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PHARMACOGNOSTIC STUDIES ON *DIOSPYROS MELANOXYLON*

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ABSTRACT

Diospyros melanoxylon is a medium-sized tree or shrub which belongs to the family Ebenaceae. The whole plant is medicinally important and cures several diseases. Therefore, in this context the detailed pharmacognostic study of various parts like leaf, stem, petiole and root has been carried out with the aim to establish its Pharmacognostical standards. The parameters selected were microscopical studies, proximate analysis, fluorescence analysis and preliminary phytochemical screening. The microscopical studies of leaf,stem and root revealed the presence of branched trichomes,spiral xylem vessel. In physicochemical evaluation the ash values and extractive values were studied. Fluorescence analysis performed showed the wide range of fluorescence colors for the crude powder. The *Diospyros melanoxylon* powder was successively extracted with hexane, benzene, chloroform, ethylacetate, ethanol and water for the identification of the best solvent. Preliminary phytochemical screening was carried out for all the extracts and maximum chemical constituents were observed in the ethanolic extract.

INTRODUCTION: Standardisation of a drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical observations. Standardisation and evaluation of herbal drugs mainly done by two methods they are organoleptic and botanical characters. In the organoleptic studies like color, odor, taste and texture where as in botanical microscopic and macroscopic characters areobserved [1, 2]. The generic name is derived from the Greek 'dios' (divine), and 'pyros' (fruit), referring to the excellent fruit of the genus. The specific name is Greek and means 'dark wood'. *Diospyros melanoxylon* is a medium-sized tree or shrub up to 25 m, and 1.9 m girth. The roots form vertical loops in sucker-generated plants. Leaves opposite or alternate and coriaceus, up to 35 cm long, tomentose on both sides when young, becoming glabrous above when fully grown. Male flowers are mauve in colour, tetramerous to sextamerous, 1-1.5 cm long, sessile or nearly sessile in short peduncles, mostly 3-flowered. Female flowers mauve, mostly extra-axillary or sometimes solitary, axillary generally 2, opposite each other, larger than the male flowers. Fruits olive green, ovoid or globose 3-4 cm across; 1-, 2-, 3-, 4-, 5-, 6-, or 8-seeded berries.

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Pulp is yellow, soft and sweet. Seeds compressed, oblong, shiny, often banded. This plant, commonly known as kendu, has been used for treatment of various ailments like diarrhoea and dyspepsia .The bark is also diuretic, carminative, laxative, styptic and used as an astringent lotion for eyes. So far there is no pharmacognostic report so this paper is aimed to report the pharmacognostic and phytochemical studies [3-5].

MATERIALS AND METHODS: The plant was collected from mulugu Warangal. It was identified and authenticated by Prof. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant herbarium was prepared and deposited in the Dept.of Pharmacognosy for further reference. The plant was identified as *Diospyros Melanoxylon* Roxb.(Ebenaceae) under the voucher no: SSCP/2010-2011-001.

Instruments and Chemicals used: Micro senior precision rotary microtome (latest Spencer 820 type), Sisco muffle furnace (3003137), Rotary vacuum evaporator, Hot air oven. The solvents used for extraction were, Benzene, Chloroform, Ethylacetate, Ethanol and Distilled Water. Other reagents used were of laboratory grade and obtained from various other commercial sources. All the reagents used were of laboratory and analytical grade. The reagents used for analysis were Phloroglucinol, Chloralhydrate, Hydrochloric acid, Iodine solution, Glycerine water, 50%HNO_3, 5%NaOH, Methanolic NaOH, 1N KOH, 5%KOH, 5% $FeCl_3$, Methanol, Conc. HCl, Conc. H_2SO_4, Aluminium chloride, Ammonia, Conc. HNO3, Sodium chloride, Solvents obtained from SD Fine-Chem Ltd. (Mumbai), Virat Lab company (Hyderabad), Accord labs (Secunderabad). Rolex laboratory reagent (Mumbai), Nova Biotech (Kolkata).

PHARMACOGNOSTIC STUDIES:

Transverse Section of Leaf, Stem and Root [6-12]: Microtome Section was done for leaf, stem and root to obtain a thin section. The sections were stained with phluroglucinol and hydrochloric acid in the ratio 1:1. Photo micrographs of different magnifications were taken to study the anatomical features.

Powder Microscopy [6,7]: Shade dried Leaf was powdered with the help of an electric grinder till a fine powder was obtained. This fine powder was subjected to powder microscopy studies, as per standard procedures mentioned.

Measurement of Cell Structure and Content [6-8]: The Length and Width of Trichomes, Width of Xylem Vessels, were measured using stage micrometer and the eyepiece micrometer by standard methods.

Determination of Physicochemical Parameters [6, 10, 11]: Total ash, acid insoluble ash, water soluble ash, crude fiber content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and petroleum ether soluble extractive values of leaves of *Diospyros melanoxylon* were determined as per standard procedures

Leaf Constants [6, 12]: The stomatal number, stomatal index, vein islet number and vein termination number were determined as per standard references.

Powder drug with different chemical reagents [13]: Powder leaf of *Diospyros melanoxylo*. Roxb was subjected to analysis with various chemical and organic reagents. Three parameters were taken into account of normal day light.

Extraction: The collected aerial parts of the plant were washed and dried under the shade. Around 25 g of the coarsely powdered aerial parts of the plant was packed in a Soxhlet apparatus and exhaustively extracted with the solvents of increasing polarity. The extract so obtained was concentrated under vacuum using rotary vacuum evaporator and dried in dessicator until use.

Preliminary Phtyochemical Screening [6, 11, 14-18]: The extract so obtained were subjected to various chemical tests as per the procedure mentioned in the standard reference books to determine the nature of chemical constituents present the in the plant .

RESULTS AND DISCUSSIONS:

Macroscopical studies: *Diospyros melanoxylon* is a medium-sized tree or shrub up to 25 m, and 1.9 m girth. Leaves opposite or alternate and coriaceus, up to 35 cm long, tomentose on both sides. Male flowers are mauve in colour, tetramerous to sextamerous, Female flowers mauve, mostly extra-axillary or sometimes

solitary, Fruits olive green, ovoid or globose Seeds compressed, oblong, shiny, often banded **(Figure -1).**

FIG. 1: PHOTOGRAPH OF *DIOSPYROS MELANOXYLON*. ROXB

Microscopical studies:

Transverse section of leaf of *Diospyros melanoxylon*.Roxb(Figure-2)

The transverse section of the leaf showed dorsiventral nature. The leaf was divided into Upper epidermis, Lower epidermis, Mesophyll and Vascular bundles.

- **Upper epidermis**: Upper epidermis was single layer of thin walled closely arranged cells and covered externally by a layer of cuticle. The upper epidermis is a continuous layer
- **Lower epidermis**: Lower epidermis was single layer of parenchymatous cells
- **Mesophyll**: The region between the upper and lower epidermis is constituted by mesophyll. The mesophyll was formed of chlorenchymatous cells and is the seat of photosynthesis. Mesophyll was differentiated in to upper palisade parenchyma and lower spongy parenchyma. Palisade parenchyma consists of two or three layers of compactly arranged cells in perpendicular to the surface. Spongy parenchyma consists of loosely arranged cells having air filled spaces in between. The chloroplast is most abundant in Palisade cells than spongy parenchyma.
- **Vascular bundles**: Vascular bundles are in conjoint and collateral in nature. Xylem towards the epidermis and phloem in between the xylem.

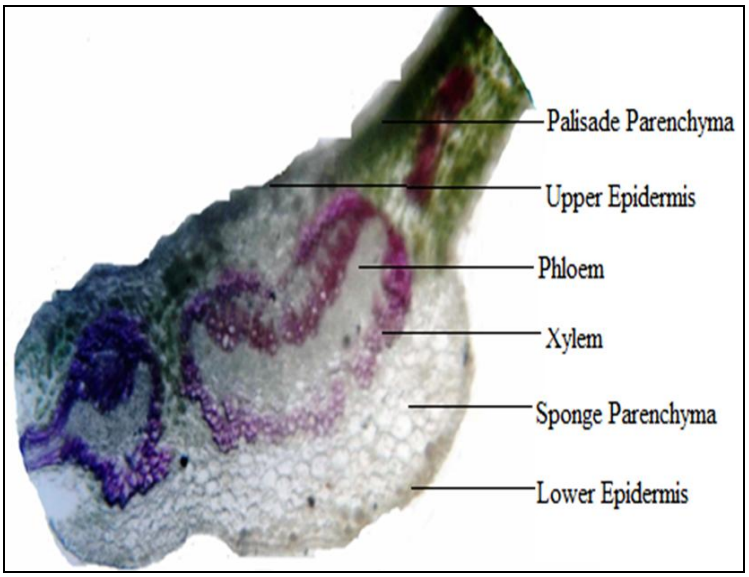

FIG. 2: T.S. OF *DIOSPYROS MELANOXYLON*.ROXB LEAF

Powder Microscopy:

FIG. 3: BRANCHED TRICHOME

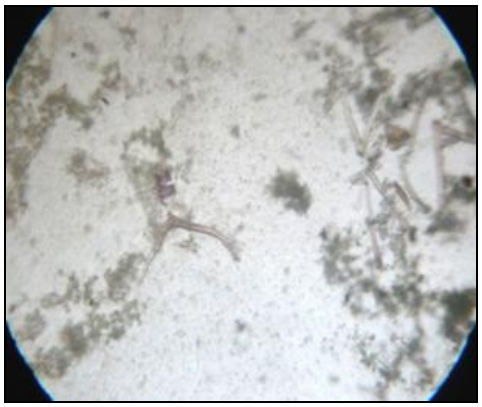

FIG. 4: BRANCHES TRICHOME WITH SPIRAL XYLEM VESSEL

a. Trichomes: trichomes are seen.They are Branched in type (Figure-3)
b. Xylem vessels are lignified,spirally thickened vessels which were seen (Figure-4)

MEASUREMENTS OF TRICHOMES, XYLEM VESSELS OF *DIOSPYROS ELANOXYLON* ROXB. LEAF POWDER: (Table 1): This helps in identification of adulteration.

TABLE 1: MEASUREMENTS OF TRICHOMES, XYLEM VESSELS

PARAMETERS	LENGTH µm	WIDTH µm
Trichome	32.5-215.35-460.5	12.5-32.5-62.5
Xylem vessel	-	25-38.125-75

Determination of leaf constants (Table 2): Leaf constants like stomatal index, vein islet number, vein islet termination and palisade ratio were determined according to the standard procedure. Leaf constants aid to determine the adulteration and substitution of the drug, because these parameters were fixed to the particular plant (**Figure 5, 6**).

TABLE 2: LEAF CONSTANTS OF *DIOSPYROS MELANOYLON*

PARAMETERS	UPPER SURFACE	LOWER SURFACE
Stomatal number	-	15/sq.mm
Stomatal index	-	27.7
Vein Islet number	9	
Vein Termination number	8	

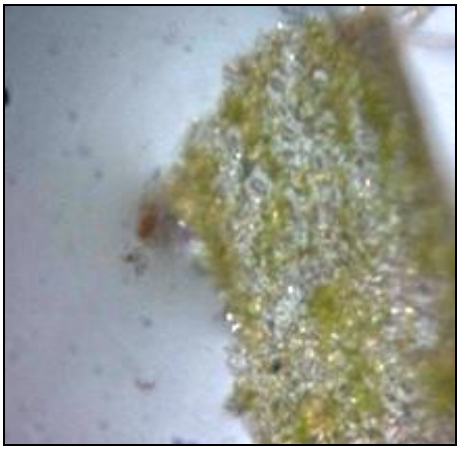

FIG. 5: ANMOCYTIC STOMATA

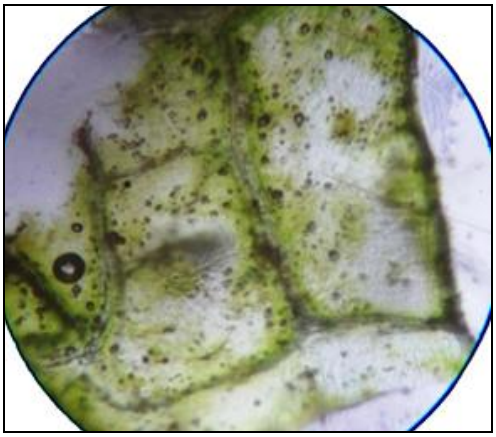

FIG. 6: VEIN ISLET AND VEIN TERMINATION

Transverse section of Stem of *Diospyros melanoxylon* Roxb. (Figure 7):

- **Epidermis**: Epidermis was outer most layers of compactly arranged cells. The cells are elongated and rectangular and covered externally by cuticle.
- **Hypodermis**: The hypodermis was inner to the epidermis and is formed of a few layers of dead, thick walled sclerenchymatous cells.
- **Cortex**: The entire region inner to the hypodermis is called Cortex. It is formed of thin-walled parenchymatous cells and having the large intercellular spaces in between them.
- **Vascular bundles**: They are arranged as collateral and conjoint. Phloem towards the epidermis and xylem towards the centre of ground tissue And Phloem and Xylem are differentiated by the endodermis and the cambium.

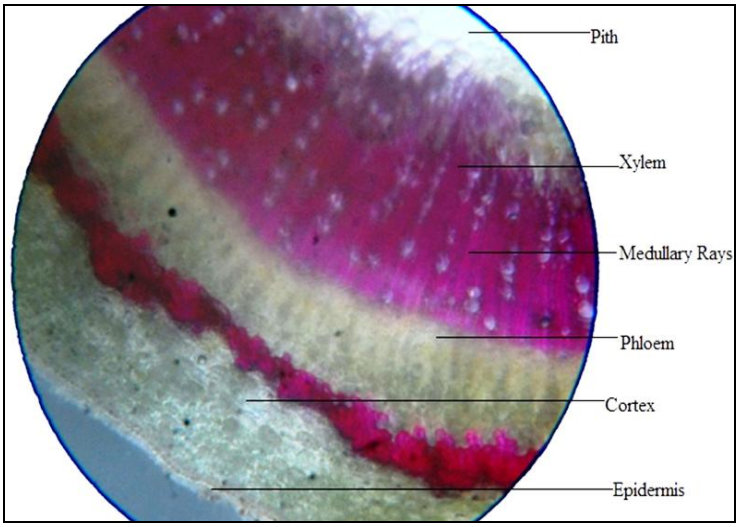

FIG. 7: T.S. OF *DIOSPYROS MELANOXYLON* ROXB. STEM

Transverse section of Root of *Diospyros melanoxylon Roxb* (Figure 8) :

- **Cork**: It was the outer most region of the root and is one cell thick in nature.The cells are thin walled on the surface.
- **Cortex**: The region inner to Cork was formed of several layers of loosely arranged thin walled parenchymatous cells.
- **Pholem**: Below the Cortex pholem is present. Pholem consists of sieve tubes and phloem fibres.
- **Xylem**: It is present in the middle region and xylem consists of Xylem vessels, Xylem parenchyma and xylem fibres. They are radially arranged.and the medullary rays are also present in the xylem.

Xylem vessels are in different radii. Xylem bundles are present alternately on different radii number of each bundle ranges from 2 to 6 and the condition is called tetrarch and pith is usually absent. The diameter of the vessels in the xylem ranges from 113.6-28.4µ.

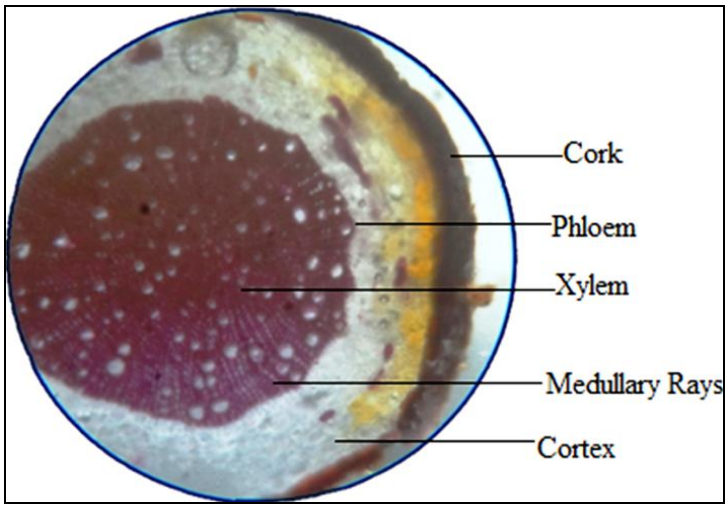

FIG. 8: T.S. OF *DIOSPYROS MELANOXYLON* ROXB. OF ROOT

Determination of Physicochemical Values of *Diospyros Melanoxylon* Roxb. Aerial Parts: The physicochemical properties help to estimate the amount of impurities like soil and particle present in the drug. It also helps to assess the calculi salts present in the drug sample. Ash values were used to detect the presence of any siliceous contamination and presence of any water soluble salts. Alcohol and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. While petroleum ether soluble extractive value indicates percent of lipid content present in the crude drug. Crude fibre content is useful technique for differentiation of similar drugs **(Table 3).**

TABLE 3: PROXIMATE ANALYSIS VALUES OF *DIOSPYROS MELANOXYLON* ROXB. AERIAL PARTS

PARAMETERS	VALUES IN % W/W
Total ash	7.2
Acid insoluble ash	18
Water soluble ash	46.5
Sulphated ash	14.6
Water soluble Extractive	10.56
Ethanol soluble Extractive	5.36
Chloroform soluble Extractive	3.2
Moisture content	1.46
Crude Fibre Content	6.05

TABLE 4: POWDER ANALYSIS BY DIFFERENT CHEMICAL REAGENTS

Reagents	Colour observed
Powder as such	Olive Green
Powder with NH_3	Yellowish Green
Powder with Conc HCl	Greenish Yellow
Powder with Conc H_2SO_4	Greenish Brown
Powder with Conc HNO_3	Pinkish Yellow
Powder with Glacial acetic acid	Yellowish Green
Powder with Picric acid solution	Light Green
Powder with 5% NaOH	Brownish Yellow
Powder with 5% KOH	Yellowish Brown
Powder with 5% $Fecl_3$	Yellowish Orange

Extraction: Extraction was carried out by the Soxhlation based on the increasing order of polarity as follows **(Table 5).**

Preliminary phytochemical screening of *Diospyros melanoxylon* Roxb: Chemical test helps in the confirmation of the chemical nature of the active principles present in the plant extract **(Table 6).**

Nature and %yield of different extracts:

TABLE 5: NATURE AND %YIELD OF DIFFERENT EXTRACTS

EXTRACTS	COLOUR	CONSISTENCY	%W/W
Benzene	Dark Green	Amophorous	5.71
Chloroform	Bottle Green	Fine Powder	1.04
Ethylacetate	Yellowish Brown	Powder Form	1.86
Ethanol	Reddish Brown	Gummy	9.14
Water	Brown	Amophorous	1.87

TABLE 6:

TEST	BENZENE	CHLOROFOM	ETHYLACETAE	ETHANOL	WATER
Carbohydrates	+	+	+	+	-
Proteins	-	-	-	-	-
Aminoacids	-	-	-	-	-
Fats and Oils	-	-	-	+	-
Steroids	+	-	-	-	-
Glycosides	+	+	+	+	-
Anthraquinone	-	-	-	-	-
Flavonoids	-	+	+	+	+
Alkaloids	+	+	-	-	-
Saponins	-	-	-	-	+
Tannins and Phenols Compounds	+	+	+	+	+

The results from the above table showed that the Leaves extracts of *Diospyros melanoxylon*.Roxb contains Carbohydrates, Fats and oils, Steriods, Glycosides, Tannins, Flavonoids and Saponins

CONCLUSION: *Diospyros melanoxylon* is widely cultivated in India for its culinary purposes. The microscopical studies, physico chemical parameters, and chemical tests performed will guide in the proper identification of the plant species from other species of *Diospyros* as well as help in authentication of the purity of the plant. By above all these parameters we can build up a suitable plant profile.

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