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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *KIGELIA PINNATA* (JACQ) DC. (BIGNONIACEAE) - ROOT

Dabhi Swati[1]*, Pandya Preeti[2], Parkhi Vishwatej[1], Patel Bhupesh[3]

*[1]PhD Scholar, [2]Laboratory Assistant, [3]Head of Department, Department of Dravyaguna, Institute of Teaching and Research in Ayurveda, INI, Jamnagar, India.

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ABSTRACT

Kigelia pinnata (Jacq) DC. (Family Bignoniaceae) commonly known as "Sausage tree" is reputed African folklore drug and abundantly found throughout India. Its root is used traditionally in cancer, diabetes, gastrointestinal problems, constipation, gynecological complaints etc. The present paper highlights the pharmacognostical and phytochemical studies along with HPTLC fingerprint profile of *K.pinnata* root for its botanical identification and standardization. Pharmacognostical analysis of *K. pinnata* root comprises of organoleptic characters, transverse section of root along with powder microscopy. It was analyzed through relevant physicochemical parameters and qualitative tests for various functional groups. HPTLC was carried out after organizing appropriate solvent system. TS and powder microscopy reveals presence of large amount of fibres, parenchymas and vessels filled with tannin content. Preliminary qualitative analysis proves the presence of flavanoids, tannins, saponins, steroids and phenolic compounds. The preliminary physico-chemical analysis shows water-soluble extract 22.78% w/w, alcohol-soluble extract 21.10% w/w and loss on drying at 105°C was 10.40% w/w with 5.2 pH. HPTLC were carried out in which maximum 4 & 5 peaks at 254nm & 366nm respectively. The anatomical characters and values obtained from analytical study can help in standardization.

INTRODUCTION

Plants have been used for various purposes since prehistoric times. *Kigelia pinnata* DC. (Balam Kheera), Bignoniaceae family, is a tropical African plant also cultivated widely in many parts of India. It is commonly called the "Sausage tree" because of its huge fruits and also known as Balamkheera in Hindi.[1]The tree is evergreen where rainfall occurs throughout the year, but deciduous where there is a long dry season. *K. pinnata* DC.is a medium-sized spreading tree of rapid growth, with short trunk and long distorted branches with greyish brown bark.[2] Leaves imparipinnate; Leaflets 7-9, elliptic-oblong or obovate, entire or serrate, 3-6 in. long; Flowers complete, deep chocolate-red, in long pendulous panicles; Fruit a woody berry, gourd-like, up to 18 in. long*5 in. diam., hanging by a rope-like peduncle up to 7 ft. long; Seeds many, embedded in fibrous pulp.[3] Different plant parts of *K. pinnata* like stem bark, fruit, seed and rootbark have been used for various medicinal purposes all around the world traditionally. Root of the plant is important because it is a ground anchoring part of plant that harbors various bioactive molecules which are distributed in other plant parts also. These have curative possibilities for many human ailments.[4] *K. pinnata* root is used in diseases like cancer, diabetes, ulcer etc.[5] Pharmacological activities of K. pinnata root including anti-malarial root decoction of *K. pinnata* is drunk to treat gastrointestinal problems, constipation, tape worm infection gynecological complaints like ante and post-natal disorders, uterus cancer, fibroid, conception. The root bark is also recommended for the treatment of venereal diseases, hemorrhoid and rheumatism.[6] Pharmacological activities including anti-malarial, anti-oxidant, anti-cancer, anti-protozoal anti-diarrheal also have been reported.[7,8,9]

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As the *K. pinnata* root is credited with many important ethnomedicinal claims, there is need of hour for its standardization. The pharmacognostical and phyto-physico chemical studies of *K. pinnata* root have not been undertaken. Therefore its detailed investigation in fresh as well as in powder form was carried out for botanical identification and standardization of the drug. HPTLC fingerprint profile along with preliminary physicochemical analysis was done. It can be useful standardization index for the correct identification of this well known drug.

MATERIAL AND METHODS

Drug collection, Identification and authentication: The plant was collected at an appropriate stage of its growth from the peripheral area around the Jamnagar. (Fig. 1) Sample specimens were botanically authenticated by expert of pharmacognosy laboratory of ITRA, INI, Jamnagar. Some of them were stored in FAA (Formalin 90: Acetic acid 7: Alcohol 3) solution[10] for microscopic investigation. Herbarium was also prepared and submitted to Pharmacognosy museum of I.T.R.A., Jamnagar, vide Herbarium no. 6370, for future reference. (Fig. 2)

Preparation and Preservation of the Test Drugs

The collected plant samples were shaken to remove adherent soil and dirt. The roots were separated from the stem, washed thoroughly with running water, shade dried and studied macroscopically to reveal their respective characters. For powder microscopy small pieces were powdered into mechanical grinder and obtained powder was passed through the mesh 40# powder was prepared and stored in well closed containers away from the light for further analysis.Free hand sections were taken and observed as such to see their cell contents and then stained with phloroglucinol and hydrochloric acid to observe the lignifications of the cell wall. Preliminary qualitative tests were also performed to detect primary and secondary metabolites.[11] The powder was subjected to determine various physico-chemical constants by the standard procedures mentioned in API.[12]

HPTLC Study

Methonalic extract of root was exposed to HPTLC study.

The solvent system used for the study is toluene: ethyl acetate (9:1)

Chromatographic Conditions

Application mode: Camag Linomat V

Development Chamber: Camag Twin trough Chamber.

Plates: Precoated Silica Gel $_{GF254}$ Plates.

Chamber Saturation: 30 min

Development Time: 30 min

Scanner: Camag Scanner III

Detection: Deuterium lamp, Tungstan Lamp

Data System: Win cats software

RESULTS AND DISCUSSION

Macroscopy: Root of the plant is

- Drug occurs in cut pieces of varying sizes, up to 1 cm thick, slightly recurved and very slightly channeled
- Surface- rough longitudinally grooved, furrowed and cracked exhibiting few lateral root scars
- Colour- camel brown externally and creamish internally
- Fracture- outer short, inner splintery (Fig. 3)

Microscopic Characters

Diagrammatic transverse section of the Root shows outermost cork inner to that narrow cortex and wide zone of central wood encircled with phloem traversed at places with medullary rays.

Detail section of TS of bark shows

- Thick and several layered dark brown coloured cork made up transversely elongated almost rectangular thin walled cork cells at places
- Cortical region wide, made up of oval to roundish thin walled parenchymatous embedded with scatterdly distributed pericyclic fibres either isolated or in groups.
- Phloem encircling the central wide wood region and made up of fibre, sieve tubes, companion cells and parenchymas. Medullary rays mostly uni to multiseriated
- Wood the central wide region made up of fibres, parenchymas and vessels.
- Prismatic crystals of calcium oxalate and simple and compound starch grains found throughout the section. (Fig. 4)

Powder Microscopy

Organoleptic Characters– Powder was rough in touch, light brown in colour with astringent- bitter taste and characteristic odour.

Diagnostic powder characters observe under microscope are Simple and compound starch grains, Prismatic crystals of calcium oxalate, thick walled fibres, simple parenchyma cells with starch grain, pitted vessel, Cork in tangential and surface view. (Fig. 5)

Physico-Chemical Parameters

Various physical-chemical tests were performed as per the standard procedures mentioned in Ayurvedic Pharmacopoeia and their results are as shown in the Table 1.

Table 1: Physicochemical Parameters of *K. pinnata* root

S. No.	Parameters	Results
1.	Loss on drying (%w/w)	10.40
2.	Total Ash value (%w/w)	3.80
3.	Water soluble extract (%w/w)	22.78
4.	Acid insoluble Ash (%w/w)	0.36
5.	Alcohol soluble extract (%w/w)	21.10
6.	Ph	5.5

Preliminary qualitative analysis

Preliminary qualitative analysis for the presence of various functional groups was carried out on the methanol soluble extractive and the result is shown in Table 2. The study revealed that the plant may contain saponins, flavanoids, alkaloid, steroids, tannin and phenols.

Table 2: Results of Preliminary qualitative analysis of *K. pinnata* root

Material	Test/ Reagent	Functional group	Observation	Result
Alcoholic extract of dried stem powder	Dragendorff's reagent	Alkaloids	Orange Brown ppt	+ve
	Wagner's reagent	Alkaloids	Reddish brown ppt	+ve
	5% fecl3	Tannin & Phenolic compound	Deep blue black color	+ve
	Gelatin solution	Tannin & Phenolic compound	White ppt	+ve
	Borntrager's test	Antnraquinone glycosides	No color change	-ve
	Biuret reagent	Protein	No color change	-ve
	Molisch's test	Carbohydrate	Violet ring was observed at the junction	+ve
	Fehling's test	Carbohydrate	First yellow, then brick red ppt observed	+ve
	Salkowoki	Steroids	Greenish yellow fluorescence	+ve
	Liebermann-buchard	Steroids	First red, then blue and finally green color appears	+ve
	Lead Acetate	Flavonoids	Yellow ppt	+ve
	Shaking in test-tube	Saponins	Frothing with honeycomb appearance	-ve

'+ve'= Present, '-ve' = Absent

HPTLC Study

The methanolic extract of *K. pinnata* root shows 4 & 5 peaks at 254nm & 366nm respectively. Among them, 0.05 and 0.45 are common Rf. values in short and long wave lengths. This indicates the presences of some chemical components are more in quantity. The Rf. values are presented in table no. 4 and the photographs of TLC plates along with peak display are shown in Fig 6, Fig. 7.

Table 3: HPTLC profile of Methanolic Extracts of KPR at 254nm & 366nm

S.no	Track code	Wave lengths	No of spot	Rf value
1	Track no.15- *Kigelia pinnata* Root	Short 254 nm	4	**0.05**, 0.09, **0.45**, 0.82
2		Long 366 nm	5	**0.05**, 0.10, **0.45**, 0.72, 0.83

FIGURES

Fig. 1 Natural habitat of Plant

Fig.2 Herbarium sheet

Fig.3 External morphology of fresh root

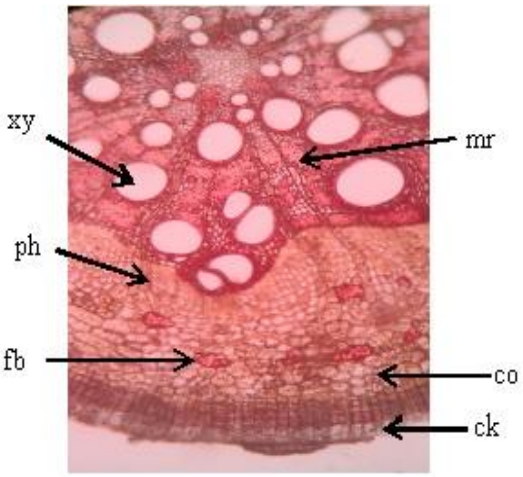
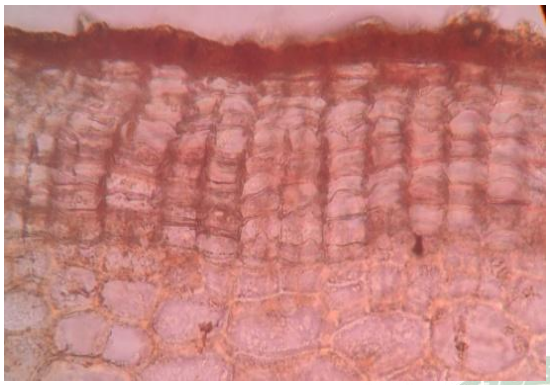
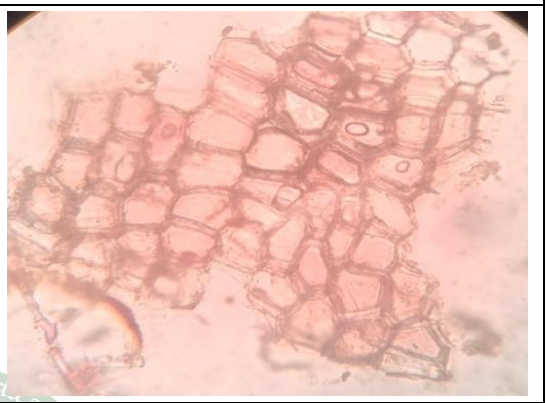


Fig.4 Transverse section of root
(ck=Cork, co=Cortex, mr=Medullary rays, fb = Fibres, ph = Phloem, xy = Xylem)

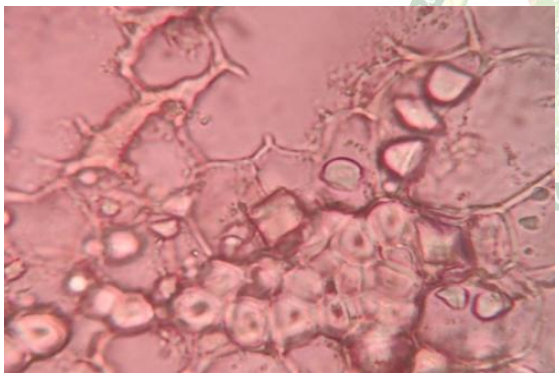
Root powder



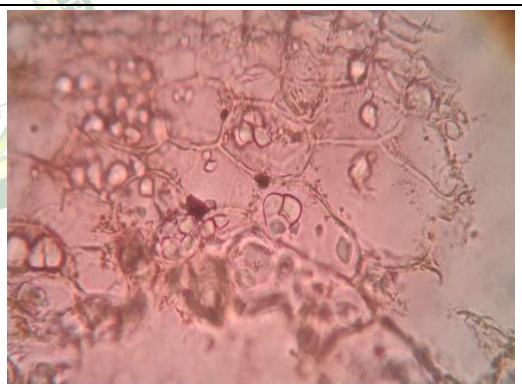
Cork in Tangential view



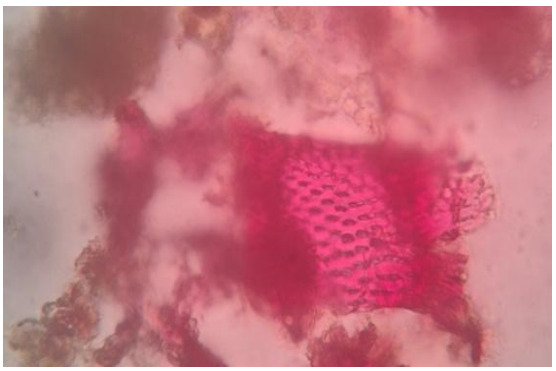
Cork in surface view



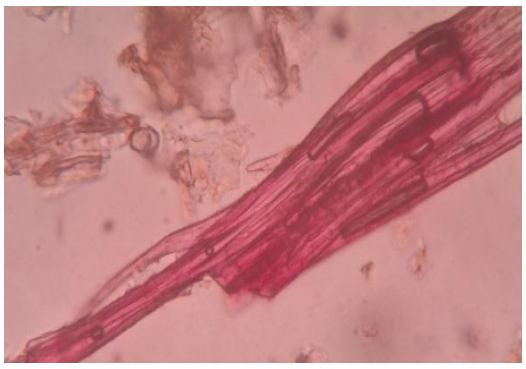
Prismatic crystals



Starch grains



Pitted vessel



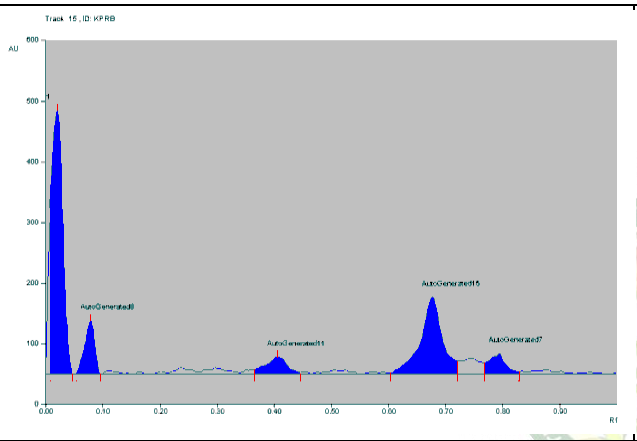
Fibres

Fig.5: Powder characters of Root

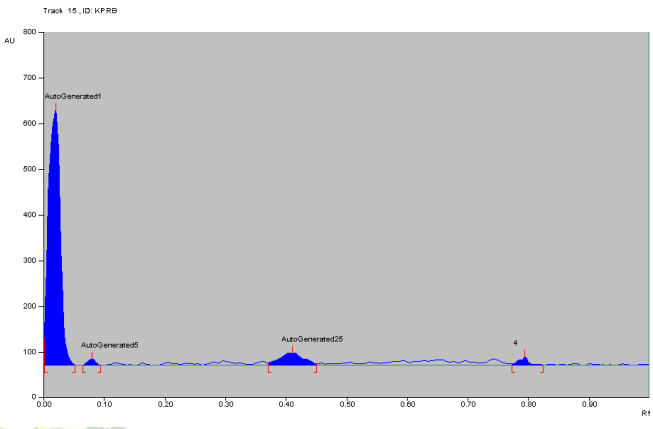
254 nm

366 nm

Fig.6 TLC Plate views



KPR- Peak Display at 254 nm



KPR- Peak Display at 366 nm

Fig 7: Peak display at 254 nm and 366 nm

CONCLUSION

In these present investigations, various pharmacognostical standardization parameters such as macroscopy, microscopy, and preliminary phytochemical screening were carried out which could be helpful in authentication of *Kigelia pinnata* DC. The result of the present study will also serve as reference material in the preparation of herbal monograph.

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Abbreviations Used

KPR: *Kigelia pinnata* Root**; T.S.:** Transverse section; **cm:** centimeter;

g: gram/s; **w/w:** weight by weight; **nm:** nanometer; **HPTLC:** high performance thin layer chromatography; **Rf:** retardation factor **ppt:** Precipitation

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***Address for correspondence**
Dr. Dabhi Swati
Phd Scholar,
Department of Dravyaguna,
Institute of Teaching and Research in Ayurveda, INI, Jamnagar, India.
Email: swatidabhi1110@gmail.com
Cell: 9773174307