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Pharmacognostic Study of Leaves of *Kigelia pinnata* (Lam) Benth

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ABSTRACT

The *Kigelia pinnata* (Lam) Benth is a species of the Bignoniaceae family commonly known as *Kigelia africana* is locally known as waghul phul. The adult sausage tree has spectabular fruits these can weigh several kilogram hence the tree English common name worsboom. Wherever the trees grows it is usually evergreen especially places where rainfall occurs throughout the year but deciduous in places of long dry season. *Kigelia pinnata* is renowned for its traditional application as anti-inflammatory, anti-diabetic, anti-oxidant, anti –microbial, and anti-cancer effects. It contains chemical constituent like kigenol, kigelinone7, kigenol12, iso-kigenolls. In the present work pharmacognostic studies are carried out to investigate its medicinal properties. The study consists of macroscopic character of the plant *Kigelia pinnata* physico-chemical parameters like total ash, acid insoluble ash, thin layer chromatography etc.

Keywords- *Kigelia pinnata,* pharmacognostic studies, total ash, acid insoluble ash, thin layer chromatography

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INTRODUCTION:-

The tree grows usually evergreen in places where rainfall occurs throughout the year. It is reasonably large trees attaining 20 m in height and grows in moist places such as river banks. The trees bark is grey and flakes. The flowers are pendulous sprays of 5-12 flowers, petals are a deep velvety red with yellow veining on the outside. The cylindrical fruit is pendulous on a long fruit stalk; the fruit can grow up to 1m long and 20cm wide and rounded at the apex. The sausage tree can grow rapidly and mature in 4-5 years mature fruit can weight 12kg. In view to its bulky characteristically shaped fruit has a variety of traditional uses where it grows as an endemic species in different habitats (Haughton P J, Gabriel O A, Olubunmi A) as well as in India and the middle East where the trees have been cultivated (Willam carey, M.Rao N.V.,Kumar B.R,Mohan). *Kigelia pinnata* is also called as Balam kheera. Anti –inflammatory and analgesic activities of methanolic extract of kigelia pinnata (Saini S, Kaur H et al) .Much of the studies on the biological activity of *Kigelia pinnata* have been concentrated on its anti-microbial (Higgains CA, Bell T, Delbederi Z). The research interest is realeted to the traditional uses of fruit extract as remedies for sexually transmitted diseases, dysentery, leprosy, miscellaneous microbial, parasitic infection (M Southi JD, MangomboD 1983). Pharmacological effects including anti-fungal, anti protozoal, hepatoproective, anti-fertility, anti-diarrheal, anti –diabetic (Moideen S.V, Haughton P J, Binutu O A, Okogunj Olaleye MT, Rocha B T). Stem bark methanolic extract in a panel of human tumor cell lines including leucamia, lymphoma derived cells. By means of bioactivity guided fraction nation it has been found that the extract contains cytotoxic agents such as lapachol regarded as a potential anti-cancer drug (Norviburatinal, Jackson). The extract of the leaves of *Kigelia pinnata* has been confirmed to possess anti-diarrhoeal activity (Akah 1996). The extract of root and bark is used for the treatment of cancer of Uterus (Msouthi, 1983). Especially it has a being effective against solar keratosis which may develop into skin cancer (Hutchings et al 1996). Thus seems some evidence that the extracts of the roots and stem bark *Kigelia pinnata* against gentio-urinary infection. The scale of a potential marketing opportunity is large with new ethnic ingredients in cosmetics so-called cosmeceuticals being launched into western market. 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

(Novesp, Imatomi, Parmer, Wagner H, Haughton P.J.). Chemical investigation has showed the aqueous extract of stembark of *Kigelia pinnata* contain irridoids, napthoquinone, isokigenol, kigenol12, kigenol7 etc.

MATERIALS AND METHODS:-

Microscopic Evaluation- For microscopical studies fresh and preserved material of rhizome was used. Hand section of leaves were taken, double stained with saffranin and light green and mounted in 50% glycerine. Observations were done under compound microscope (10x, 45x).

Histochemical Studies - Free hand cut sections of the leaves of *Kigelia pinnata* were taken and tested with respective reagents for the detection and localization of active constituents such as starch, proteins, tannins, fats, sugars, saponins, alkaloids, and glycosides.

Test for starch – 0.3g of Iodine and 1.5 g of potassium iodide were dissolved in 100 c.c. of distilled water. A drop of this solution is added on a section, washed with water and observed under microscope.

Test for proteins – 1g of potassium ferrocynide added to 20 c.c. of water and 10 c.c. glacial acetic acid. Sections were kept in this solution for 1 hour and then washed with 60% alcohol and few drops of aqueous $FeCl_3$ were added. Blue color indicates presence of proteins.

Test for tannins – Sections were treated by acidic $FeCl_3$ (0.5 – 1%) and mounted in clove oil color reactions shows presence of tannins.

Test for saponins – Sections were kept in 1 drop of concentrated sulfuric acid on a slide. It gives a characteristic sequence of color reaction starting from yellow to red within 30 min. finally it becomes violet or blue green in short time.

Test for glucoside (Grignard's test) – Sections were immersed in 1% of aqueous picric acid for 30 min. washed with water and placed in 1 drop of 10% aqueous sodium carbonate on slide. The red color of the section appearing presence of glucoside.

Test for alkaloids –Sections were treated with the following alkaloid reagents.

Mayer's reagent – Potassium mercuric iodide solution – 13.55g of mercuric chloride and 50g of potassium iodide were dissolved in 1 lit. of distilled water.

Wagner's reagent – 1g iodine and potassium iodide 2g were dissolved in 50 mi distilled water.

Dragendorff's reagent – Solution A – 0.8g of basic bismuth nitrite was dissolved in mixture of 10ml of acetic acid&40ml of distilled water. Solution B – dissolve 8g of KI in 20ml of distilled water. The stock solutions A and B were mixed together.

Hager's reagent- Saturated aqueous solution of picric acid was diluted with an equal volume of water. Formation of precipitate or development of turbidity in the section clearly indicated presence of alkaloids.

Qualitative tests for starch - Iodine solution of 2% in aqueous KI is prepared and treated on water extract of the sample.

Qualitative tests for proteins (Million's test) – Dissolve 150g mercuric chloride in 1 lit. of 15% sulfuric acid. This solution is treated on the water extract of sample. White coloration shows presence of proteins.

Qualitative tests for tannins – The water extract of sample is treated with the acidic ferric chloride solution.

Qualitative tests for saponins – Water extract is vigorously shaken (with few drops of neutral water). Permanent leathery foam indicates presence of saponins.

Qualitative tests for Flavonoids – To 1ml ethanol extract, add few drops of HCl and Mg The pink or magnate coloration indicates presence of Flavonoids.

Qualitative test for Glycoside – To 1 ml ethanol extract, add few drops of benzene solution. The brown coloration indicates presence of Glycoside.

Qualitative tests for alkaloids – Precipitation of alkaloids can be obtained with a variety of inorganic and organic reagents.

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. this reagent gives brown precipitate with alkaloids.

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

Ash analysis – About 3g of accurately weighed powdered drug was taken into porcelin 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 .

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 .

Thin layer chromatography:

Procedure:

•Extraction of alkaloids –

•4 gm tissue crushed in mixture of Ethanol: Acetic acid (90:10) stand for 40 min.

•Filter through Whatman filter paper no. 1.

•Addition of filterate solution and ammonium solution kept (1:2) keep over night.

•Centrifuge and take precipitation dissolve in methanol (1 ml).

•Spotted on precoated TLC plate.

•Thin layer chromatographic technique was performed on a precoated silica gel plate. (TLC silica gel 60 F254).

.The solvent system used for the detection of alkaloids was taken in the ratio 100:2. The solvent system used for the following process is Methanol: Ammonium solution.

•Sample loaded on the precoated plate is the chloroform extract both comparative of hot and cold chloroform extract was used.

•The silica plate introduced with the sample was then introduced in the chromatographic chamber. Before introducing the plate in the chamber the chamber is saturated with the solvent system using Whattman paper for 10-15 minutes.

•After saturation of chamber the silica plate with spotted sample was introduced in the chamber and then further the solvent is allowed to run till the complete press carry and the band separation is seen.

•The solvent is allowed to run till the solvent reaches 2 cm before the end of silica plate.

•The plate is further removed from the chamber and kept for drying in hot air oven at 600c.

•As the plate is removed it is marked with the pencil where the solvent front ends.

•Plate is then further removed from the oven and cooled for 5-10 min.

•Spraying or flaming reagent used is prepared .For the above analysis, I2KI reagent was used.

•After cooling the plate is sprayed or flame with I2KI reagent and observed for spot localization.

•After localization of spot the solute front is marked and thus, determination of retardation factor is calculated.

•On calculation of the retardation factor the presence and type of alkaloid is confirmed with the standard Rf available.

RESULTS

Microscopic evaluation:

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

The thin section of leaves shows the upper and lower epidermis .The palisade tissue is present below the upper epidermis stomata are present for transpiration. The endodermis is present below the palisade, below endodermis vascular bundle (xylem and phloem) are present, then spongy tissue are present.

Quantative microscopy:

Table 1:-Histochemical test:-

Test	Reagent	Leaves
Starch	I_2KI	+
Tannins	Acidic $Fecl_3$	+
Fat	Sudan 3	+
Glycoside	Benzene	+
Protein	Potassium	+
Saponins	Conc.H_2SO_4	+
Alkaloids	Mayer's reagent	+
	Wagner's reagent	+
	Dragendroff's reagent	+
	Hager's reagent	+

Designation:-+ indicates presence of compound

- indicates absence of compound.

Phytochemical Test

Test	Reagent	Leaves
Starch	I_2KI	+
Saponins	Conc.H_2SO_4	+
Protein	Potassium ferrocynide	+
Tannins	Acidic $FeCl_3$	+
Alkaloids	Mayer's reagent	_
	Wagner's reagent	+
	Dragendorff's reagent	+
	Hager's reagent	+
	Mayer's reagent	_

Designation; + indicates presents of compounds and

– indicates absences of compounds

Thin layer chromatography:

For the chromatographic technique the calculated

1. Solvent front is found to be = 9

2. Solute front is found to be = 6.5

Thus, the retardation factor is found to be 0.72

Total Ash and Acid insoluble ash of Rhizome of *K.pinnata*

Organ	Total Ash	Acid-insoluble Ash
Leaf powder	58.64%w/w	35.65%w/w

DISCUSSION:

In this context some reliable characters like quantitative microscopic of Physico-chemical parameters is helpful Ash value and acid insoluble value for the leaves of Kigelia pinnata was found to be 58.64%w/w and 35.64%w/w respectively. Extractive value is calculated with respect to dried powder. The determination from above studies established the macro and microscopic parameters, Physico-chemical parameters for the characterization of correct source of Kigelia pinnata. The standard value for Kigenol was found to be 0.7978 which is very close to the value obtained by calculating the Rf value after performing the TLC which indicates that the leaves of Kigelia pinnata.

CONCLUSION:

In the pharmacognostic study of *Kigelia pinnata* the micro and macroscopic characters will be helpful in determining correct taxonomic identification of the plant and also helpful for standardization of drug. The Physico-chemical parameters including the total ash and acid insoluble ash is found to be 58.64%w/w and 35.66%w/w respectively. The standard value for Kigenol was found to be 0.7978 which is very close to the value obtained by calculating the Rf value after performing the TLC which indicates that the leaves of *Kigelia pinnata*. These investigations will be useful for the correct botanical identification of the drug. The present study may be useful to supplement the information with regards to standardization and identification.

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