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, cardiovascular, antimicrobial, diuretic and analgesic. In the present study, pharmacognostical investigation on roots was carried out by determining the morphological, microscopical 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*, pharmacognosy, 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-apiofuranosyl-β-D-glucopyranoside), prunetin-4-O-β-D-galactoside, dalspinosin-7-O-β-D-glucopyranoside were reported from root (Gandida san, *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) [14, 15]. 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 analytical grade.

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., 1949; Kokoshi, et al., 1964) [28]. Cleared sections were then mounted in glycerin for micro-scopical observation (Sass, 1940) [21]. 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) [8, 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) [11].

Fluorescence analysis

Organic solvents of hexane, benzene, chloroform, alcohol and acetone, water, 1N HCl and 50% H2SO4 and alkaline solutions of aqueous and alcoholic IN 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 CHCl3). 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, reniform, 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 phellem, 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 uniseriate, 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 uniseriate, 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 histochemical 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 fibre consists of the material other than ash which cannot be dissolved in water and cannot be digested by boiling with H2SO4 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 may be determined by ascertaining the presence of lignin and cellulose. 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 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].

Phytochemical screening

| Reagents                        | Phytoconstituents | Colour      | Zone                  | Degree of intensity |
|--------------------------------|-------------------|-------------|-----------------------|---------------------|
| Phloroglucinol+HCl             | Lignin            | Reddish violet | Secondary xylem, tracheids | +++                |
| Fast Green+Safarin            | 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          | -               | +       | +       | +       | +           |
| Tammins           | -               | +       | +       | +       | +           |
| 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% HNO₃             | Yellowish brown | Green        |
| Powder + 50% H₂SO₄            | Light brown   | Light green       |
| Powder + Methanol             | Light brown   | Light green       |
| Powder + NH₃                  | Reddish brown | Brownish green    |
| Powder + I₂ solution          | Light brown   | Green             |
| Powder + FeCl₃                | 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)
Conclusion
The pharmacognostical 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 pharmacognostic 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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