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Pharmacognostical studies on leaves of *Kigelia pinnata* Linn

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Abstract
Kigelia pinnata Linn. (Balam Kheera) belongs to the family of Bignoniaceae and commonly called the "sausage" tree because of its hugs fruits. This species can reach 20 meters in height, Sausage like in appearance with long cord -like stalks. It is widely applied in the treatment of genital infections, gynecological disorders, renal ailments, fainting, epilepsy, rheumatism, sickle - cell anemia, psoriasis, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and kidney stones. The current communication provides a detailed account of the pharmacognostical investigation carried out on *Kigelia pinnata* Linn. leaves. The study has immense value in the botanical identification and standardization of the drug in the crude form HPTLC fingerprint profile and preliminary -phytochemical analysis were done along with fluorescence study and physicochemical analysis which may serve as useful indices for the correct identification of the powered drug. This study would be useful evidences for further investigations of the medicinal plant.

Keywords: *Kigelia pinnata*, powder microscopy, physicochemical study, pharmacognosy, phyto-chemical analysis

Introduction
Kigelia pinnata Linn. (Balam Kheera) belongs to the family of Bignoniaceae and commonly called the "Sausage" tree because of its hugs fruits. This species can reach 20 meters in height, Sausage like in appearance with long cord -like stalks. It is also known as Balam Kheera in Hindi (Meena *et al.,* 2009) [1]. This plant is commonly found throughout in western and southern India and a few species in the Himalayas. It is a large evergreen glabrous tree measuring 8-10 min height, stem, trunk straight with branches in all direction. Bark is thick black. Leaves opposite, crowded near the ends of branches, compound, with 3-5 pairs of leaflets plus a terminal leaflets oblong up to 6-10 cm, roughly hairy on both surfaces. Flowers colour in dark maroon with heavy yellow veining in the outside. Cup shape asymmetric, unpleasant, and smelling. Fruits, Sausage shaped up to 1m- 18cm grayish-brown heavily dotted with lenticels, weighing up to 12 kg. Flowering - August to October and fruiting from December to June (Hemamalini *et al,* 2012; Dubey *et al.,* 2004) [2, 3]. This plant has traditional used which include anticancer, antimicrobial, antioxidant, anti- inflammatory and anti malarial properties. It is also widely applied in the treatment of genital infections, gynecological disorders, renal ailments,
fainting, epilepsy, rheumatism, sickle - cell anemia, psoriasis, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and kidney stones (Meena *et al.,* 2010; Milan, 2011, Govindachari *et al.,* 1971; Inouye, 1981; Joshi *et al.,* 1982) [4-8]. The current communication provides a detailed account of the pharmacognostical investigation carried out on *Kigelia pinnata* Linn. leaves. The study has immense value in the botanical identification and standardization of the drug in the crude form HPTLC fingerprint profile and preliminary phyto- chemical analysis were done along with fluorescence study and physic-chemical analysis which may serve as useful indices for the correct identification of the powered drug. This study would be useful evidences for further investigations of the medicinal plant.

Material and methods Collection of specimens
The fresh plant leaves were collected from Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.), India in the month of November. The plant was identified and authenticated. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for powder microscopy, preliminary phyto-chemical screening, florescence study physicochemical analysis and HPTLC fingerprints profile.

Macroscopy
Macroscopic or organoleptic characters like appearance, colour, odour, and taste were evaluated.

Powder microscopy
The dried leaves were subjected to powdered and completely passes through 355 um IS Sieve (old sieve number 44). About 2 gm of powder washed thoroughly with potable water, was poured out without loss of material. Several slides were prepared as follows: mounted a small portion in glycerin, warmed a few mg with chloral hydrate solution, washed and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, about 1 gm of powder warmed over water bath with 50% conc. Nitric acid till brown fumes appear, cooled and washed with water thoroughly and mounted a small portion in glycerin and seen under microscope at 40x
X 10x magnification of the Trinocular Research Microscope (Anonymous 2001, Tripathi & Sikarwar 2014, Kokate 2016) [9-11].

Physico- chemical parameters
Physico-chemical parameters such as moisture content (loss on drying at 105 oC), alcohol soluble extractive extractive value, water soluble extractive value, total ash value and acid insoluble ash value were calculated (Harborne 1973) [12].

Preliminary phyto-chemical screening
Preliminary phytochemical screening were carried out on ethanolic and water extract for the presence /absence of phyto-constituents like alkaloids, Flavonoid, tannins, resins carbohydrates, proteins and saponins (Ansari *et al.* 2013) [13].

Florescence study
Fluorescence characteristic of powder drug with different reagent were observed under day light and U.V. light with different reagents.

HPTLC finger print profile
For HPTLC, 5 gm of the powdered leaves sample was extracted with 100 ml of ethanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica gel aluminium plate 60 F254 (5x10 cm with 0.2 mm layer thickening Merck Germany) using CAMAG Linomat-5 sample applicator and a 100 ulHamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of Toluene: Ethyl acetate (7:3 v/v). Linear ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. At room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, TLC plates was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with Camera photo documentation system CAMAG Reprostar 3. Visualization of spot was made before and after derivatization (with
Dragendorff's regent) at 254nm, 366nm and day light with Win Cat software anf Rf values noted (Anonymous 2007) [14].

Result and Discussion Macroscopic characters
It is a large evergreen glabrous tree measuring 8-10min height Leaves opposite, crowded near the ends of branches, compound, with 3-5 pairs of leaflets plus a terminal leaflets oblong up to 6-10 cm, leathery, roughly hairy on both surfaces leaves colour green, whereas dried leaves powder colour is brownish green, odour characteristics and taste is slightly bitter or astringent.

Powder microscopic characters
The powder colour is brownish green, odour characteristics and taste is slightly bitter or astringent. Under microscope examined powder shows fibres, prismatic crystals of calcium oxalate, upper epidermal cells, sclereids, lower epidermal cells with stomata, parenchymatous cells filled with prismatic crystals and starch grains, pitted parenchymatous cell, group of pitted elongated stone cells and palisade cells (Fig. 1-9).

Plate 1: Powder Microscopy of *Kigelia pinnata* leaves powder

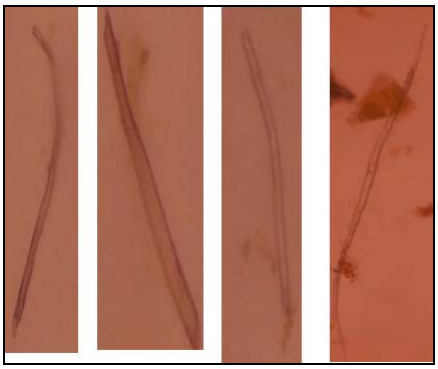

Fig 1: Fibres

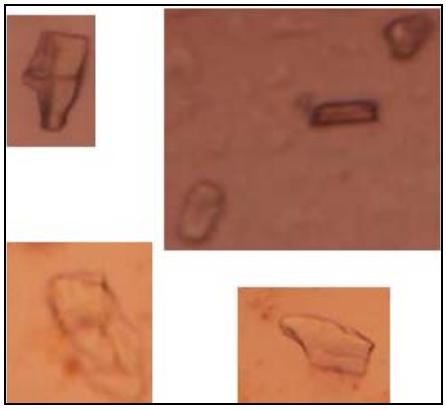

Fig 2: Prismatic crystals of calcium oxalate

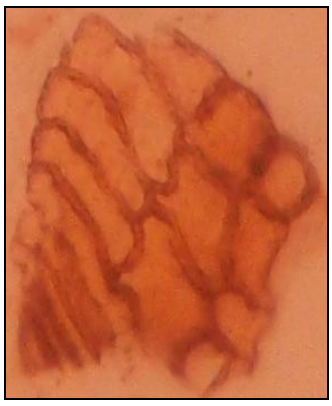

Fig 3: Upper epidermal

Fig 4: Sclereids

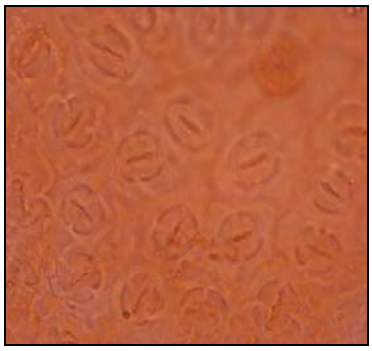

Fig 5: Lower Epidermal cells with stomata

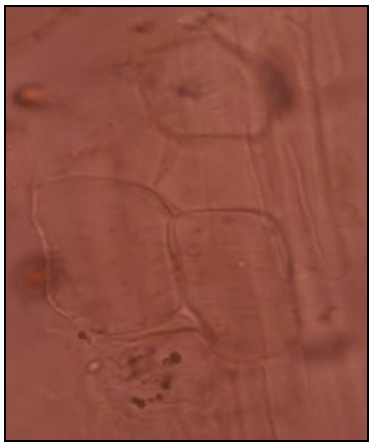

Fig 6: Parenchymatous cells filled with prismatic crystals and starch grains

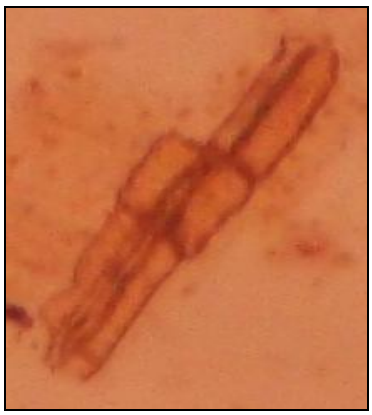

Fig 7: Pitted parenchymatous cell

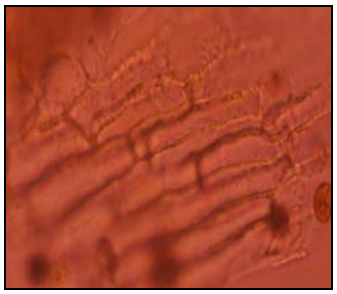

Fig 8: Group of pitted elongated stone cells

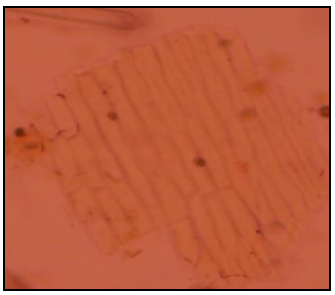

Fig 9: Palisade cells

Physico-chemical analysis

The physic-chemical parameters such as loss on drying at 1050C, water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. Results are given in (Table 1).

Florescence study

The fluorescence analysis is a tool for the determination of constituents present in the plant that gives an idea on its chemical nature. Therefore fluorescence analysis of the powder was carried out and data has been presented in (Table 2)

Preliminary phyto-chemical studies

Qualitative phyto chemicals were screened in the extract of water and alcohol. The results are given in (Table 3).

HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the ethanolic extract two spots of the sample extracts applied in the TLC plate. Major spots Rf values with colour were recorded under 254nm, 366nm, UV light, and after derivatization at 254nm, 366nm and UV light, Chromatogram profile and Rf values are given (Table 4 & Fig. 10-13)

The macroscopic, powder microscopic diagnostic characters has been established to identify *Kigelia pinnata* Linn leaves. The pharmacognostic and physic-chemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phyto-chemical constituents present in the crude drug These findings could be helpful in identification and authentication.

Table 1: Psycho-chemical analysis of *Kigelia pinnata* (leaf)

S. No.	Name of tests	Result
1	Loss on drying (at 105oC)	6.30%
2	Water soluble extractive value	23.28 %
3	Alcoholic soluble extractive value	10.97%
4	Total ash value	10.42%
5	Acid insoluble ash value	0.20%

Table 2: Fluorescence Study of *Kigelia pinnata* (leaf)

S. No.	Drug powder Chemical	Observation at UV light	Observation at 366nm
1.	Powder	Green	Blue wish white
2.	Powder +50% KOH	Black	Green
3.	Powder + 50%H2SO4	Dark green	Dark green
4.	Powder+50% HNO3	Yellowish brown	Black
5.	Powder +acetic acid	Green	Green
6.	Powder+ NaOH (water)	Black	Green
7.	Powder +iodine water	Green	Dark Green
8.	Powder + 1N NaOH (methanol)	Black	Green
9.	Powder + distilled water	Green	Black
10.	Powder + con. H2SO4	Black	Brown
11	Powder + dil. NH3	Dark green	Dark green
12	Powder + con. HNO3	Green	Light green
13	Powder + con. HCl	Dark green	Dark green

Table 3: Phyto-chemical analysis of *Kigelia pinnata* (leaf)

S. No.	Name of phyto- constituents	Result
1	Alkaloids	Present
2	Carbohydrates	Present
3	Protein	Present
4	Saponin	Absent
5	Steroids	Absent
6	Resin	Absent
7	Tannin	Present
8	Flavonoid	Present

Table 4: Rf values of HPTLC finger print profile of *Kigelia pinnata* (leaf)

Rf values	At 254nm	At 366nm	At 366nm (after derivatization)	UV light (after derivatization)
Rf1	0.81(black)	0.35(pink)	0.15(brown)	0.15(light brown)
Rf2	-	0.40(pink)	0.31(brown)	0.35(light brown)
Rf3	-	0.46(pink)	0.35(brown)	0.52(brown)
Rf4	-	0.54(brown)	0.80(brownish red)	0.68(green)
Rf5	-	0.80(red)	-	-

Plate 2: HPTLC finger print profile of *Kigelia pinnata* (leaf)

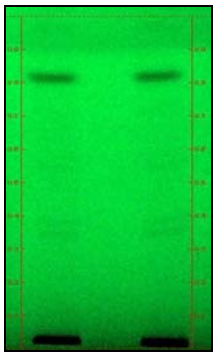

Fig 10: HPTLC Finger print profile at 254nm

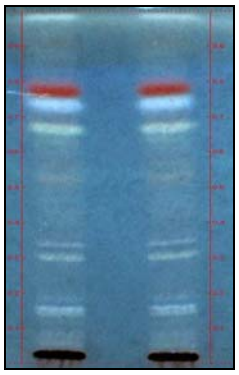

Fig 12: HPTLC Finger print profile at 366nm after derivatization

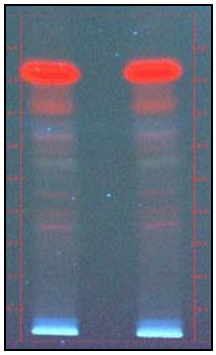

Fig 11: HPTLC Finger print profile at 366nm

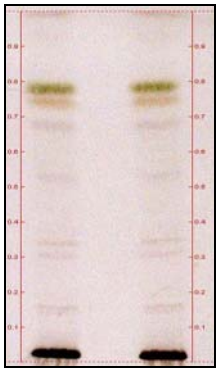

Fig 13: HPTLC Finger print profile at UV light after derivatization

Conclusion

Kigelia pinnata Linn has numerous uses in traditional medicine to treat several ailments plant has traditional used which include anticancer, antimicrobial, antioxidant, anti-inflammatory, and anti-malarial properties. It is also widely applied in the treatment of genital infections, gynecological disorders, renal ailments, fainting, epilepsy, rheumatism, sickle - cell anemia, psoriasis, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and kidney stones. Due to its wide therapeutic importance it is worthwhile to standardize it for use as drug. The present study reveals standardization profile of drug *Kigelia pinnata* Linn, which would be immense value in botanical identification and authentication of plant drug may help us in preventing its adulteration.

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