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PHARMACOGNOSTICAL INVESTIGATION AND TOTAL PHENOLIC CONTENT OF *DALBERGIA LATIFOLIA* (ROXB.) BARK

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Keywords:

Dalbergia latifolia, Physicochemical parameter, Phenolic and flavonoid content

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ABSTRACT: *Dalbergia latifolia* commonly known as bilayatti shisham (Fabaceae) is a medicinal plant. The dry bark is brown colored astringent characters. Traditionally various species are reported to be used as aphrodisiac, abortifacient, expectorant, anthelmintic, antipyretic, appetizer, allays thirst, vomiting, burning sensation, cure skin diseases, ulcers Ayurvedic practice, bark is used as leucoderma, leprosy edema and bladder disorder. The bark minerals, acids, phenolic compounds, flavonoids, have characteristic smell astringent taste. This study deals with the pharmacognostical evaluation of the dried bark of *Dalbergia latifolia* which includes macro and microscopic studies, determination of physicochemical.

INTRODUCTION: The genus consists of 300 species and about 25 species occur in India. Many species of *Dalbergia* are important timber trees, valued for their decorative and often fragrant wood, rich in aromatic oils [1, 2].

Traditionally various species are reported to be used as aphrodisiac, abortifacient, expectorant, anthelmintic, antipyretic, allays thirst, vomiting, burning sensation, cures skin diseases, ulcers, diseases of the blood, reduces obesity, used in leucoderma, dyspepsia, dysentery, for diseases of the eye and nose, syphilis, stomach troubles, leprosy, leucoderma, scabies and ringworm [3, 4, 5]. The present paper is a compilation of the phytoconstituents that have been identified in this genus and the traditional and reported biological activities.

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Some phytoconstituents namely flavonoids, isoflavonoids, glycosides, steroids, *etc*.; have been isolated from the various species of the genus. *Dalbergia latifolia* (Roxb.) Family- Fabaceae [5], a larg glabrous tree a single stem with characteristic smells [6]. The tree has grey bark that peels in long fibers, compound leaves and bunches of small flowers [7]. The bark is grey, thin with irregular short cracks, exfoliating in fibrous longitudinal flakes [8]. It is distributed in Bihar, Bundelkhand and Central India [9].

It contains dalbinol a new 12a-hydroxyrotenoid [10], sisafolin coumarin from seeds, β-sitosterol, also contain dalbergichromene, lupeol, latifolin, and dalbergin from the bark of the tree, heartwood contains latinone, neoflavonoid dalcriodon [11], [12] and latinone, a substituted phenanthrene-1, 4-quinone was isolated from *Dalbergia latifolia* [13]. Ethnomedicinally, the stem bark contains tannin is used for the treatment of leprosy, obesity, and worm. The genus consists of 300 species, and about 25 species occur in India. Many species of *Dalbergia* are important timber trees, valued for their decorative and often fragrant wood, rich in aromatic oils [14].

Traditionally various species are reported to be used as aphrodisiac, abortifacient, expectorant, anthelmintic, antipyretic, appetizer, allays thirst, vomiting, burning sensation, cures skin diseases, ulcers, diseases of the blood, reduces obesity, used in leucoderma, dyspepsia, dysentery, for diseases of the eye and nose, syphilis, stomach troubles, leprosy, leucoderma, scabies and ringworm [9], [15].

MATERIAL AND METHODS:

Materials: The bark material was collected in October from the Delhi market and identified from the Hamdard University New Delhi. Folin-Ciocalteus's phenol reagent and sodium carbonate were from Merck chemical supplies (Darmstadt, Germany), routine and gallic acid purchase from Qualigens chemical suppliers.

Methods:

Microscopy: Transverse section (TS) of the root was cut by free hand sectioning and stained with different safranin and aniline blue. The various histological parts examined and drawn with the help of camera lucida [16] were performed for the presence of lignin, suberin, tannins, mucilage, starch grains and types of crystal present. Histochemical color reactions of the powdered drug were carried out with ruthenium red, iodine solution, Millon's reagent, and Dragendorff's reagent for the detection of mucilage, starch, protein, and alkaloids respectively [17].

Physico-Chemical and Fluorescence Analysis: Loss on drying, total ash, insoluble acid ash, water-soluble ash, and crude fibers contents was performed as per Indian Pharmacopoeia [18]. The extract of the powdered fruit was prepared with different polar and non-polar solvents for the study of successive extractive values. Fluorescence analysis of the powder drug was carried out with different chemical reagents in the day (254 nm) and UV light (365 nm). The dry powder drug was studied on glass slide whereas the different extracts were studied by adsorbing the extracts on Whatmann filter paper [19].

Quantitative Estimation: For the quantitative estimation, 100 g of the powdered drug was successively extracted in a Soxhlet apparatus with various solvents like petroleum ether, chloroform, ethyl acetate, methanol and water [20]. The extracts were dried on a water bath, weighed and the color of the extracts was also observed. The different extracts were subjected to qualitative estimation for the presence of various phytoconstituents [21].

Determination of Total Phenolic Content: A total phenolic content in the *B. diffusa* extract was determined by the modified Folin-Ciocalteu method [22]. An aliquot of the extracts was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The mixtures were allowed to stand for 30 min at 40 °C for color development. Reagent blank using distilled water was prepared. The total phenolic content was calculated with the help of calibration curve prepared by repeating the operation using 1 ml of gallic acid solutions at concentrations (50,100, 150, 200, 250, 300 µg/ml) in distilled water.

Determination of Total Flavonoid Content: Total flavonoid content of *B. diffusa* was estimated by colorimetric method [23]. The extract was added in a volumetric flask (1 ml containing 10 mg/ml) of each followed by distilling water. The extract was mixed with 5% solution of sodium nitrite. After 5 min 0.3 ml of 10% $AlCl_3$ and after 6 min 2 ml of 1M-NaOH was added. Made up the volume to 10 ml with distilled water and the mixture of the volumetric flask were mixed thoroughly. The Absorbance of the mixture was measured at 510 nm against blank. The total flavonoid content was calculated with the help of the calibration curve and prepared standard rutin solutions at concentrations (50, 100, 200, 300, 400, 500 µg/ml) in distilled water.

RESULTS AND DISCUSSION:

Macroscopic & Microscopic Characters: The surface of the bark is rough, flat; fibrous, reddish brown in color.

FIG. 1: *DALBERGIA LATIFOLIA* BARK

They have longitudinal fissures. It is fibrous in texture, and the cut surface is smooth with a slight odor. The bark has 2 cm thick, 10-15 inch wide and 5-10 cm long **Fig. 1**.

It is differentiated into outer cork cell and inner cortex. The outer bark consists of simple stratified consist of 4-7 layers of rectangular cork cells. The cells are uniform tangentially oblong, and walls are suberized. The phelloderm is broad and prominent consisting of seven to ten layers in radial cells. The phelloderm cells are thin-walled with collapsed phloem zone is the abundance of prismatic calcium oxalate crystals disturbed in the parenchymatous cell **Fig. 2**.

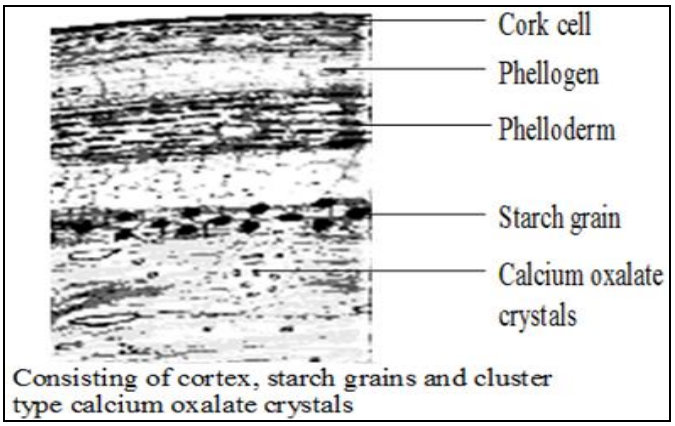
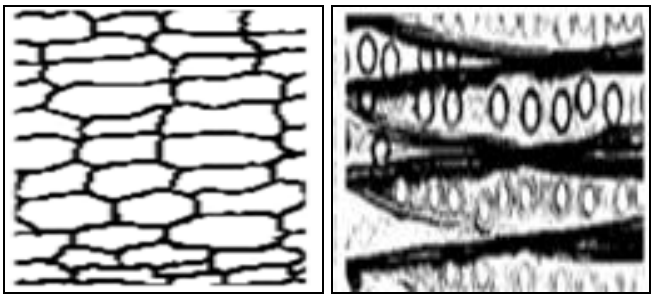


FIG. 2: TRANSVERSE SECTION *DALBERGIA LATIFOLIA* BARK

Analysis of the Powder Bark: The powdered bark reveals fibrous light brown with slight odor and mucilaginous astringent taste. Simple hexagonal types of cork cells are found. Cork cells are stratified, appears like benzene ring in surface view.



Cork cells **Phloem fibres with vessel**

FIG. 3: POWDER MICROSCOPY OF *DALBERGIA LATIFOLIA* BARK

Stone cells are containing with parenchymatous cells in the groups. Phloem fibers with linear fusiform arrangement are visible as highly lignified and pitted, pinkish colored, Calcium oxalate crystals are also present in prisms shaped scattered all over the powder. Prominent modularly rays are also observed in tangential longitudinal view. Black blue color starch grains are also seen inside the cells that are scattered over the slide observed when treated with iodine [24] **Fig. 3**.

Histochemical Color Reactions: The powdered drug was pressing between filter paper mechanically no greasy stains were observed indicating the absence of fatty oil. When the powdered drug was mixed with water in a test tube and shake well frothing was not observed indicating saponins was absent. Powdered bark was pass through 60 mesh and mounted with different chemical reagents ruthenium red solution, Dragendorff reagent, conc. NaOH, anisaldehyde, chloral hydrate, iodine, and phloroglucinol + HCl were used for detection of the color of the powdered drug respectively **Table 1**.

TABLE 1: HISTOCHEMICAL COLOUR REACTION OF POWDER DRUG OF *DALBERGIA LATIFOLIA*

Reagents + Powder Drug	Colour
Phloroglucinol + Conc. HCl	Pink colour lignified cell
Anisaldehyde	Bright yellow color lignified sclerites
Ruthenium red solution	Pink color
Iodine solution	No coloration
Dragendorff reagent	brown color
Conc. NaOH	Golden yellow color flavonoids

Physicochemical and Fluorescence Analyses: Physicochemical analyses of a powdered drug like a loss on drying, ash values, crude fibers and successive extractive values with different solvents of powdered root were analyzed. The percentage of all values in triplicate and their mean values ± SEM were calculated concerning the air-dried drug **Table 2**.

The changes in the color of *D. latifolia* bark powder under UV radiation about daylight were observed with different chemical reagents; it showed different colors reaction of powder indicating the presence or absence of chemical constituents **Table 3**. The fluorescence analyses of powdered drug play a vital role in the determination of quality and purity of the drug.

TABLE 2: QUANTITATIVE STANDARDS FOR THE *DALBERGIA LATIFOLIA* BARK

Parameters	Values of 3 Replicates (%) w/w	Mean ± S.D.
Loss on Drying	8%	
	7.13%	7.90±0.42
	8.57%	
Ash Value		
	13.17%	
Total ash	12.70%	13.83 ±0.91
	15.63%	
	1.72%	
Acid insoluble ash	1.63%	1.71 ± 0.04
	1.77%	
	2.09%	
Water soluble ash	2.17%	2.12 ± 0.03
	2.09%	

TABLE 3: FLUORESCENCE ANALYSIS OF POWDER *DALBERGIA LATIFOLIA* BARK

Treatment	Colour in daylight	Colour in shorter UV (254nm)	Colour in longer UV (365nm)
Dry powder	Light Brown	Dark Brown	Particles give a brown color
Powder +Alcoholic HCl	Matted	Light green	Black
Powder + Aqueous 0.1NHCl	Light yellow	White	Black
Powder+ Aqueous NaOH	White	Whitish	Blackish brown
Powder + Alcoholic NaOH	Light green	Light green	Blackish brown
Powder + 50% H_2So_4	Green	Light green	Black

Extractive Values: The qualitative phytochemical analysis of *D. latifolia* bark extract was found to be 0.75 ± 0.14, 0.18 ± 0.02, 1.13 ± 0.03, 5.58 ± 0.14 and 5.56 ± 0.15 in petroleum, chloroform, ethyl acetate, alcohol, and aqueous extract respectively. All values in triplicate and their mean values ± SEM were calculated concerning the air-dried drug **Table 4**.

TABLE 4: EXTRACTIVE VALUES FOR CRUDE BARK OF *DALBERGIA LATIFOLIA*

Parameters	Colour of consisting	Value of 3 replicates (%) w/w	Mean ± SD
Pet. ether	Light yellow	0.52%	0.75 ±0.14
		1.01.%	
		0.73.%	
Chloroform	Yellow-brown	0.15%	0.18 ± 0.02
		0.21%	
		0.18%	
Ethyl acetate	Dark brown	1.09%	1.13 ±0.03
		1.13%	
		1.18%	
Methanol	Dark brown	5.32%	5.58±0.14
		5.59%	
		5.83%	
Water	Dark brown	5.25%	5.56± 0.15
		5.73%	
		5.69%	

Qualitative Analysis: The presence or absence of different phytoconstituents *viz.* carbohydrate, glycoside, alkaloids, protein, tannins, flavonoids and terpenoids were detected by the phytochemical screening methods with different chemical reagents [17]. Ethanolic and water extracts of the roots powder showed positive results for carbohydrate, glycoside, alkaloids, protein, tannins, saponins, flavonoids and terpenoids. The chloroform and ethyl acetate extract show positive results for terpenoids. Petroleum ether extracts have resinous matter which was not dissolved in other solvents **Table 5**.

Determination of Total Phenolics and Flavonoids Contents: **Table 6** indicated that the total phenolic and flavonoids content in *D. latifolia* bark of ethanolic extract had a higher level of

phenolic compounds. The maximum absorbance of 0.73 was observed at a concentration of 210±1.51 µg/ml of extract. Gallic acid used as a standard which gave a maximum absorbance of 0.92 nm was observed at a concentration of 100 µg/ml and flavonoid observed in this plant 46 ± 3.61 µg/g which is equivalent to 175 µg/ml of rutin standard **Table 6**.

TABLE 5: QUALITATIVE CHEMICAL ANALYSIS OF *DALBERGIA LATIFOLIA* BARK EXTRACT

Test	Pet ether extract	Chloroform extract	Ethyl acetate extract	Alcoholic extract	Aqueous extract
Carbohydrate	-	-	-	+	+
Glycoside	-	-	-	+	+
Alkaloid	-	-	-	-	-
Protein	-	-	-	+	-
Tannin	-	-	-	-	-
Flavonoid	-	-	-	+	+

(+) Present, (-) Absent

The antioxidant activity mainly due to the redox properties [25], which showed an important activity in adsorbing and neutralizing free radicals, entrapments of singlet and triplet oxygen, or oxidizing peroxides. The results of this study suggested that phenolics are important components of these plants.

TABLE 6: POLYPHENOL CONTENT OF *DALBERGIA LATIFOLIA* BARK EXTRACT

Parameters	Ethanolic fraction (µg/mL)
Total Phenolic	210 ± 1.56
Total Flavonoid	46 ± 3.61

CONCLUSION: The present study of the bark powdered indicated the presence of carbohydrate, glycoside, alkaloid, protein, tannin, flavonoid and terpenoid. Pharmacognostical studied of the plant, in this study it was found that the *D. latifolia* bark had minerals, organic acids, flavonoids and phenolic compounds which has to found possesses antioxidant, mast cells stabilizing effects. The constituents of this plant have a tremendous impact on the health care system and may provide medical health benefits including the prevention and or treatment of diseases. Polyphenols traditionally have been considered to possess an anti-nutrient effect.

Recent interest in food phenolics has also increased greatly owing to their antioxidant capacity and possible beneficial implication in human health such as in treatment and prevention of cancer, cardiovascular diseases, and other pathological conditions. The powder drug was tested for the presence of various inorganic elements such as potassium, magnesium, phosphorus, iodine respectively which have good neutraceuticals potentiality and can be used as a food supplement, preventive medicine provide medical health benefits including the prevention or treatment of diseases.

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