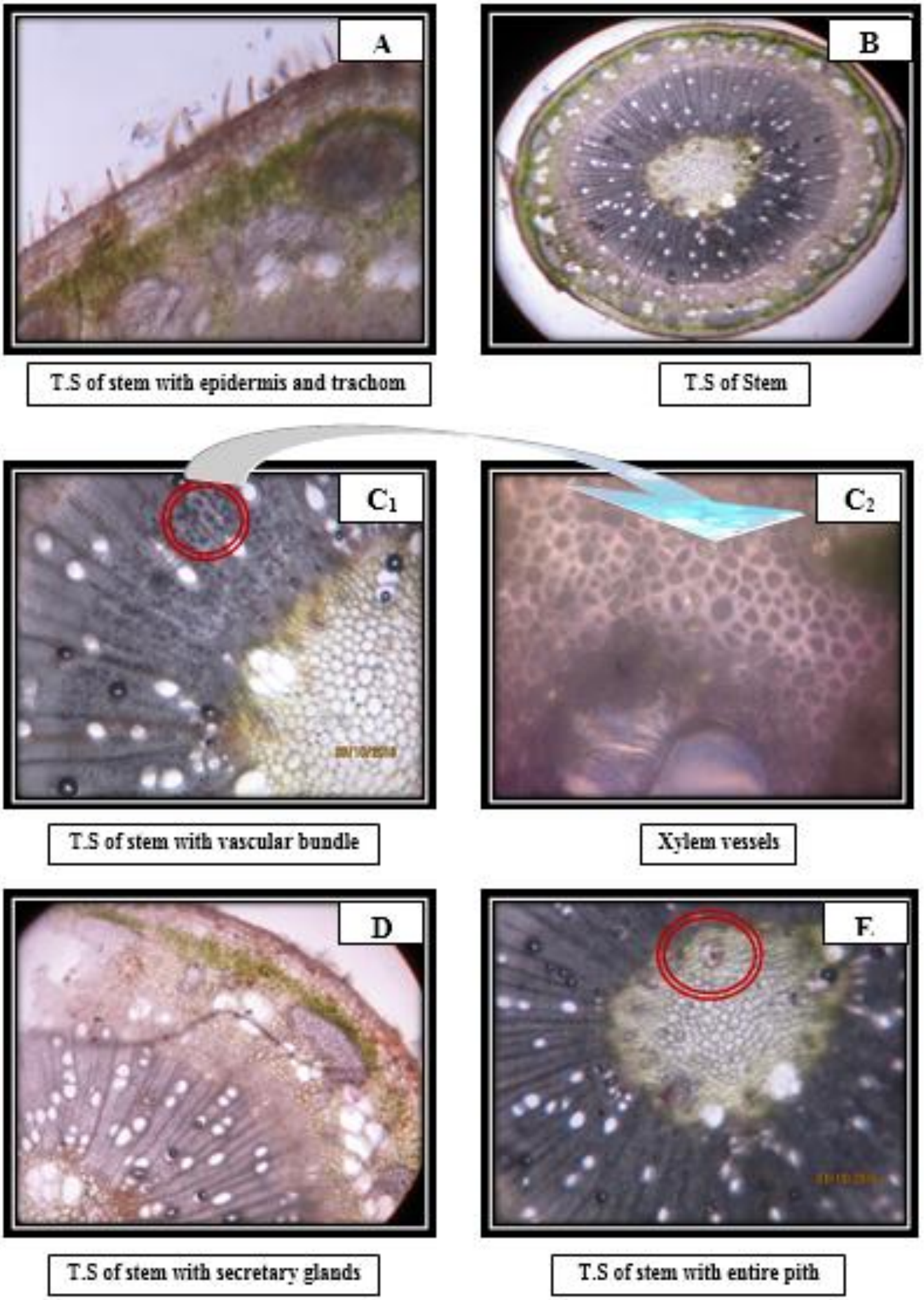
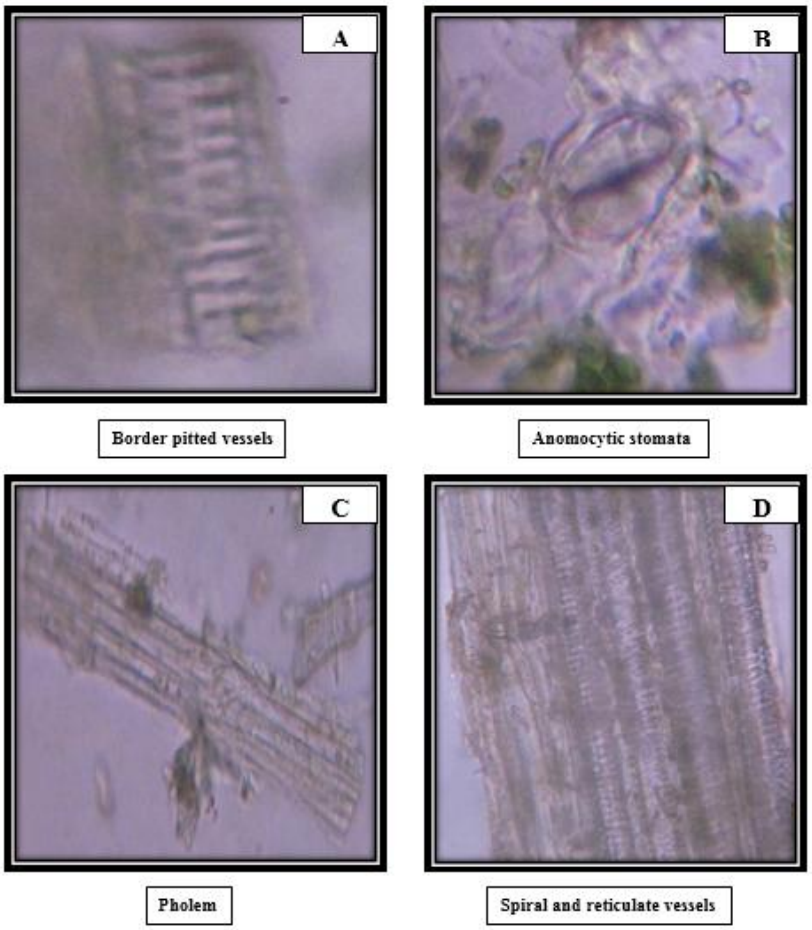


Fig 3: Microscopic characteristics of *Pterocarpus santalinus* stem

Leaf



Stem

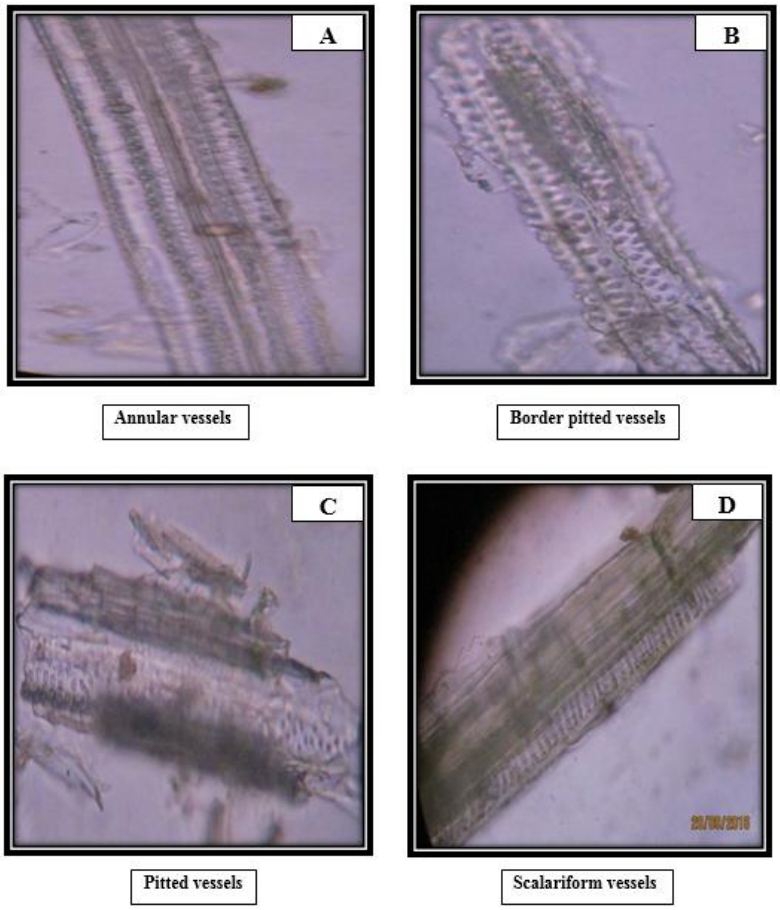


Fig 4: Microscopic characteristic of powder of *Pterocarpus santalinus* stem

Table 1: Organoleptic features of *Pterocarpus santalinus* leaf and stem

Parts	**Observation**	
Part	**Leaves**	**Stem**
Arrangement	**Alternate**	-
Size	15.5 cm long, 13.7 cm wide(trifolite leaf) 10.5cm long and 8.9cm wide(singal leaf)	50 to 51cm diameter in trunk and 8 to 9 meter (26feet) tall
Shape	Lanceolate to ovate	Cilindrical
Colour	Green	Redish brown
Odour	Fragrant scent	Fragrant scent
Taste	Fragrant taste	Aesthetics (Rasa)
Appearance	Smooth	Rough & Scabrous
Margin	Entire	-
Apex	Subacute	-
Base	Symmetrical	-
Petiole	Long (5.5 cm)	-
Texture	Semi smooth	Dark Redish brown colour.
Veination	Reticulate veination	-
Outer surface	Smooth	Rough surface

Table 2: Qualitative phytochemical analysis of leaf, stem and bark

No.	**Phytochemical**	**Leaf**	**Stem**	**Bark**
1	Alkaloids			
	i) Mayer's Reagent	-	-	-
	ii) Dragondroff's Reagent	+	-	-
	iii) Wagner's Reagent	-	-	+
2	Flavonoids	+	++	++++
3	Tannins	++	++	+
4	Phlobatannis	-	-	-
5	Saponins	+++	+++	-
6	Steroids	+	-	++++
7	Cardiac glycosides	+	-	-
8	Triterpenes	++	+++	++
9	Anthocyanin	+	-	-

Note: (++++) - High amount, (+++)-Moderate amount, (++)-Less amount, (+)-Very less amount, (-)-Absent

Table 3: Physiochemical analysis

No	**Parameters**	**%Value (w/w) Leaf**	**. %Value (w/w) Stem**	**. %Value (w/w) Bark**
1	Loss on drying	8.5	11	5.75
2	Total ash	8.91	9.25	3.33
3	Water soluble ash	5.8	1.33	1.33
4	Acid insoluble ash	1	0.83	0.50
5	Sulphated ash	10.16	5.83	3
6	Extractive value -PE	0.76	0.79	0.76
7	Extractive value -TO	1.49	1.13	4.20
8	Extractive value -EA	1.37	1.46	11.41
9	Extractive value -AC	4.37	2.88	17.46
10	Extractive value -ME	13.11	6.20	17.39
11	Extractive value -AQ	10.80	5.87	3.37

(PE-Petroleum ether, TO-Toluene, EA-Ethyl acetate, AC-Acetone, ME-Methanol, AQ-Water)

Table 4: Fluorescence analysis of *Pterocarpus santalinus* leaf powder

Treatment	**Visible light**	**Under UV light Short Wavelength (254 nm)**	**Long Wavelength (365 nm)**
1 N NaOH (aq)	Green	Black	Green
1 N NaOH (alc)	Green	Black	Green
Ammonia	Green	Black	Brown
Picric acid	Green	Black	Green
Petroleum ether	Green	Black	Green
50% HCl	Light Green	Black	Brown
50% H_2SO_4	Green	Black	Green
Ethyl acetate	Green	Black	Brown
Ethyl alcohol	Green	Black	Brown
Methanol	Green	Black	Dark brown

Table 5: Fluorescence analysis of *Pterocarpus santalinus* stem powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long Wavelength (365 nm)
1 N NaOH (aq)	Green	Black	Green
1 N NaOH (alc)	Brown	Black	Green
Ammonia	Brown	Black	Green
Picric acid	Yellow	Black	Green
Petroleum ether	Brown	Black	Green
50% HCl	Brown	Black	Green
50% H_2SO_4	Brown	Black	Brown
Ethyl acetate	Brown	Black	Green
Ethyl alcohol	Brown	Black	Green
Methanol	Light Brown	Black	Light Yellow

Table 6: Fluorescence analysis of *Pterocarpus santalinus* bark powder

Treatment	Visible light	Under UV light Short Wavelength (254 nm)	Long Wavelength (365 nm)
1 N NaOH (aq)	Wine Red	Black	Dark Red
1 N NaOH (alc)	Wine Red	Black	Green
Ammonia	Red	Black	Brown
Picric acid	Red	Black	Red
Petroleum ether	Red	Black	Red
50% HCl	Red	Black	Brown
50% H_2SO_4	Red	Black	Red
Ethyl acetate	Brown	Green	Yellow
Ethyl alcohol	Red	Black	Brown
Methanol	Reddish brown	Black	Brown

Conclusion

The present study was done to evaluate the pharmacognostic, phytochemical, physicochemical parameters of leaf, stem and bark of *P. santalinus*. The parameters evaluated are diagnostic characters of this plant and help in its correct identification even from crude powder. It will help in checking adulterants and substituents so that the efficacy of the plant parts will be maintained. The parameters obtained in this study will also help in establishing the monograph of the plant. The information collected will be of use to researchers for further pharmacological and therapeutic evaluation of the plant.

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