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Pharmacognostical studies on *Dalbergia spinosa* root

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Abstract
Dalbergia spinosa Roxb. (Family: Leguminosae-Papilionoideae), a large climbing shrub commonly found in mangroves along the Coromandel coasts of south India. Root is bitter taste, used to treat inflammations, urinary problems, pain and fever and also reported for various pharmacological properties such as hypothermic, spermicidal, semen coagulant, hypoglycemic, cardio vascular, antimicrobial, diuretic and analgesic. In the present study, pharmacognostical investigation on roots was carried out by determining the morphological, micro scopical and physicochemical parameters. It was found that the root is cylindrical, elongated, tuberous in nature with lateral branches, yellowish brown in colour, slightly sweet taste with aromatic odour. Microscopical evaluation reveals that the presence of brown coloured cork cells and the periderm is distinguished into phellum, phellogen and phelloderm, made up of parenchyma followed by secondary phloem and xylem consists of vessels, fibres and lignified parenchyma. Histochemical studies of the root exhibits the presence of polyphenols, lignins and total proteins in the cortical cells, vessels and phloem respectively.

Keywords: *Dalbergia spinosa*, pharma cognosy, standardization, herbal drug

Introduction
Dalbergia spinosa Roxb., (Leguminosae-Papilionoideae), a Climbing shrub locally known as Jantri Kanta or *Nechitanchedi* in Tamil language (John Britto, 1989) [15] distributed in mangroves of India, Bangladesh, Myanmar and Malaysia (Tangavelou, 2011) [33]. Medicinally, the plant is used for the treatment of inflammations, urinary excretion problems, pain and fever (Kirthikar, 1994) [16]. Kurz, *et al*., (1881) [22] reported that a spoonful of root powder in a glass of water is sufficient to destroy the effects of alcohol within half an hour even in cases bordering on delirium tremens. Phyto-chemically, several isoflavone compounds such as dalspinin, dalspinosin, caviunin and 5-hydroxy-6-methoxy-3',4'-methylenedioxy-7-[(6-O-β-D-apiofuranosyl-β-D-glucopyranosyl)oxy] isoflavone (dalspinin-7-O-β-D-[apiofuranosy (1->6)] gluco pyranoside), prunetin-4-O-β-D-galactoside, dalspinosin-7-O-β-glucopyranoside were reported from root (Gandidasan, *et al*., 1998; Radha, *et al*, 2015) [7, 8, 30]. Several biological activities on root extracts were reported such as analgesic, anti-inflammatory, antimicrobial, anti-nociceptive, antioxidant, cytotoxicity, cardio vascular effects, diuretic, hypoglycemic, hypothermic, semen coagulant and spermicidal activities (Dhawan, *et al*., 1977; Senthamarai, *et al*., 2003; Jaiganesh, *et al*., 2009a & b; Jaiganesh, *et al*., 2010; Bala, *et al*., 2011) [4, 2, 11, 12, 13, 32]. But Pharmacognostical studies have not yet been reported for standardization and quality control of this plant. To fulfill this gap, the present study is to perform the Pharmacognostical investigation and preliminary phytochemical screening on *Dalbergia spinosa* Roxb root.

Materials and Methods
Plant material
Roots of *Dalbergia spinosa* were collected from the mangrove forests Thandavarayan Solanganpettai, near Chidambaram, Tamil Nadu, India. The plant was botanically identified by plant taxonomist of Raphinat Herbarium, St. Joseph's College, and Tiruchirappalli and compared with herbarium (Plate No. 381, RHT 12844) and a voucher specimen was deposited in the department for further reference. The collected root was separated, washed completely with water and then shade-dried for further studies.

Collection and Preservation of materials
Fresh root pieces were collected and fixed in the field immediately in the fixatives FAA (Formalin: Acetic acid: Alcohol) and kept in FAA for more than two days. Dehydration was carried out by employing graded stages of tertiary butyl alcohol and ethyl alcohol mixtures as per the standard method (Sass, 1940; Johansen, 1940) [31, 14]. After dehydration, paraffin infiltration was carried out till super saturation of tertiary butyl alcohol was achieved.

Morphological studies
The fresh roots were spread on a dry plastic sheet for investigating the morphological characters with the help of field lens and dissection microscope.

Reagents and Chemicals
All the reagents and chemicals used for analyzing various parameters were obtained from Merck Pvt. Limited, Mumbai, India, of analyticalgrade.

Microtoming
The paraffin embedded specimens were sectioned with the help of a rotary microtome 10-12 µm thickness of sections was made. However, dewaxing of the sections was done by using customary procedure (Johansen, 1940) [14]. The sections were later stained with O-Toluidine blue (O'Brien, *et al*., 1964) [28]. Cleared sections were then mounted in glycerin for micro-scopical observation (Sass, 1940) [31]. Glycerin mounted temporary preparations were made for macerated/ cleared materials. Powdered plant parts were cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell components were studied and measured (Krishnamoorthy, 1998) [20]. Microphotographs were taken by using NIKON trinocular research microscope. Descriptive terms of the anatomical features are given as per the standard anatomy books (Esau, 1964; Metcalf and Chalk, 1950 & 1979) [5, 24, 25].

Physicochemical studies
Air-dried, coarsely powdered roots were subjected to physicochemical studies such as ash values, extractive values, loss on drying and crude fibre content (Anonymous, 1996) [1].

Fluorescence analysis
Organic solvents of hexane, benzene, chloroform, alcohol and acetone, water, 1N HCl and 50% H_2SO_4 and alkaline solutions of aqueous and alcoholic 1N NaOH were taken and treated individually with desired quantity (1 g) of the plant material. After 24 h, fluorescence of each extraction was observed and recorded both under daylight and UV light (Chase and Pratt, 1949; Kokoshi, *et al*., 1958; Prasad, *et al*., 1960) [3, 19].

Preliminary phytochemical screening
The air dried and powdered root was extracted successively with petroleum ether, benzene and ethanol (70%) by continuous hot percolation method in a soxhlet apparatus. Finally the marc was macerated with chloroform water (0.25% v/v $CHCl_3$). Each extract was concentrated by distilling off the solvent and were subjected to various qualitative tests for an identification of chemical constituents present in the plant material (Farnsworth, 1966; Harborne, 1998; Evans, 2006; Sarker, *et al*., 2006) [10, 6] and the observations were recorded.

Results and Discussion
Morphological studies
Large climbing shrub. Root, taproot, branched, cylindrical, elongated, soft, tuberous, yellowish brown, slightly aromatic and characteristic odour with slightly sweet taste. Leaves, compound, alternate; leaflets 5-11, obovate or elliptic-ovate, apex obtuse, margin emarginate. Flowers white with yellowish stripes. Pod, compressed, reni-form, smooth, glabrous, coriaceous, one-seeded (Fig.1).Organoleptic evaluation is based on the study of morphological and sensory profiles of whole drugs (Kokate, *et al*., 2007) [18]. It is therefore considered as a primary step in the qualitative assessment of crude drugs. The parameters such as the structure of root, surface of roots, the typical tongue sensation and the odour are some important diagnostic as well as qualitative organoleptic indicators of root drugs. For example, the characteristic aroma of leaves (or any other plant parts) is a true indicator of the presence of volatile active principles.

Microscopical characters
The transverse section of the root is circular in outline with wavy margin, covered by brown coloured cork cells (Fig. 2). Periderm is distinguished into phellum, phellogen, 4-5 layered below the cork; phelloderm, 4-6 layered, made up of broader parenchymatous cells with intercellular spaces. Secondary phloem is made up of sieve cells and parenchyma with starch grains. Cambium is distinct, 4 layered and arranged in radial rows. Secondary xylem consists of prominent vessels, fibres and lignified wood parenchyma. Medullary rays are uni seriate, distinct and extended radially from pith to secondary phloem. The pith is composed of large parenchymatous cells with intercellular spaces. The roots are yellowish brown in colour, with aromatic odour and sweet taste. The transverse section of the root shows secondary tissues such as periderm, secondary phloem, secondary xylem with prominent vessels and uni-seriate, medullary rays. The primary tissues were crushed due to the development of secondary tissues.
Histochemical studies revealed that the presence of lignins, proteins and polyphenols in the transverse section (Fig. 2 & 3) of the root and the results were tabulated (Table 1). In histo-chemical studies, the root section was stained with different reagents which revealed that the secondary xylem, tracheids indicate more intense reddish-violet colour, due to the presence of lignin. Secondary phloem and cambial region shows more intense green colour due to the presence of proteins. The cortical cells and vessels are showing moderately blue green colour with TBO solution, indicates the presence of polyphenols and lignins.

Physicochemical studies
The results of physicochemical studies were exhibited (Table 2), that water soluble ash value (9.52%w/w) is slightly greater than acid insoluble ash value (7.40%w/w). Alcohol yielded higher extractive value (19.92%w/w) when compared with water (13.11%w/w).Ash is a residue of sand and soil adhering to the plant material (i.e.) carbonized residue and it includes carbonates, phosphates, silicates and silica of sodium, potassium, magnesium, calcium or added purposefully for adulteration. The acid insoluble ash is a part of ash is imposed, especially in case where silica and calcium oxalate content of the drug is very high due to the presence of earthy material (Evans, 2006) [6]. Alcohol soluble extractive value is higher than water soluble extractive value, because of the unique feature of alcohol, is capable of dissolving all polar and nearly low polar constituents.

The crude fibre content (10.43%w/w) exhibited the presence of cellulose and lignin. Estimation of crude fiber denotes the measurement of the content of cellulose, lignin and cork cell in the plant tissue. The crude fiber consists of the material other than ash which cannot be dissolved in water and cannot be digested by boiling with H_2SO_4 or NaOH. Thus it represents the more resistant part of the plant cells as well as some less resistant cell wall component like cellulose and pectin. The presence of adulteration containing sclerenchyma or other resistant tissue than is permissible for the crude drug under examination may be determined by ascertaining the crude fiber of that sample (Mukherjee, 2005) [26]. Environmental factors such as moderate water stress may interrupt the progression towards maturity and therefore maintain low plant fibre (Griffin, *et al*., 2015) [9].

The moisture content of the root powder was reported (20.42%w/w).The moisture content (% Loss on Drying - LOD) of the powdered root drug was found to be 20.45% w/w which indicates that the drug was properly dried and stored. The determination of moisture content is important for the plant drugs because insufficient drying may lead to possible enzymatic deterioration of active principles (Kokate, *et al*., 2007) [18]. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Excess of moisture content will enhance the microbial growth and leads to the determination of the plant material by the loss of biological activity (Poos and Varju, 2017) [29]. This parameter is therefore essentially used to control the quality of crude drugs and/or herbal drugs/drug products.

Phytochemical screening

Qualitative analysis revealed (Table 3) that all the root extracts gave positive reaction for flavonoids and lignins. Petroleum ether extract exhibited negative reaction while the other extracts gave positive reaction for carbohydrates and glycosides. Preliminary phytochemical screening shows the presence of flavonoids, lignins, carbohydrates and glycosides.

Fluorescence analysis

In the present study, root powder was treated with various reagents as well as root extracts showed characteristic fluorescence at 254 nm wavelength (Table 4).

The pharmacognostical study is one of the major criteria for identification of plant drugs. The present study on *Dalbergia spinosa* root will provide useful information such as morphology, microscopy, physicochemical standards, fluorescence analytical data and phytoconstituents of diagnostic values. Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. The ultra violet light produces fluorescence in many natural products which do not visibly fluorescence in daylight. If substance themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents, is used to determine the presence of chromophores. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence crude drugs are often assessed qualitatively in this way and it is an important parameter for pharmacognostic evaluation of crude drugs (Kumar and Kumar, 2012; Zhao, *et al*., 2011; Muhammad Zia-Ul-Haq, *et al*., 2013) [21, 23, 34].

Table 1: Histochemical studies of *Dalbergia spinosa* root

Reagents	Phytoconstituents	Colour	Zone	Degree of intensity
Phloroglucinol+HCl	Lignin	Reddish violet	Secondary xylem, tracheids	+++
Fast Green+Safranin	Sulphated, Carboxylated Polysaccharides	Reddish purple	Cortical cells	++
TBO Solution	Polyphenols	Blue green	Cortical cells, vessels	++
TBO Solution	Lignin	Blue green	Cortical cells, vessels	++
Fast Green	Total proteins	Bright green	Secondary phloem, cambial region	+++
Sudan dye	Total lipids	Dark purple	Tracheids, medullary rays	++

Table 2: Physico-chemical studies of *Dalbergia spinosa* root

Parameters	Average value (% w/w)
Total ash	16.42
Water soluble ash	9.52
Acid insoluble ash	7.40
Sulphated ash	4.29
Loss on drying	20.45
Crude fibre content	10.43
Alcohol soluble extractive value	19.92
Water soluble extractive value	13.11

Table 3: Preliminary phytochemical screening of various extracts of *Dalbergia spinosa* root

Phytoconstituents	Petroleum ether	Benzene	Ethanol	Aqueous	Root Powder
Alkaloids	-	-	-	-	-
Carbohydrates & Glycosides	-	+	+	+	+
Phytosterols	-	-	-	-	-
Fixed oils & fats	-	-	-	-	-
Saponins	-	+	+	+	+
Tannins	-	+	+	+	+
Proteins & Amino acids	-	-	-	-	-
Mucilages& Gums	-	-	-	-	-
Flavonoids	+	+	+	+	+
Lignins	+	+	+	+	+

(+) = Presence of phytoconstituents; (-) = Absence of phytoconstituents

Table 4: Fluorescence behavior of *Dalbergia spinosa* root powder with different chemical reagents

Reagents	Day light	UV light (254 nm)
Powder as such	Light brown	Light green
Powder + 1N NaOH (Aq)	Reddish brown	Brownish yellow
Powder + 1N NaOH (Alc)	Dark brown	Green
Powder + 1N HCl	Yellowish brown	Pale green
Powder + 50% HNO_3	Yellowish brown	Green
Powder + 50% H_2So_4	Light brown	Light green
Powder + Methanol	Light brown	Light green
Powder + NH_3	Reddish brown	Brownish green
Powder + I_2 solution	Light brown	Green
Powder + $FeCl_3$	Greenish black	Dark green

Table 5: Fluorescence analysis of *Dalbergia spinosa* root extracts

Extracts	Day light	UV light (254 nm)
Petroleum ether	Pale brown	Pale green
Benzene	Dark brown	Green
Chloroform	Dark brown	Light green
Acetone	Brown	Dark green
Alcohol	Reddish brown	Green
Aqueous	Brown	Dark green

Fig 1: Habit of *Dalbergia spinosa* Roxb

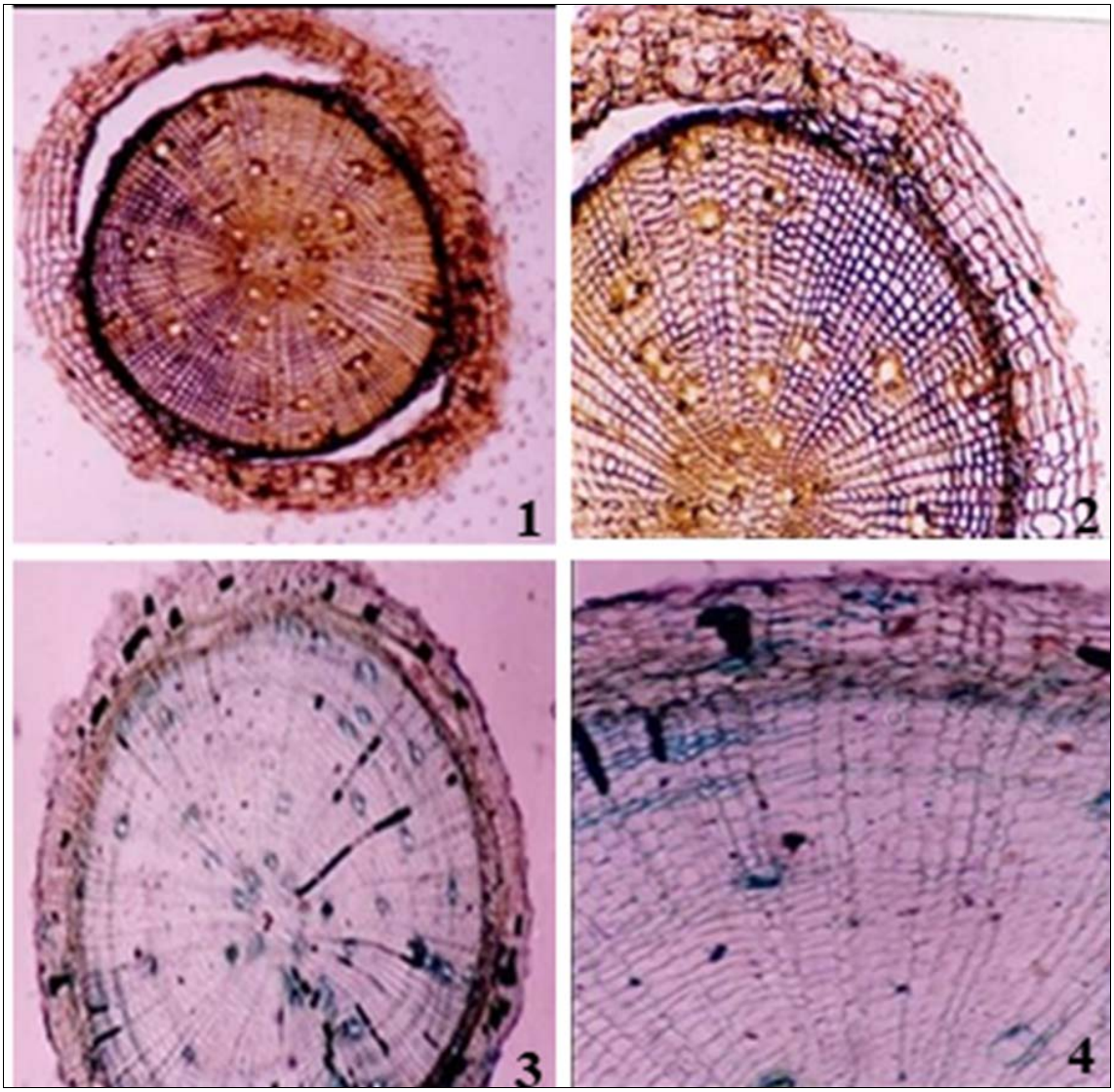

Fig 2: 1 & 2 Phloroglucinol-HCl-Lignins (10x X 4x) &(10x X 10x) 3 & 4. Toluidine Blue-O-Polyphenols (10x X 4x) &(10x X 10x)

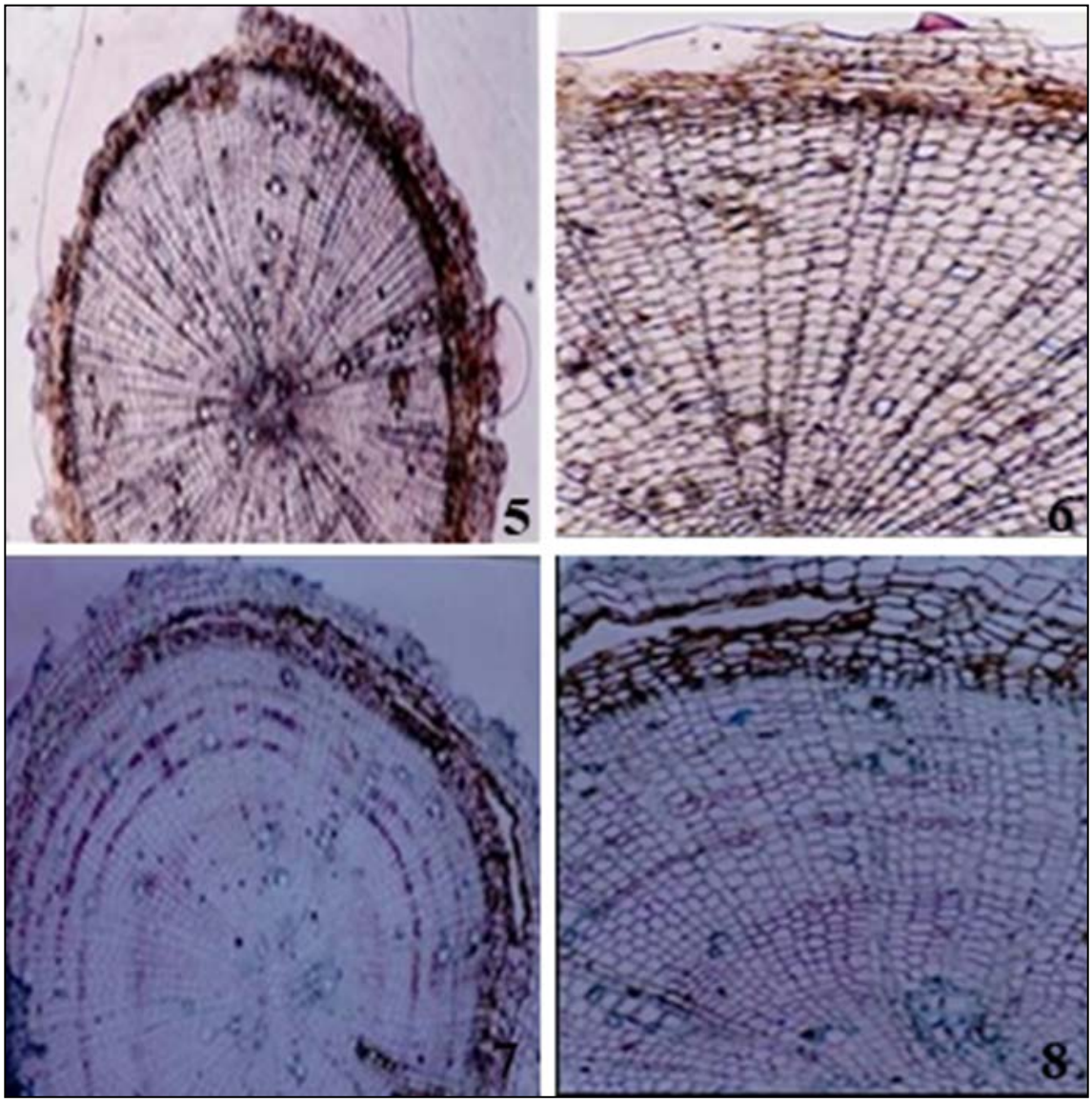

Fig 3: 5 & 6. Sudan dye-Total lipids (10x X 4x) &(10x X 10x) 7&8. Fast Green-Saffranin-Lignins (10x X 4x) &(10x X 10x)

Conclusion

The pharma cognsotical parameters established in this study such as morphology, microscopy, physicochemical standards, fluorescence analysis and phytochemical screening will be useful in standardizing the crude drug, develop a monograph and also used to differentiate the closely related species. The pharmacognostical parameters can be considered as a distinctive character for the plant which is good enough to authenticate the plant in herbal industry to prevent adulteration and also facilitate the quality assurance of the starting material. These studies revealed the presence of various important bioactive constituents and proved that these plant drugs are also medicinally important.

The present investigation on the root of *Dalbergia spinosa* Roxb, reveals a pharma cognostic identity. This study will be helpful for manufacturers for assessing the purity of raw material. Briefly, the aspects described here can be considered as characteristic to identify and authenticate this drug.

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