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Original Article

PHARMACOGNOSTIC STUDIES OF DALBERGIA SISSO ROXB.

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ABSTRACT

Objective: Present study was aimed to standardize the leaves, pods, and bark of *Dalbergia sisso* Roxb (Fabaceae) plant which is one of the most important species of Pakistan and used in different ailments.

Methods: Powders of dried parts of this plant were used for macroscopic and microscopic, histological, fluorescence, micro chemical, proximate, infra-red spectroscopic examinations and extract were used for preliminary phytochemical examination. These entire tests were performed as per World Health Organization (WHO) standards.

Results: In preliminary phytochemical analysis *D. sisso* carbohydrates, alkaloids, and tannins were detected in the pod while leaves contain carbohydrates alkaloids and flavonoids. The different cellular structure provides the basis of different parts identifications like stomata in leave (A), schlerides in pods (B) and tissues in bark (C) parts of the powdered plant. Proximate analysis showed the high level of moisture content and ash values of A, B and C samples. The fluorescence behavior of powdered material of A, B and C revealed the coloration of these samples under different wavelength. Fourier transform infrared spectroscopy (FTIR) established the spectrum include aromatic and aldehyde based functional groups for the all three powdered samples of *D. sisso* Roxb.

Conclusion: This research work was performed for the standardization of the plant *D. sisso* Roxb. as per WHO recommendations and we established the proper identification profile of the plant and its parts.

Keywords: Powder microscopy, *Dalbergia sisso Roxb,* Phytochemical analysis, Fabaceae, Histology

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INTRODUCTION

Large genus *Dalbergia* is widely distributed in the tropical region of the world including central and South America, Africa Madagascar, and Southern Asia having approximately 300 species and belongs to the pea family *Fabaceae* formerly named as *Pappilionacea* [1]. The most important species *Dalbergia sisso* Roxb is native to foothills of Himalayas India, Pakistan and Nepal. Basically, it is cultivated in forest plantations but also growing along the river bank, roadsides, railway lines, water channels and Borders of the agricultural fields [2]. In Pakistan, it is widely distributed in the mega cities of Punjab and Sindh province and plays its role in reducing the pollution.

The various chemical constituents have been isolated from the different parts of this plant. Leaves contain Isoflavone-O-glycoside, flowers have Biochanin A, tectorigenin, 7, 4 dimethyl tectorigenin and 7-O-methyle tectorigenin. Immature and mature pods are full of Mesoinisitol, 7-O-methyle tectorigenin and 4'-rhamnoglucoside, Isocaviumin, tectorigenin, Dahlberg in, caviunin and tannins type compounds. Dalberginone, dalbergin, methyl dalbergin and dalbergichromene were isolated from its stem bark whereas, Dalbergin, nordalberginones, dalbergichromene, fixid oil and essential oils from heartwood. Wood of this plant is used for fuel and furniture making purpose. The plant has been cultivated as a venue tree and also has a great medicinal value.

Ethanolic leave extract show osteogenic activity in calvarial osteoblast cultures and also proved efficacious in isoproterenol-induced myocardial injury in rats [3, 4]. Aqueous and methanolic extracts of stem bark of this plant have shown antioxidant and Antidiabetic activity [5, 6]. It is used as folk remedy for excoriation, gonorrhea and skin ailments [7]. Ayurveda prescribed the leaf juice for eye ailments, considering whereas the wood and bark are used as an abortifacient, anthelmintic, antipyretic, aphrodisiac, expectorant and refrigerant. Wood has also used in Unani medicine for blood disorders, burning sensations, eye and nose diseases scabies, scalding urine stomach problems and syphilis.

The aim of this study is to provide such information that can proceed as reference information for correct identification of exacting plant and also will be valuable in making a monograph of the *Dibergia sisso* plant. Moreover, it will operate the sample as a tool to perceive adulterants and substituent. The detailed authentic sample identification as per WHO requirement for the standardization of different parts of *Dibergia sisso* not reported therefore it will provide the basis for safe, effective equality products to the market by the utilization of standard herbal drug as raw material or as an active herbal drug.

MATERIALS AND METHODS

Collection and Identification of plant materials

The leaves (A) pods (B) and bark (C) of *Dalbergia sisso* Roxb. were collected in the month of December 2014 from the premises of University of Karachi. The plant was identified by Prof. Dr. Ghazala. H. Rizwani, Department of Pharmacognosy, University of Karachi. The voucher specimens with numbered 104A, 104B and 104C respectively were deposited in the herbal museum of Department of Pharmacognosy, Faculty of the Pharmacy University of Karachi.

Chemicals and reagents

All chemicals were used of analytical grade and reagents were prepared in the distilled water. methanol, ethanol, n-butanol, ethyl acetate, chloroform, sulphuric acid, acetic acid, hydrochloric acid, nitric acid, water, vanillin, iodine, chloral hydrate, glycerin, ferric chloride, Canada balsam,saffranine, malachite green, clove oil, nitrocellulose, amylacetae, sodium hydroxide, ammonium ceric sulphate, Molisch's reagent, Salwaski's reagent, Dragndroff's reagent, and Liebermann Burchard's reagent were used.

Extraction

Sample A, B and C of *Dalbergia sisso Roxb*. were separated, cleaned and weighed (10Kg each) properly,chopped into small pieces. Then these parts were percolated separately in absolute methanol (Merck,

Germany) at room temperature for fifteen days and evaporated under reduced pressure and controlled temperature on the rotary evaporator (Eyela, Japan). After obtaining the extracts, the materials were again percolated in the same way. This procedure was repeated thrice. The 55g (A) 46g (B) and 50g (C) residues were obtained.

Preliminary phytochemical examination

The portion of all methanolic extracts was utilized for the detection of various primary and secondary compounds in A, B and C parts of the plant. Preliminary phytochemical analysis including carbohydrate (Molisch's Reagent), terpenoids (Salwaski's reagent), alkaloids (Dragndroff's reagent) steroids (Liebermann Burchard's reagent) tannins and flavonoids were performed on each methanolic extract, according to standard procedures [8].

Proximate examination

Proximate parameters including loss on drying, moisture content, and dry matter weight were determined in drying oven (DHG-9053A). Percentage of total ash was also determined at 800°C by using Muffle Furnace (A product of PCSIR, MF-102) [9].

Pharmacognostic examination

The macroscopic and microscopic evaluations of sample A, B and C were carried out according to WHO recommendations.

Macroscopy

Macroscopic or organoleptic study of sample A,B and C were evaluated after washing of plant with distilled water, dried it in hot air oven at 35 ˚C and grinded with the help of electric grinder. Color, odor, and taste of powdered drug were performed by sensory organ. [10]

Microscopy

Microscopic examination of powdered sample A, B and C were performed by using a microscope (Nikon Co. Ltd., Japan). A small amount of fine powdered of A, B, and C were placed on a microscopic slide, mixed with 10%w/v aqueous chloral hydrate solution and covered with a coverslip then was placed microscope and observed carefully for identification of different cellular diagnostic features. Then same procedure was repeated by using 5%w/v aqueous solution of iodine and 50% w/v aqueous solution of glycerin [11,12].

Histological examinations

The histological examination of sample A, B, C was carried out by cutting the transverse sections of each part and examined one by one under a microscope. [13]

Fluorescence examination

Fluorescence analysis was performed with a powdered form of the plant. In this analysis, organic compounds absorb radiations of a specific wavelength and many of them also re-emit some radiations. The characters of powdered parts of the plant were studied by using UV radiations at two different wavelengths i. e 254 nm and 336 nm [14].

Microchemical examinations

The color examination was performed for powdered parts of the plant (A, B, C). Behaviors of the powders were examined by treating them with chemical reagents such as water 5% Ferric chloride, 10% Sulphuric acid, 4% Sodium hydro oxide and Chloroform [11].

FT-IR fingerprinting

The fine powder of plant parts A, B and C was analyzed on FT-IR Spectrophotometer (Thermo Spectronic Model, Helios Alpha No UVA 090714, England) and the spectrum was recorded.

RESULTS

Preliminary phytochemical examination

The preliminary phytochemical examination of plants parts A, B and C was showed in table 1. It is revealed that carbohydrates, alkaloids gives positive in all parts of plant whereas steroids and terpenoids were absent in all these three parts.

Proximate examination

The percentages of moisture content, ash value of the samples A, B and C were present in a fig. 1.

Pharmacognostic examination

The color, odor and taste of sample A, B and C are presented in table 2 and their diagnostic features were enlisted in table 3. Whereas, the diagrammatic presentations are shown in fig. 2 and 3.

Table 1: Preliminary phytochemical examination of different parts of *D. Sisso* Roxb

S. No.	Test	A	B	C
1.	Carbohydrate	Positive	Positive	Positive
2.	Alkaloids	Positive	Positive	Positive
3.	Steroids	Negative	Negative	Negative
4.	Tannins	Negative	Positive	Positive
5.	Terpenoids	Negative	Negative	Negative
6.	Flavonoids	Positive	Positive	Negative

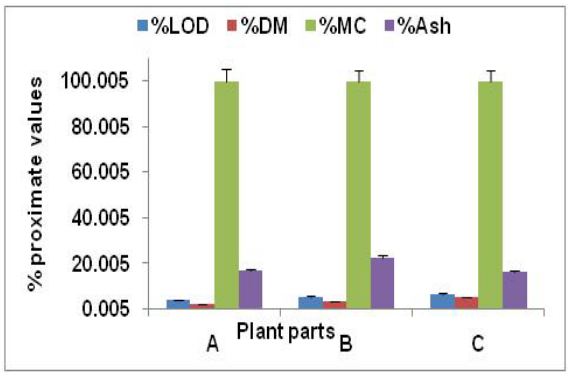

Fig. 1: Proximate Examination of different parts of *D. Sisso* Roxb, Data are expressed as means±standard deviation where n = 3

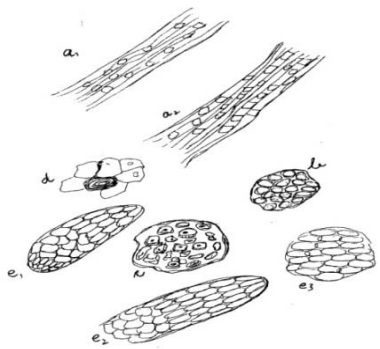

Fig. 2: Powder microscopy of parts B of *Dalbergia sisso* Roxb, (a) Fibers (b) Cells containing starch grains (c) Calcium oxalate prism-like cells (d) Ground tissues (e) Different types of Schlerides

Table 2: Macroscopic features of A, B and C parts of *D. sisso* Roxb

Properties	A	B	C
Color	Lush green	Yellowish Brown	Light Brown to Dark grey
Odor	Slightly waxy	No Characteristics	Dusty Pungent
Fracture	Leathery/Shiny	Complete	Tough and Rough
External Making	Apiculate apex	Shiny, wrinkled, sharp lateral edges, with pointed at one end and slightly blunt at another end. Ventral and dorsal sides having prominent, 3 marks which shows seeds embracing cavities at everything distance with dark brown mark.	Ridges, Shed in narrow strips
Internal Making	Polished surface.	2-4 cavities are present and a seed is present in each cavity.	Slightly Smooth
Size	15 cm long.	4-8 cm long and 1 cm wide	2.5 cm Long
Shape	Suborbicular	Lanceolate	Rectangular
Taste	Slightly sweet and slimy	Insipid	Slimy

Table 3: Diagnostic features of the powdered parts of *D. Sisso* Roxb

S. No.	A		B		C	
1	Lamina	The isobilateral shape of diacytic stomata's are present.	Calcium oxalate	Rare Calcium oxalate crystals are seen in powders of pods	Starch Grains	Free starch grains are present
2	Epidermis	Upper and lower epidermis with rectangular thin epidermal cells, lower epidermis, contain oil contents vigorously	Schlerides	Schlerides found to contain high deposition of lignin along with tannin	Ground Tissues	Some Ground tissues are seen
3	Calcium Oxalate	Crystals or prism-like cells are present more abundantly in the drug.	Fibers	Elongated lignified and no lignified fibers are present in the powders of the pods	Fibers	Compacted fibers are present
4	Fibers	Groups of fibers contain some lignin content are present some of the fibers contain calcium oxalate crystals.	Starch Grains	Free starch grains and compacted grains containing cells are also seen	Hexagonal Cells	Hexagonal epidermal cells
5	Lignified Cells	Elongated thickly lignified fragment are found abundantly	Ground tissue	Rounded ground tissue are occasionally found.	Rectangular Cells	Some rectangular cells seen

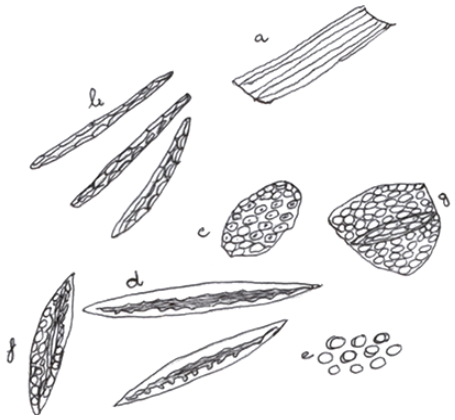

Fig. 3: Powder microscopy of A of *D. sisso* Roxb, (a) Group of fibers (b) Epidermis (c) Calcium oxalate (d) Fibers (e) Lignified cells (f) Starch grains

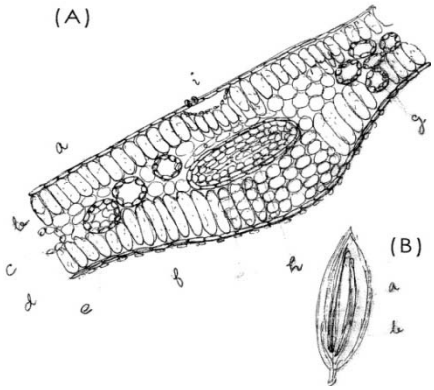

Fig. 4: Transverse section of A and B of *D. Sisso* Roxb, A. (a) Epidermal layer (b) Palisade Parenchyma (c) Collenchyma (d) Palisade (e)Pericycle fiber (f) Oil contents (g)Fibrovascular bundle (h)Pith (i)Stomata, B. (a)Embryo (b) Endospermic cell

Histological examination

The plant histology showed different types of cellular structures which are enlisted in table 4 and their cellular shapes can be identified in the fig. 4 and 5.

Florescence examination

The fluorescence behavior of powdered material of A, B and C was established under a day, with UV minimum and maximum wavelengths of lights and the color of powdered drugs are revealed green to brown as indicated in table 5.

Micro Chemical Examination

Different chemical reagents give particular behavior with a powdered sample of A, B, and C and only 5% Ferric chloride gives no color with all three samples table 6.

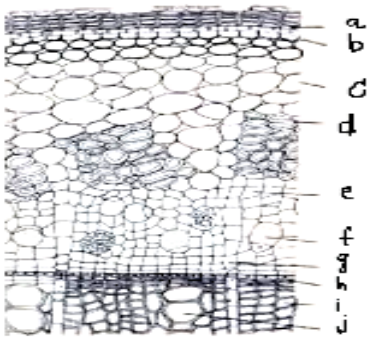

Fig. 5: Transverse section of part C of *D. Sisso* Roxb, (a) Cork (b) Cork cambium (c) Schaleren chyma (d)Parenchyma (e) Sieve tube (f) Ray (g) Vascular Cambium (h) Xylem fibers (i) Vessels

Table 4: Transverse section of A, B and C of *D. Sisso* Roxb

A	B	C
Epidermal Layer	**Embryo**	**Cork**
Upper and lower	A thread like linear Embryo is present more prominently.	Outermost layer of rectangular cells
Stomata Diacytic	**Endospermic cells**	**Cork cambium**
Stomata seen in the wide stomatal chamber composed of small thin cells.	Epidermal cells containing oil globules and portentous matter along with rich lignified fibers present.	Underlying the cork cells Cork cambium is present
Palisade Parenchyma		**Schaleren chyma**
Underlying the epidermis elongated parenchyma columnar cells with elongated chloroplasts.	_____	Bundles of Phloem fibers are seen.
Collenchyma		**Collenchyma**
Central region of midrib composed of moderate size spherical collenchyma cells	_____	Consisting of phloem cells and sieve-tube.
Fibers Vascular Bundles		**Ray**
Rounded Fibro-vascular bundles on the both sides towards laminal region are present	_____	It consists of a layer of square shaped cells.

Table 5: Florescence analysis of A, B and C parts of *D. Sisso* Roxb

Treatment with reagent	Observations								
	A			B			C		
	At ordinary light	At 254 nm	At 336 nm	At ordinary light	At 254 nm	At 336 nm	At ordinary light	At 254 nm	At 336 nm
Powder as such	Green	Dull Green	Dark Green	Brown	Light Brown	Dark brown	Brown	Light Brown	Dark brown
Powder treated with 1 N NaOH in MeOH	Green	Dull Green	Dull green	Light Brown	Off white	Dark brown	Light Brown	Off white	Dark brown
Powder treated with 1 N HCl	Bright Green	Green	Light Green	Brown	Light Brown	Brown	Brown	Light Brown	Brown
Powder treated with 50% H_2SO_4	Dark Green	Dark Green	Blue	Slight turbid	Dull Brown	Dark Brown	Slight turbid	Dull Brown	Dark Brown
Powder treated with 50% HNO_3	Light Green	Dark Green	Dark Green	Light Brown	Light Red	Red	Light Brown	Light Red	Red
Powder mounted in nitrocellulose in amyl acetate	Green	Dark Green	Dull Green	Dull Brown	Brown	Dark Brown	Dull Brown	Brown	Dark Brown

Table 6: Microchemical analysis of A, B and C parts of *D. Sisso* Roxb

Reagents	Color observations		
	A	B	C
Absolute alcohol	Light green	No color change was seen	No color change was seen
Ferric Chloride 5%	No color change	No color change was seen	No color change was seen
Sulphuric acid 10%	Turbidity seen	No color change was seen	Turbidity seen
Sodium Hydroxide 4%	Light green color seen	Light brown color seen	Light brown color seen
Chloroform	Light green color	No color change was seen	No color change was seen

FT-IR spectrum

The IR spectroscopy was developed for the identification of different functional groups present in the samples A, B and C. Spectra are shown in fig. 6 (A, B and C) and their spectral behavior is illustrated in table 7.

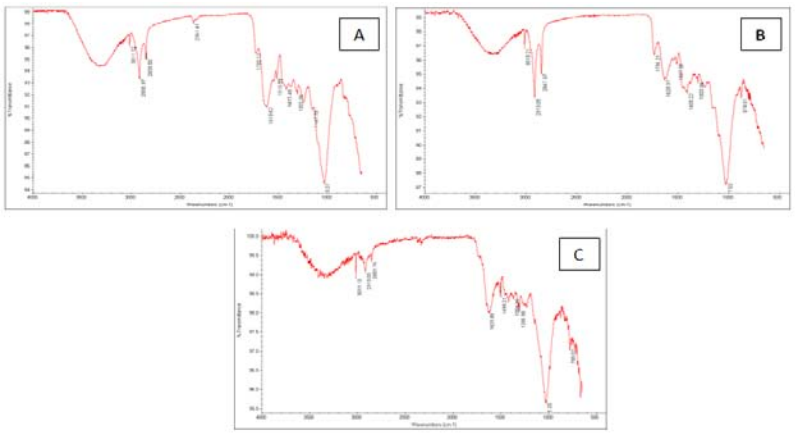

Fig. 6: FT-IR spectrum of A, B and C parts of *D. sisso* Roxb

Table 7: FT-IR spectroscopic analysis if A, B and C of *D. sisso* Roxb

Frequency (cm^{-1})	Bonding	Absorption	Interference	A	B	C
3011.12	C-H	s	Aromatic	+	-	+
3015.21				-	+	-
2908.99	C-H	w-m	Alkane	+	-	-
2913.05				-	+	+
2839.50	C-H	w	Aldehyde	+	-	-
2847.67				-	+	-
2851.76				-	-	+
1732.12	C= O	s	Aldehyde	+	-	-
1736. 21				-	+	-
1613.62	C=C	w-m	Alkene	+	-	-
1625.88				-	-	+
1629.97				-	+	-
1515.55	C=C	w-m	Aromatic	+	-	-
1499.21	C=C	w-m	Aromatic	-	-	+
1417.48	C-H	w-m	$-CH_3-$	+	-	-
1147.79	C-O	s	Alcohol	+	-	-
874.01	CH	s	-	-	+	-
780.02				-	-	+

DISCUSSION

Plants are the significant portion of the world to ensure reproducible quality and enhance the consumer confidence for the utilization of herbs as pharmaceutical and commercial industries for raw materials and as well as an active principal [14]. Use of herbal remedies is growing tremendously throughout the world, and this trend is also obvious in Pakistan. Standardization is the key for safety and efficacy of herbal preparations, and this can be achieved only by pharmacognostic studies. On the basis of these studies identification and authentication of herbal drugs has become more appropriate which is also helpful for making monograph of plants. Phytochemical analysis (table 1) revealed that plant parts contain the different types of compounds which authenticated the use of this plant in various ailments and that can be of the valuable therapeutic index and these phytochemicals have been attributed the protective effect of herbal plants [15]. The Moisture content of sample A, B and C of *D. sisso* were high 99.93%, 99.86% and 99.87% respectively, hence there is more chance of degradation during the storage, in addition, highest moisture content also worked as moisture aid in stabilizing the plant by maintaining the protoplasmic content of the cells and make it perishable [16]. Total ash value in A and B are 16.15% and 16.69% respectively indicating the presence of normal complexes of organic and inorganic components, as maximum accepted limit is 22%, whereas bark showed 22.87% pointing towards abnormal complexes, higher mineral, metallic salts and silicates, etc. [17].

All three parts of the drug i. e leaves (A) pods (B) and bark (C) exhibit distinguished prominent differentiating organoleptic and histological characters including fibers, calcium oxalate crystals, lignified cells, ground tissues and vessels. All these major macroscopic, microscopic and histological features may be a useful tool for the establishment of standards regarding the apparent morphology, cell shapes, size and arrangement of the cellular structure of the plant. It is one of the economical procedures and necessary for the quantitative evaluation of closely allied herbal raw materials [18]. Fluorescence analysis under the UV at a wavelength of 254–365 nm showing the presence of different primary and secondary metabolites and their UV activations. The different colors of the fluorescence rings are due to different atoms present in the compound having different wavelengths. UV spectrophotometric techniques is one of the most preferred methods for routine analytical work of herbal drugs because of its simplicity and reasonable sensitivity [19]. Functional groups were determined by the use of infrared spectroscopy and reported the different aldehyde alkenes and alcohol etc. these findings are important for the determination of reactivity of samples, which plays an important role in the standardization of the drug. FT-IR spectrum is the scenery of chemical constituents with their functional groups in a complex system and is the most credible method to validate and recognize the material in traditional medicine or herbal medicine [20].

CONCLUSION

In conclusion, this standardization can serve as a basis for proper identification, collection and investigation of the *D. sisso* Roxb plant and their utilization as a therapeutic agent. Plant *D. sisso* and its parts A, B and C is first time developed as a standard drug and in addition side to side have important economic advantages that can be utilized as a raw material for the commercial intention, pharmaceutical aid and also in the different formulation for various diseases.

CONFLICTS OF INTERESTS

Declared none

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