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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *OUGEINIA OOJEINENSIS* (ROXB) HOCHR. BARK

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ABSTRACT

Ougeinia oojeinensis (Roxb) Hochr, an ethanomedicinal plant of Western Ghats seen in deciduous forests. The bark is used to treat various ailments in folk medicine. There are no previous reports on pharmacognostical study on this plant. As a promising ethanomedicinal plant, systematic and detailed pharmacognostical studies were carried out. The present study focused on its macro and micro morphological characters of the bark. An attempt was made on to evaluate the physico-chemical parameters like ash values, extractive values, loss on drying and fluorescence analysis of the bark powder are performed. The chemical composition of the ethanolic extract of *Ougeinia oojeinensis* was determined by GC-MS analysis. The present investigation revealed the immense value of standardization and botanical identification of the plant material for further investigations and forms an important aspect of drug studies.

INTRODUCTION: *Ougeinia oojeinensis* (Roxb) Hochr. (Fabaceae) is a medium deciduous pretty tree (**Fig. 1**) seen in the Himalayan region extending through the whole of northern, central India and is distributed in Western Ghats [1]. It is upto 12 meter in height with short crooked trunk, leaves pinnately trifoliate, leaflets large, rigidity coriaceous, terminal broadly elliptic or roundish, some times trapezoidal, prominent. Flowers white or pink in short fascilate racemes. Pod linear, elongate and flat. Seeds compressed reniform, light brown in color. The bark was deeply cracked, outer surface greenish white, shallow fissured, fissures longitudinal narrow and long. Inner surface is vertically grooved, pale yellow, small circular spots of reddish resin secretion along the surface [2-3].

FIG. 1: ***OUGEINIA OOJEINENSIS***

Texture-bark fibers are with reddish twigle in the outer surface, bark having an acrid with a sharp cooling taste, and astringent, specific odor. The barks are intended for jaundice, anti-inflammatory, antihelmintic, febrifuge, leprosy and anemia [4]. However, the pharmacognostical and physico-chemical parameters of the plant have not been published. Therefore the present investigation was under taken to standardize the bark. As a promising ethanomedicinal drug the morphological, anatomical, physico-chemical constants, preliminary phyto-chemical and GC-MS analysis of the bark were carried out and the results are presented.

MATERIALS AND METHODS:

Plant material: Plant materials collected from forests of Munnar, Kerala, India was processed as herbarium specimens and voucher specimens were deposited in the crude drug museum in Department of Pharmacognosy, K.P. College of Pharmacy, Thiruvanamalai. The plant material was identified following local floras and authenticated by plant anatomy research centre (PARC), Chennai (PARC/2010/581).

Chemicals and instruments: GC-MS, Compound microscope, Camera lucida (mirror type), Stage and eye piece micrometer and other basic equipments and glasswares are used for the present study. Photographs of different magnifications of plant tissues and cells, starch grains, calcium oxalate crystals were taken with Nikon Lab phot2 and polarized light microscope. Solvents like hexane, alcohol, aqua and reagents formalin, acetic acid, 70% alcohol, Toluidine blue, Phloroglucinol and Carrier gas helium are used.

Pharmacognostical studies:

Morphological studies: Morphological studies were done using a compound binocular microscope. The shape, size, color, taste and odor of roots were determined.

Microscopical studies: Microscopic studies were done by preparing free hand sections of root. The sections of the root was cleared with chloral hydrate solution and then stained with phloroglucinol and hydrochloric acid and mounted in glycerin. Separate sections were prepared and stained with iodine solution for identification of starch grains. Powders (#60) of the roots were used for observation of powder microscopical

characters. The powdered drug was separately treated with Phloroglucinol, Hydrochloric acid solution, glycerin and iodine solution to determine the presence of lignified cells, calcium oxalate crystals and starch grains.[5]

Fluorescence studies: Fluorescence study is an essential parameter for first line standardization of crude drug. The powder material was treated separately with different reagents and exposed to visible and ultraviolet light (short & long) to study their fluorescence behavior [6-7].

Phytochemical studies: The powdered bark of *Ougeinia oojeinensis* (Roxb) Hochr. extracted by cold maceration method using alcohol and distilled water [8-9].

GC-MS analysis: GC analysis was carried out at Clarus 500, Perkin Elmer and computer mass spectral library NIST version. The capillary column was ELITE-1 (100% methyl polysiloxane).The extracts were diluted in alcohol and the injection volume of samples was 1ml. They were injected in the split mode with 10:1 ratio. Electron Ionization (E_1) mass spectra were measured at 70 eV over the mass range (m/z) 45-450. The chromatographic conditions are: helium was used as a carrier gas, Injector temperature 250^0. The column oven temperature was maintained at 110^0 for 2min, then increased to 280^0 at a rate of 5^omin and maintained for 9min, MS total time 36 min. The constituents were identified after comparison with those available in computer library attached with the GC-MS instrument.[10]

RESULTS: The bark powder of *Ougeinia oojeinensis* (Roxb) Hochr [1] has been investigated in a systematic way covering pharmacognostical, preliminary phyto-chemical and GC-MS analysis were carried out and the results are presented.

Pharmacognostical studies: Macroscopical studies of bark showed that outer surface grayish white, shallow fissured fissures longitudinal, narrow and long. Vertically grooved, pale yellow, small circular spots of reddish resin seen along the inner surface (**fig. 2**)

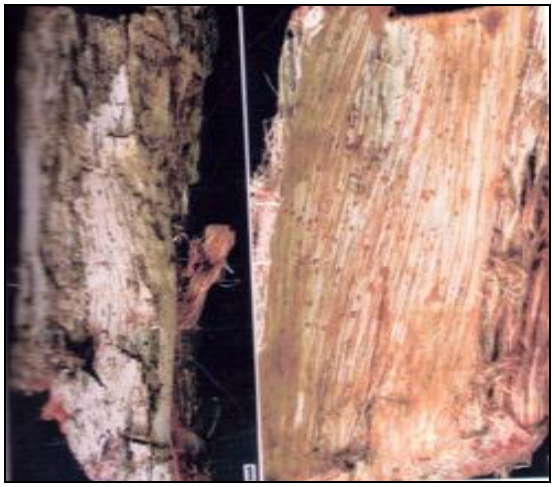


FIG. 2: EXOMORPHIC FEATURES OF BARK

Microscopical studies showed the bark consists of dark zones rhytidome and inner wider zones of secondary phloem. In collapsed phloem some of the phloem rays are narrow and parallel to each other, sclerenchyama, calcim oxalate crystals and tanninferous idioblasts and ray dilation were not seen. In TLS view ”ripple marks” and in RLS view “ flat ribbous” were seen. Crystal oxalates were abundant in the bark (**fig. 3-6**).

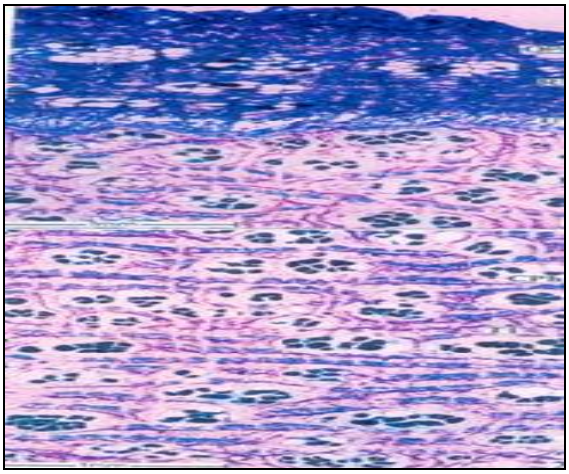


FIG. 3: T. S. OF BARK

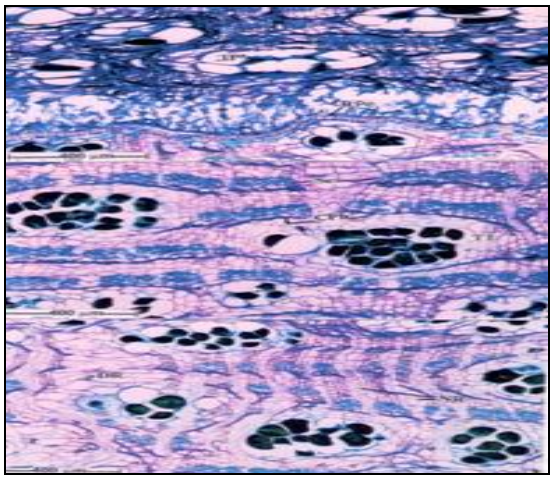


FIG. 4: STRUCTURE OF PERIDERM

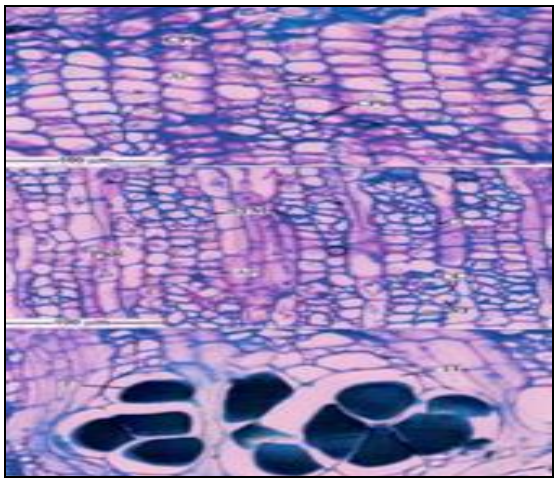


FIG. 5: COLLAPSED AND NON COLLAPSED

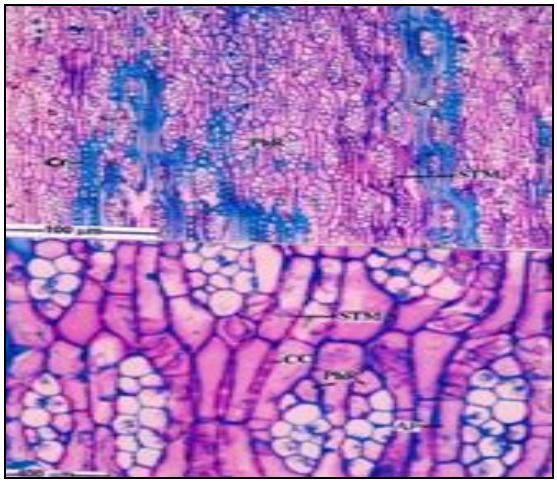


FIG. 6: TLS VIEW OF SECONDARY\COLLAPSED PHLOEM

In the bark powder analysis, (**fig. 7-9**), the phloem sclerenchyma and axial parenchyma bearing crystals were observed. The fibers are thin walled; pits are not evident in the fibers. There are also long parenchyma cells in the powder. Some of the parenchyma cells have crystals forming that are known as crystal strand. The crystals are exclusively prismatic type. They range from rectangular, cuboidal and pyramidal to irregular shape (**Fig. 9**). They are 20-35µm long

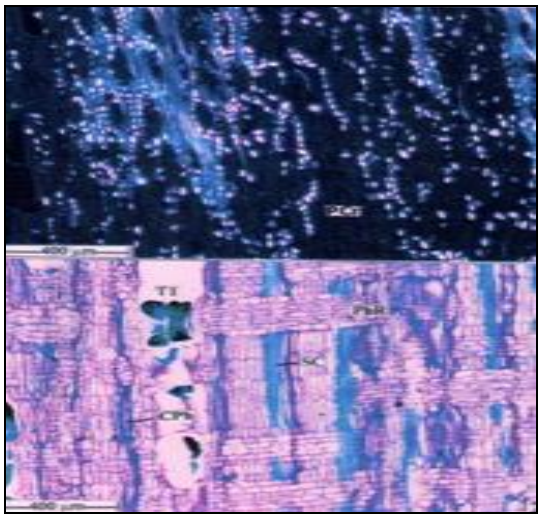


FIG. 7: CRYSTAL IN THE PHLOEM

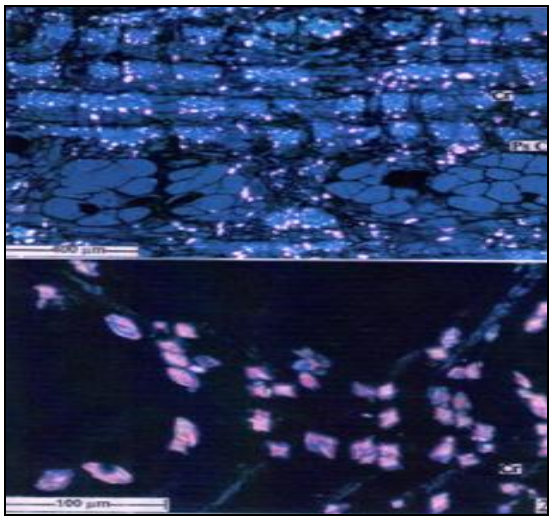


FIG. 8: CRYSTAL DISTRIBUTION IN BARK

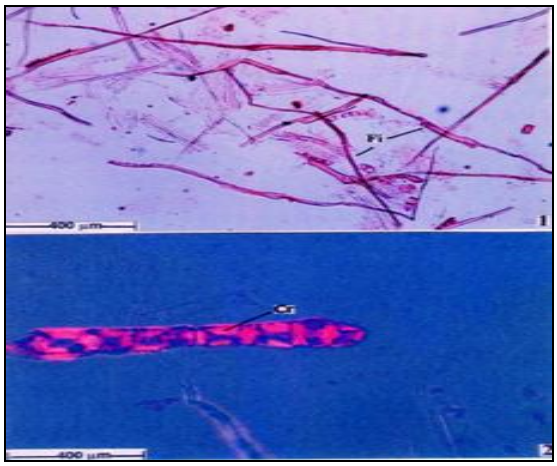


FIG. 9: POWDER MICROSCOPY OF BARK

Further analytical parameters like Fluorescence studies (**Table 1**), Ash value (**Table 2**), Extractive values and total extractive values (**Table 3 & 4**), loss on drying (**table 5**) and phytochemical analysis (**Table 6**) were carried out. The above studies enable the identification of the plant material for further investigations and forms an important aspect of drug studies.

TABLE 1: DATA FOR FLOURSCENCE ANALYSIS OF *OUGEINIA OOJEINENSIS* (ROXB.) HOCHR BARK

Chemical Treatment	Day Light	UV Light
Powder As Such	Brown red	Light brick red
Powder + 1N HCL	Reddish orange	Greenish orange
Powder + aqueous 1NNaoH	Greenish Black	Black
Powder + alcoholic 1NNaoH	Black	Greenish black
Powder + 50% HNO_3	Light orange	Greenish orange
Powder + 50% H_2SO_4	Light brown	Light green
Powder + Methanol	Pale brown	Greenish yellow
Powder + water	Brick red	Brown

TABLE 2: DATA FOR ASH VALUES OF *OUGEINIA OOJEINENSIS* (ROXB.) HOCHR BARK

Analytical Parameter	%w/w of bark
Total ash	16
Acid in soluble ash	1.2
Water soluble ash	2.3
Sulphate ash	4.2

TABLE 3: DATA FOR EXTRACTIVE VALUES FOR *OUGEINIA OOJEINENSIS* BARK

Analytical Parameters	%w/w in bark
Alcohol soluble extractives	5.80
Water soluble extractives	4.72
Ether soluble extractives	1.46

TABLE 4: DATA FOR EXTRACTIVE VALUES FOR *OUGEINIA OOJEINENSIS* (ROXB.) HOCHR BARK BY COLD MACERATION METHOD

Extracts	Yield (gms)	% yield (w/w)
Ethanolic extract	56.25	5.62
Aqueous extract	46.25	4.62

TABLE 5: DATA FOR LOSS ON DRYING FOR *OUGEINIA OOJEINENSIS* (ROXB.) HOCHR BARK

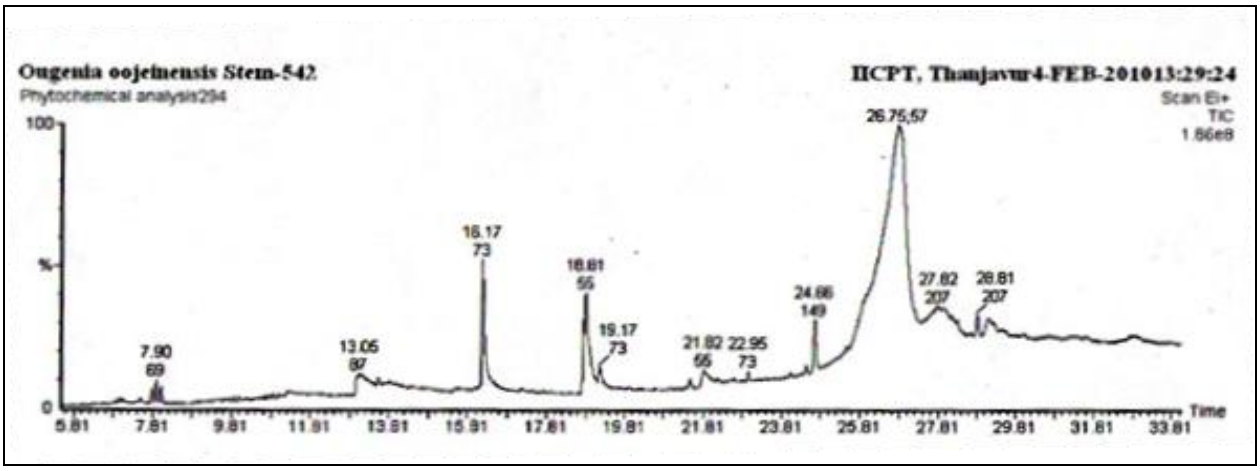
Loss on Drying	5.33% w/w

TABLE 6: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS OF POWDERED BARK OF *OUGEINIA OOJEINENSIS*

Phytoconstituents	Ethanolic extract	Aqueous extract
Alkaloids	+	-
Saponins	+	+
Glycosides	+	+
Carbohydrates	+	+
Tannins and Phenolic compounds	+	+
Flavonoids	+	+
Steroids	-	-
Proteins and Amino acids	-	-
Triterpenoids	-	-
Fixed oils and Fats	-	-
Gums and Mucilage	+	+

(+): Present, (-): Absent

GC-MS analysis: GC-MS analysis of the ethanolic extracts showed the presence of 8 constituents the major constituents of this extract are alcoholic compounds 1-octanol, 2-butyl, sugar moiety 3-o-methyl-d-glucose, palmitic acid, linoleic acid, oleic acid, 1, 2 benzene dicarboxilic acid, fatty acid ester, triterpine squalene are present in this extracts (**spectra**) and the data given (**Table 7**).



SPECTRA

TABLE 7: GC-MS STUDY-ACTIVITY OF PHYTOCOMPONENTS IDENTIFIED IN THE BARK EXTRACT OF *OUGEINIA OOJEINENSIS* (ROXB.) HOCHR

RT	Name of the compound	Molecular formula	M.W.	Peak Area %	Compound Nature	**Activity
7.90	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	0.42	Alcoholic compound	Antimicrobial
13.05	3-0-Methyl-d-glucose	$C_7H_{14}O_6$	194	4.54	Sugar moiety	Preservative
16.17	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.88	Palmitic acid	Antioxidant, Hypocholesterolemic, Nematicide
18.81	9, 12-Octadecadienoic acid (z, z) -	$C_{18}H_{32}O_2$	280	5.85	Linoleic acid	Anti-inflammatory, cancer preventive, hepatoprotective, Antihistaminic, Antiarthritic
19.17	Oleic acid	$C_{18}H_{34}O_2$	282	1.64	Monounsaturated fatty acid	Anti-inflammatory, cancer preventive, Dermatigenic, Hypocholesterolemic, Anemiagenic
24.66	1, 2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.89	Plasticizer compound	Antimicrobial, Anti-inflammatory,
26.75	Dodecanoic acid, 1, 2, 3-propanetriyl ester	$C_{29}H_{74}O_6$	638	77.12	Fatty acid ester	Antioxidant, Antibacterial, COX-1 & COX-2 inhibitor, Antiviral Hypocholesterolemic
28.81	Squalene	$C_{30}H_{50}$	410	0.67	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant

CONCLUSION: This is the first report of pharmacognostical evaluation of *Ougeinia oojeinensis.* The pharmacognostical studies include macroscopical, microscopical, proximate analysis like ash values, extractive values and fluorescence analysis gives valuable information about the plant. It is helpful for correct identification of this plant for the future reference. Phytochemical and GC-MS analysis conformed the presence of flavonoids and triterpenoids.

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