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# PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *KIGELIA PINNATA* LINN LEAVES

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## Abstract

*Kigelia pinnata* Linn. belongs to the family of Bignoniaceae and commonly called the "Sausage" tree because of its hugs fruits. This species of tree *Kigelia pinnata* can reach 20 meters in height. Sausage like appearance with long cord like stalks. It also known as Balam Kheera in Hindi. 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 m in height, stem, and trunk straight with branches in all direction. Bark is thick black. The leaves are opposite, compound, with 3-5 pairs of leaflets plus a terminal leaflet oblong up to 6-10 cm, roughly hairy on both surfaces. In experimental work pharmacogenetic and phytochemical parameters was find out such as physicochemical, preliminary-phytochemical and quantitative estimation of primary metabolites. The results of this experimental work shows that taxonomic characters, identification and to differentiate closely related species. And also, to find out active phytochemicals for further use.

**Keywords:** *Kigelia pinnata*, Rutin, Powder microscopy, Moisture content, Extractive values, Sausage, Phytochemicals.

## Introduction

Medicinal plants are major a part of traditional medicinal system in developing countries for infectious diseases are known to be treated with herbal remedies. Medicinal plants are potential source of latest compounds having therapeutic value .In parts of medicinal plants having therapeutic property as antimicrobial ,antiviral then on. They need secondary metabolites in plant parts with their therapeutic value Secondary metabolites as flavonoids glycosides, steroids, alkaloids etc., are found in leaves, fruits, roots, flowers and stem of medicinal plants. The alkaloids are one among the foremost divorcees groups of secondary metabolites found in living organisms and have an array of structure type, biosynthetic pathway and

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pharmacological activities. Although alkaloids are traditionally isolated from plants an increasing number are to be found in animals insects and marine invertebrates and microorganisms. Many alkaloids are used for many years in medicine and same are still prominent drugs today, hence this group of compounds has had great prominence in many fields of scientific endeavour and continues to be of great interest today. Plant has a limit less ability to synthesize aromatic substance mainly secondary metabolites of which a minimum of 12,000 are isolated variety estimated to be less than 10% of the total. The specific function many photochemical remains unclear how are a substantial number of studies have shown that they're involved within the interaction of plants /pests/disease. Mature fruit is applied as a dressing on the treatment of wounds, abscess and ulcers. Infusion from the root and bark is taken to treat of pneumonia. Extract of the leaves is used for the backbone, anti- malarial. The bark is used to treat syphilis and gonorrhoea and used to treat ulcers and as purgative. The chemical constituent of plant was iridoids, naphthoquinones, flavonoids [8], and other class of compounds. Isolated iridoids from this plant excreted GLUT4 translocation modulatory effect in skeletal muscle cells [9] in vitro antiamoebic activity [10], and antimicrobial activity [11]. Naphthoquinones from this plant shows antitrypanosomal activity against Trypanosoma brucei blood stream form trypomastigotes [12], also shows in vitro activity against chloroquine-sensitive (T9-96) and chloroquine resistant (K1) Plasmodium falciparum strains [13]. Verminoside, an iridoid glucoside, exhibited potent anti-inflammatory activity by inhibiting iNOS expression as well as the NO release in the LPS-induced J774.A1 macrophage cell line [14]. The plant belonging to Bignoniaceae family contains about 110 genera and 650 species could also be a family of flowering plants, commonly mentioned because of the trumpet vine family, Jacaranda family, Bignonia family, or the Catalpa family. The plant species close to this family are distributed worldwide, but most of them occur in the tropical and sub-tropical countries. The family is little , the Bignoniaceae plants are important for his or her reported bioactive constituents and diverse pharmacological activities. Bignoniaceae family plants also are widely utilized in traditional medicinal systems of variety of nations, including

Bangladesh, where folk and tribal medicinal practitioners use variety of species for treatment of diverse ailments. Since folk medicinal practitioners form the primary tier of primary health care in Bangladesh, the target of this study was to conduct a review of reported bioactive constituents from this family and compare the normal medicinal uses of Bignoniaceae family plants in various countries of the planet including Bangladesh. From observation it is found that traditional medicinal practitioners use whole of seven Bignoniaceae family species for treatment of ailments like, cancer, gastrointestinal disorders, skin disorders, respiratory tract disorders, hepatic disorders gynaecological disorders, cholera, epilepsy, pain, urinary problems, malaria, heart problems, and sexually transmitted diseases. The seven species of Bigononiaceae family plants use were calabash, Tecoma gaudichaudi, Oroxylum indicum, Stereospermum suaveolens, Tabebuia argentea, Heterophragma adenophyllum, and Tecoma stans. Since the available scientific literature validates gaudichaudi, and Tecoma stans. The available scientific literature validates the use of kind of those plants for the ailments they're prescribed for by tribal medicinal practitioners, and the Kavirajas the plants present excellent potential for further scientific studies, which can end in discovery of novel compounds of therapeutic interest.

**Objectives:**

- To Identification of phytochemical composition of Leaves of *Kigelia pinnata*
- To Establishing pharmacognostic and physicochemical parameters of Leaves of *Kigelia pinnata*
- Isolation of major chemical compounds of Leaves of Kigelia pinnata

**Plan of work:**

- Collection of plant material
- Pharmacognostic evaluation of Plant material
- Histology, Leaf constants, powder microscopy
- Physicochemical evaluation: Moisture content, Extractive values (water, ether and alcohol soluble), Ash values (Total, acid insoluble, water soluble).

- Preliminary phytochemical screening of plant material: qualitative chemical tests
- Quantitative estimation of primary metabolites

## Need And Scope

The natural medicinal drugs are of advantage having easily available, economic and less or no side effects, but disadvantage of this natural drug are victims for adulteration. The common problem in these medicinal plants having one common vernacular name entirely different species this problem is solved by this pharmacognostic study. Medicinal plants having therapeutic potential depends on quality and quantity of chemically active constituents. Chemical constituents are the active medicinal ingredients which having medicinal therapeutic activity.

So, this present study is necessary for ascertain the taxonomic characters of plant, medicinally active ingredients for further use.

## Experimental Work

**Fig 1:** *Kigilia Pinnata* tree

**Plant Profile:**

Botanical Name: *Kigelia pinnata*

Family: Bignoniaceae

Common name:

- English: Sausage tree
- Hindi : Balam khira , Jhar fanoos
- Kannada:Aanethoradu Kaayi, Mara Sowthae
- Telugu: Enuga thondamu, Kijili

**Collection Of Plant Material**:

The fresh plant leaves were collected from Shri Shivaji Science College, Amravati, 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, physicochemical evaluation, preliminary phytochemical screening, quantitative estimation, and isolation of major bioactive compounds

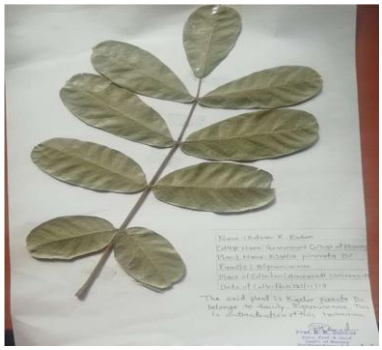

**Fig 2:** Authenticated and Identified Plant

## Pharmacognostic Evaluation of Plant Material:

**Microscopy:**

For microscopical studies fresh leaves was used. Hand section of leaves were taken, double stained with phloroglycinol and mounted in 50% glycerine. Observations were done under compound microscope (10x, 45x).

**Leaf Constants:**

**1) Determination of stomatal number:**

i. Leaf was boiled with chloral hydrate solution to clear piece of the leaf. Upper and lower epidermis was peel out separately by means of forceps. Mounted in glycerine water and kept it on slide.
ii. Camera lucida and drawing boards was used for making drawing scale.
iii. 1mm of square was drawn by means of stage micrometer.
iv. Slide were placed with clear leaf on the stage and the epidermal cells and stomata traced on the paper.
v. The number of stomata was count in the area of 1 sq.mm with including cell if at least half of its area lies within the square.

**Powder Microscopy:**

The dried leaves were subjected to powdered and completely passes through 44 no. Sieve. About 2 gm. of powder washed thoroughly with potable water, was poured out without loss of material. Several slides were prepared as follows: treat a few mg with iodine solution and mount in glycerine. And washed with water thoroughly and mounted a small portion in glycerine and seen under microscope at 40x 10x.

## Physicochemical Evaluation

**1) Moisture Content:** 10 gm. of powdered drug placed in tarred evaporating dish and dried at $105^0$ C for 5 hours and weighed. Drying and weighing was taking continue until difference
between two successive weighing corresponds to not more than 0.25 per cent. Constant weight was reached when two consecutive weighing after drying for 30 min and cooled for 30 min in a desiccator.

**2) Water Soluble Extractive Value:**

5 gm. of coarsely powdered drug was macerated in 100 ml chloroform water for 24 hours in a closed flask. Shaking frequently for 6 hours and stands for 18 hours. Filtered rapidly and evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dried at $105^0$C to constant weight.

**3) Alcohol Soluble Extractive Value:**

5 gm. of coarsely powdered drug was macerated in 100 ml alcohol for 24 hours in a closed flask. Shaking frequently for 6 hours and stands for 18 hours. Filtered rapidly and evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dried at 105°C to constant weight.

**4) Ether Soluble Extractive Value:**

Air dried powderd drug was extracted with solvent ether (or petroleum ether B.P. $40^0$ C to $60^0$ C) in soxhlet extractor for 6 hours. Extract was filtered and evaporated the solvent in water bath. And residue was dried at $105^0$C to constant weight.

**5) Ash Values:**

About 3g of accurately weighed powdered drug was taken into porcelain crucible previously ignited and weighed. The drug was evenly scattered in fine layer on bottom of the crucible. Then the crucible was heated slowly till the powder was free from carbon, cooled and weighed. The percent of total carbon free ash was calculated with reference to air dried powdered drug.

**6) Acid insoluble ash:**

The carbon free ash was boiled in crucible for 5 min, with 25ml of diluted hydrochloric acid. Filtered through ash less filter paper and insoluble matter collected on filter paper was washed with hot water, and then

the filter paper was dried in crucible previously weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried powdered drug.

**7) Water Soluble Ash Value:**

This is determined similar as acid insoluble ash, using 25 ml of water, in place of dilute hydrochloric acid.

## Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on ethanol and water extract for the presence /absence of phyto-constituents like alkaloids, Flavonoid, tannins, resins carbohydrates, proteins and saponins.

**Test for carbohydrates**:

Molisch test: To 2-3 ml of aqueous extract few drops of alpha-naphthol solution in alcohol, was added and shake and conc.$H_2SO_4$ was added from sides of test tube.

**Test for proteins**:

Biuret test: To 3 ml of test solution 4% NaOH and few drops of 1% $CuSO_4$ solution was added.

**Test for alkaloids**:

Aqueous, alcoholic and chloroform extracts was evaporate separately. And dilute HCL was added in residue with shaking and filter out. With filtrate following test were carried out.

**a.** Mayer's reagent – 1.3g mercuric chloride and 5ml of KI were dissolved separately in 60ml and 10ml of distilled water, respectively. Both the solutions were mixed and diluted to 100ml

**b**. Dragendorff's reagent – 8g bismuth nitrate was dissolved in 20ml concentrated nitric acid and 27.2g of KI in 50ml of distilled water. Both the solutions were allowed to stand till KIO3 crystallizes out. Supernatant was decanted and final volume was adjusted to 100ml.

**c**. Wagner's reagent – 1.27g iodine and 2g of KI were dissolved in distilled water and diluted to 100ml.

**d.** Hager's reagent - saturated aqueous solution of picric acid was diluted with an equal volume of water

**Test for flavonoids:**

**Shinoda Test:** To 1 ml of water extract 5 ml of 95% ethanol and few drops of HCL and 0.5 g of magnesium turnings was added.

**Test for tannins:** To 2-3 ml of aqueous extract few drops of following reagents was added:

a. 5% $FeCl_3$

b. Lead acetate solution

**Test for steroid:**

Salkowoski reaction: To 2 ml of extract 2 ml of chloroform and 2 ml of conc.$H_2SO4$ was added and shaken.

## Quantitative Estimation of Primary and Secondary Metabolites

**Quantitative Estimation of flavonoids**:

Aluminium chloride complex forming assay was used to determine the total flavonoid content of the extracts. Rutin was used as standard and flavonoid content was determined as rutin equivalent. A calibration curve for rutin was drawn for this purpose. From the standard rutin solution the dilutions of (0.1, 0.5, 1.0, 2.5 and 5mg/ml) concentrations were prepared in methanol. 0.1ml of each of the rutin dilution was mixed with 0.5ml of distilled water and then with 0.1ml of 5% Sodium nitrate and allowed to stand for 6 minutes. Then 0.15 ml of 10% Aluminium chloride solution was added and allowed to stand for 5 minutes after which 0.2 ml solution of 1M Sodium hydroxide was added sequentially. The absorbance of this reaction mixture was recorded at 510 nm on UV spectrophotometer. The same procedure was repeated for water extract of drug and total flavonoid content was calculated as rutin equivalents (mgQE/g). All the procedures were performed in triplicate.

## Results and Discussions

**Microscopic Evaluation:**

**The T.S. of leaves of *Kigelia pinnata***

The thin section of leaves of *kigelia pinnata* shows upper and lower epidermis. Below the upper epidermis palisade parenchyama was present. Two to three rows of collenchyma is present below upper and lower epidermis. The endodermis was present below the palisade parenchyma. Vascular bundle present below the endodermis.

**Fig 3: T.S. of *Kigelia pinnata* leaves**

**Leaf constants:**

The number of stomata found in per sq.mm in upper surface and lower surface of leaf was found to be 59 and 140.

**Powder Microscopy**:

The powder microscopic characters show the prismatic crystals of calcium oxalate, and fibers

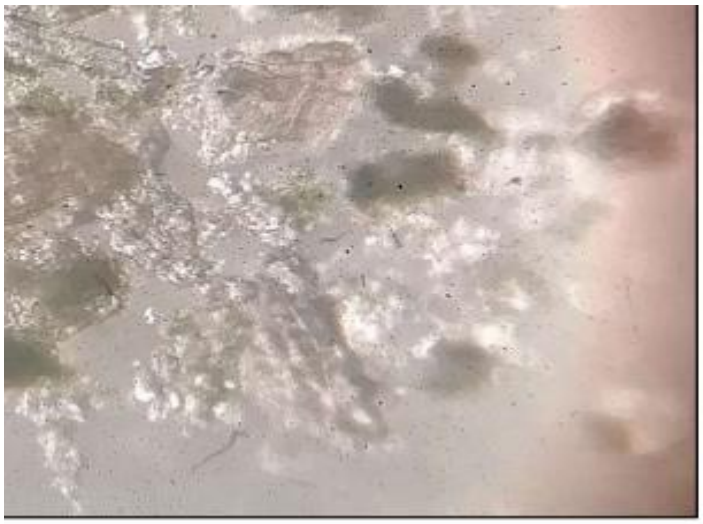

**Fig 4:** Prismatic crystals of calcium oxalate

**Physicochemical Evaluation:**

The physicochemical evaluation parameters such as moisture content at 105$_0$C, water soluble value, alcohol soluble extractive value, ether soluble extractive value, total cash value and acid insoluble ash value were performed and results are given in table 1.

**Table 1:** Physicochemical evaluation parameters of *Kigelia pinnata* leaves

| Sr.No | Parameters | Results |
|---|---|---|
| 1. | Moisture content at $105^0$C | 4.22% |
| 2. | Water soluble extractive value | 13.6% |
| 3. | Alcohol soluble extractive value | 5.6% |
| 4. | Ether soluble extractive value | 2.4% |
| 5. | Total ash value | 10.3% |
| 6. | Acid insoluble ash value | 0.30% |

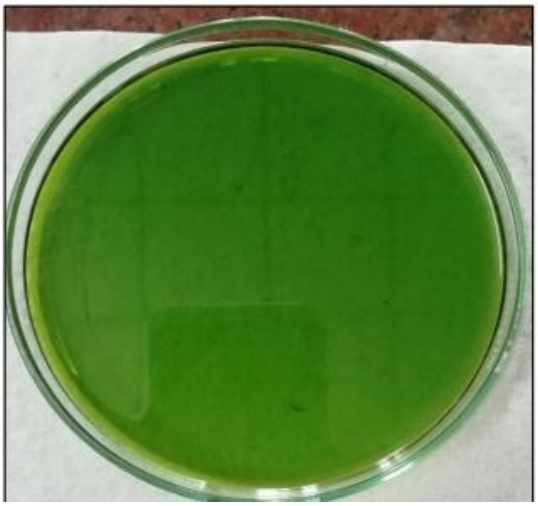

**Fig 5:** Alcohol soluble extract of leaves

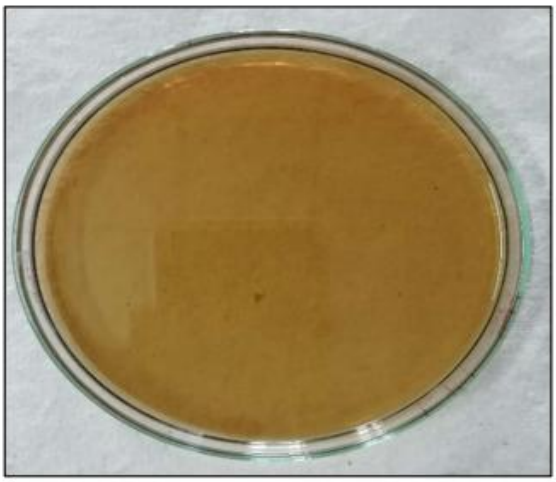

**Fig 6:** Water soluble extract of leaves

**Preliminary Phytochemical Evaluation:**

Qualitative phytochemicals were screened in the extracts of water and alcohol where alkaloids, carbohydrates, flavonoids, tannins, proteins are found present and steroids are absent. Results given in table 2.

**Table 2:** Phytochemical Evaluation of *kigelia pinnata* leaves

| Sr.No. | Name of phytoconstituents | Test | Observation | Results |
|---|---|---|---|---|
| 1. | Alkaloids | Dragondroff's Test<br>Mayer's Test<br>Wagner's Test | Orange brown ppt<br>Gives ppt<br>Reddish brown ppt | +ve |
| 2. | Carbohydrate | Molisch Test | Violet ring formed | +ve |
| 3. | Flavonoids | Shinoda Test | Orange colour appears | +ve |
| 4. | Tannins | $FeCl_3$ solution | Deep blue black colour | +ve |
| 5. | Proteins | Biuret Test | Pink colour appears | +ve |
| 6. | Steroids | Salkowski Test | No change | -ve |

## Quantitative Estimation of Primary and Secondary Metabolites:

### Quantitative estimation of Flavonoids:

For the estimation of flavonoids firstly calibration curve of rutin was calculated where rutin is standard in different concentration (mg/ml) and absorbance was measured at 510 nm. According to this calibration curve of flavonoid content in leave was determined by using following equation y =0.1765x + 0.2081, $R_2$ =0.9533, where X is the rutin equivalent and Y is the absorbance

**Table 4:** Flavonoid Content of *kigelia pinnata* leaves

| Sr.No. | Sample | Concentration (mg/ml) | Absorbance |
|---|---|---|---|
| 1. | Rutin | 0.2 | 0.235 |
| | | 0.4 | 0.291 |
| | | 0.6 | 0.305 |
| | | 0.8 | 0.364 |
| | | 1.0 | 0.375 |
| 2. | Kigelia pinnata leaves water extract | 1 | 1.545 |

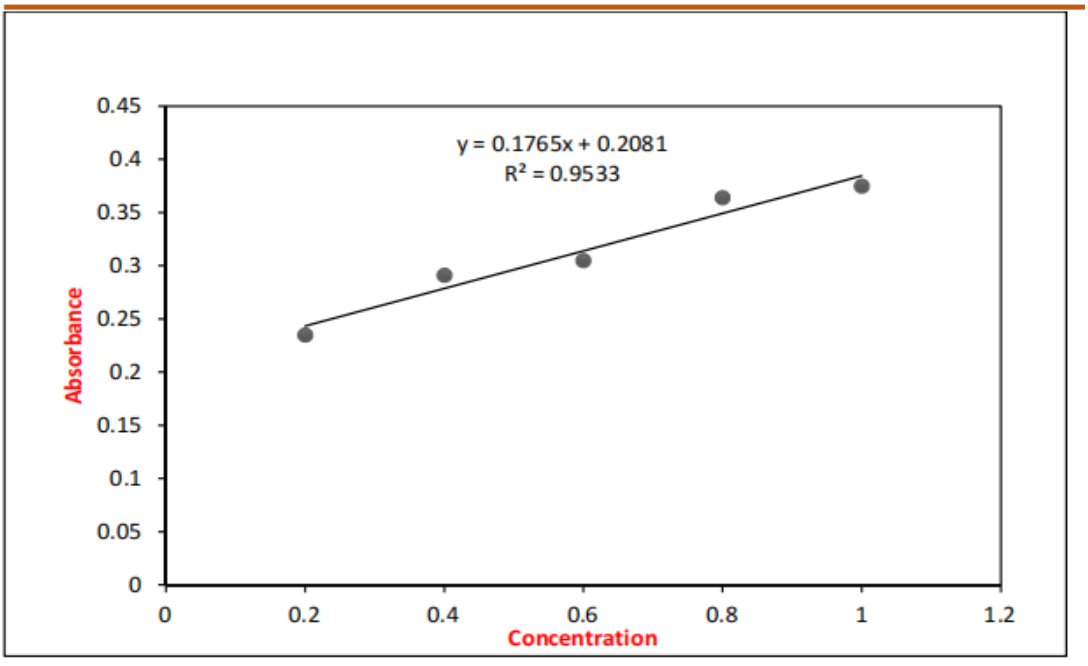


**Fig 7:** Calibration Curve of Rutin

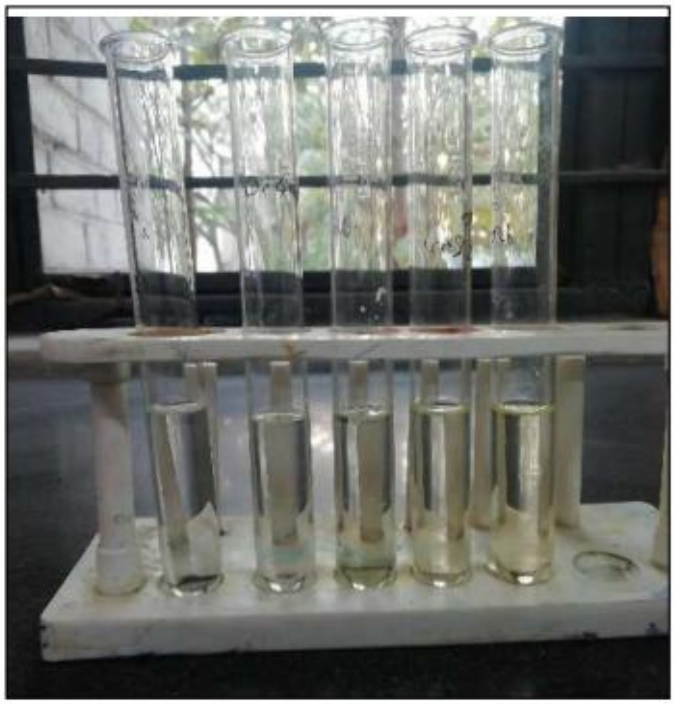

**Fig 8:** Dilutions of standard material (Rutin)

## Summary

The aim of this project work to perform pharmacognostic and phytochemical screening *Kigelia pinnata* leaves to ascertain the identification of phytochemical composition, establishing pharmacognostic and physicochemical parameters of leaves of kigelia pinnata. The plant commonly called sausage tree due to huge fruits. This plant having medicinal value to cure human aliments. In experimental work pharmacognostic and phytochemical parameters was find out such as physicochemical, preliminary-phytochemical and quantitative estimation of primary metabolites. The results of this experimental work shows that taxonomic characters, identification and to differentiate closely related species. And also to find out active phytochemicals for further use.

## Conclusion

The present study reveals the microscopic characters of *Kigelia pinnata* leaves which will be immense value in identification and authentication of the plant. Also reveals the active phytochemicals which would be medicinal therapeutic efficacy.

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