

PHARMACOGNOSTICAL STUDY, PHYTOCHEMICAL ANALYSIS AND PHENOLIC CONTENT OF KIGELIA AFRICANA LEAVES

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ABSTRACT

Objective: The present communication attempts to evaluate the pharmacognostical study, phytochemical screening & Phenolic content of methanolic extract of leaves of *Kigelia Africana* belongs to the family Bignoniaceae, which is widely distributed in africa and western to southern India.

Methods: In order to ensure the use of genuine and authentic material in the preparation of herbal formulations, pharmacognostical and phytochemical methods of standardization of the plant has been carried out in the present work. Microscopic, physico-chemical characters of the *kigelia africana* leaves was carried out. Preliminary phytochemical investigation of methanolic leaves extract of *kigelia africana* followed by their TLC profiling was carried out. Phenolic content of the methanolic leaves extract of *kigelia africana* were determined by the Folin-Ciocalteus reagent method.

Results: Phytochemical analysis reveals the presence of diverse groups of phytoconstituents in methanolic extract. Chemical constituents also show different Rf values in two different solvent systems. The total phenolic content of methanolic extract (1mg/ml) was found to be 3.52g of Gallic Acid / 100g of methanolic extract of *kigelia africana*.

Conclusion: This study confirms that methanolic leaves extract of *Kigelia africana* contains high amount of phenolic content and it may be responsible for good antioxidant activity. Further studies are necessary to isolate the active compound in the crude extract of *Kigelia africana* responsible for activity.

Keywords: *Kigelia Africana*, TLC, Pharmacognostical, Phenolic content.

INTRODUCTION

The use of medicinal plant is growing worldwide because of the increasing toxicity and allergic manifestations of the synthetic drugs. The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal is increasing in both developing & developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects, easily medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural & tribal lives of India.[1]

Kigelia is a genus with only one species, *Kigelia africana*, belonging to family *Bignoniaceae*, popularly called the **sausage tree**, is a spreading tree bearing long, pendulous racemes of mottled dark flowers which appear like a candelabra [2]. This species can reach 66 feet (20 meters) in height. Its fruits are long and woody, sausage like in appearance with long cord-like stalks. Not uncommon, it is found in several areas of the city and few can miss it because of its uniquely shaped fruit - long cylindrical with a woody epicarp. It is evergreen in areas with abundant rainfall year round, but deciduous, losing its leaves seasonally. *Kigelia africana* is also grown as an ornamental plant, especially in tropical areas of the world, for its interesting flowers and fruits. They have showy flowers, the 5-lobed calyx and corolla, and the didynamous stamens. The corollas are leathery, which resists shredding by the clawed wings of bats that visit and pollinate this species in its native habitat in tropical West Africa. The flowers and the large, sausage-like indehiscent fruits that develop from them hang on long stems below and away from the limbs of the tree. Although not edible, the fruits are used in Africa as an external medication. [3]

Kigelia is a native of W. Africa. The *K.africana* plant has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include irridoids, flavonoids,

naphthoquinones, and volatile constituent's etc. Because of these secondary metabolites present in plants, they may provide a basis for its traditional uses, particularly if they are the same as, or similar in structure to compounds from other species which display relevant activity. To some extent, the type of compounds likely to be present can be deduced from its taxonomic position and this can be seen to be the case with *Kigelia africana* which is a member of the *Bignoniaceae*. [4, 5]

MATERIALS AND METHOD

Plant Material

Sample of the leaves of *Kigelia africana* were collected from the University of Pune. The samples were authenticated from Mr. Chakraborty, Botanical survey of India, Pune, and Maharashtra. Samples were cleaned and air dried, then powdered.

Preparation of Extract

The powdered material was subjected to soxhlet extraction with various solvents ranging from non-polar to polar. The solvents used were Petroleum ether, chloroform, methanol and water. Each time before extraction with next solvents the marc was air-dried. All the extracts were concentrated by distilling the solvent at low temperature. They were then weighed and percentages of different extractive values were calculated with respect to air-dried substance.

Microscopic analysis

Microscopy of leaf of *kigelia africana* was studied. For the microscopical studies transverse sections were prepared and stained. [6]

Physicochemical parameters

The various physicochemical parameters such as Total ash, alcohol and water soluble extractive value, Moisture content, swelling factor, Foaming index were determined by the method reported by Sailor et al. with slight modification. [7]

Preliminary phytochemical Analysis

The preliminary phytochemical screening of the methanolic extract of *Kigelia africana* was carried out in order to ascertain the presence of various constituents viz. steroids, alkaloids, flavonoids, tannins, sugars and glycosides by utilizing standard conventional protocols. [8, 9, 10]

TLC analysis

Thin layer chromatographic technique was used to separate the chemical compounds present in the drug. Various solvent systems were checked to separate the maximum number of chemical compounds in the drug. After performing TLC of the methanolic extract, Rf values were calculated for the spots which were seen under UV illuminator. [11]

Determination of total phenolic contents in the plant extracts

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. The samples were thereafter incubated in a thermostat at 45°C for 45 min. The absorbance was determined using spectrophotometer at $\lambda_{max} = 765$ nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolic was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (g of GA/g of extract). [12]

RESULT AND DISCUSSION

Transverse section of leaf- The leaf has prominent midrib and even lamina. Midrib is shallowly convex on the adaxial side and broadly semicircular on the abaxial side. It shows prominent cuticle as well as radial wall. It shows the presence of vascular bundle covered with pericyclic fibre. The ground tissue has dilated circular compact parenchyma cell. It also shows the presence of collenchymas at two places below upper and lower epidermis (Figure 1).

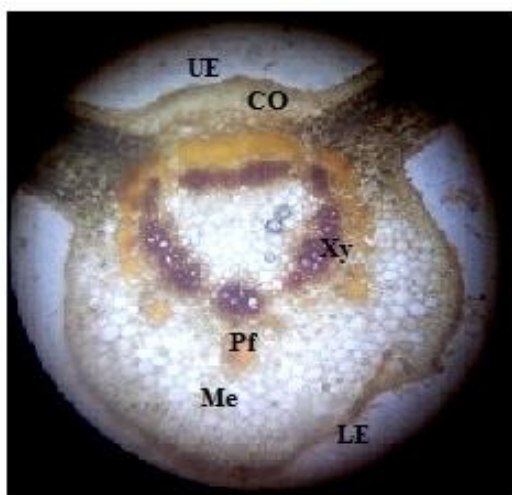


Fig. 1: T.S. of leaf through midrib with lamina (Co-Collenchyma, Xy-Xylem, Pf-Pericyclic fibre, Me-Mesophyll (spongy parenchyma), UE-upper epidermis, LE-lower epidermis)

Preliminary phytochemical results showed the presence or absence of certain phytochemical in the drug. The tests were performed using methanolic and aqueous extracts. Phytochemical test revealed the presence of flavonoids, glycosides, steroids, alkaloids, tannins, saponins and results are given in Table 1.

Table 1: Preliminary phytochemical test for different solvent extract of leaves of *kigelia africana*

S. No.	Natural product	Test performed	Result
1.	Alkaloids	Dragendroff's test	+ve
2.	Flavonoids	Shinoda test	+ve
3.	Steroids	Liebermann-Burchard reagent	+ve
4.	Saponins	Froth test	+ve
5.	Tannins	Lead acetate test	+ve
6.	Glycoside/sugars	Molish test	+ve
7.	Phenols	Ferric chloride test	+ve

Physico-chemical parameters of the leaves of *kigelia africana* are tabulated in Table 2. The loss on drying at 105°C in leaves was found to be 23.88% w/w. Total ash value of plant material indicated the amount of minerals and earthy material attached to the plant material. Analytical results showed total ash value content was 10.5%. The amount of acid-insoluble siliceous matter present in the plant was 9%. The water-soluble extractive value was indicated the presence of sugars, acids and inorganic compounds. The alcohol-soluble extractive value was indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and the results given in Table 2.

Table 2: Physico-chemical parameters of leaves of *kigelia africana*

S.No.	Parameters	Results
1.	Description	Greenish brown
2.	Foreign matter	1% w/w
3.	Loss on drying at 105°C	23.88% w/w
4.	Total Ash	10.5% w/w
5.	Acid-insoluble Ash	9% w/w
6.	Water-soluble Ash	4.5% w/w
7.	Water-soluble extractive	13.4% w/w
8.	Alcohol-soluble extractive	18% w/w
9.	Swelling index	2
10.	Foaming index	611.11%

TLC of the methanolic extract developed in the mobile phase of

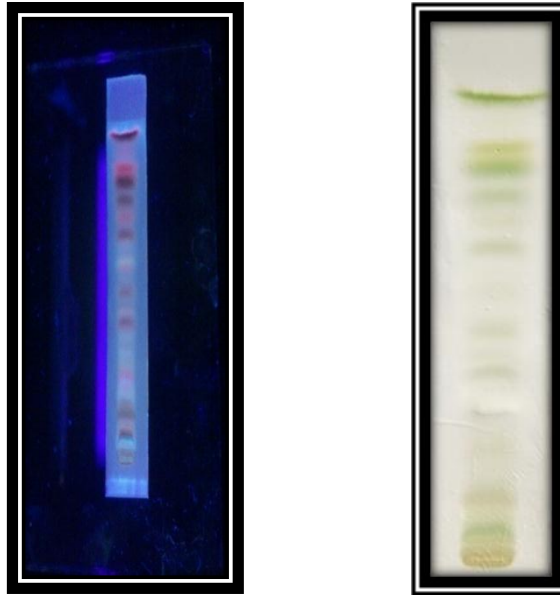
i) chloroform: acetone: formic acid:: 7.8:1.65:0.8 (Figure 2) and observed under UV 366 nm showed 12 spots at Rf value 0.06, 0.08, 0.13, 0.14, 0.23, 0.44, 0.49, 0.58, 0.65, 0.72, 0.77 and 0.82 (blue colour).

ii) petroleum ether: ethyl acetate :: 7:3 (Figure 3) and observed under UV 366 nm showed 11 spots at Rf value 0.08, 0.18, 0.30, 0.34, 0.43, 0.58, 0.63, 0.72, 0.78 and 0.81, 0.91 (blue colour).

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 27.549x + 0.3054$, $r^2 = 0.983$). The values obtained for the concentration of total phenols are expressed as g of GA/100g of extract. The total phenolic content in the examined extract was found to be 3.53g of GA/100g of methanolic extract of *kigelia africana* (Table 3 & Figure 4).

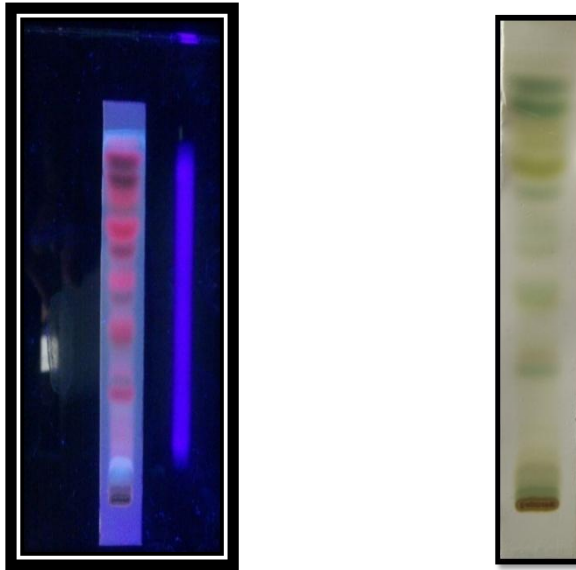
Table 3: Phenolic content of methanolic extract of *kigelia africana*

S.No.	Sample	Concentration (mg/ml)	Absorbance
1.	Gallic Acid	0.01	0.6987
		0.02	0.8835
		0.04	1.1866
		0.06	1.9953
		0.08	2.0025
2.	<i>Kigelia africana</i> Methanolic extract	0.1	3.1506
		1	1.2766



UV 366nm

Fig. 2: TLC profile of methanolic leaves extract of *kigelia africana*.



UV 366nm

Fig. 3: TLC profile of methanolic leaves extract of *kigelia africana*.

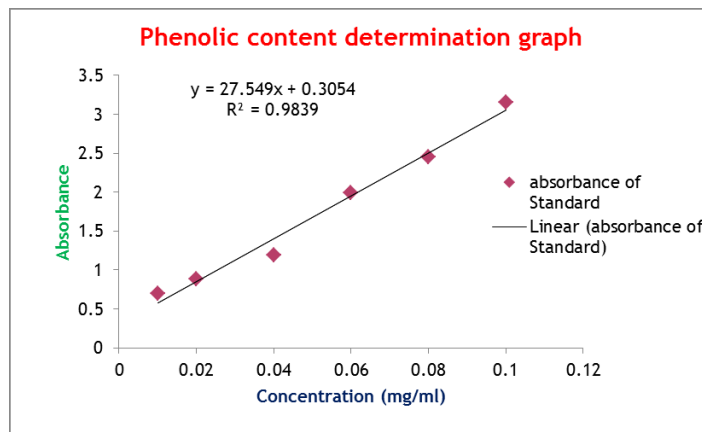


Fig. 4: Phenolic content of methanolic extract of *kigelia africana*

CONCLUSION

Effective formulations have to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials. The manufacture of plant products should be governed by standards of safety and efficacy. So finally we concluded that these pharmacognostical and phytochemical analysis of methanolic extract of *kigelia africana* discussed here can be considered as identifying parameters to substantiate and authenticate the drug. In the present work, it was confirmed that leaves of *Kigelia africana* contains high amount of phenolic compounds. Therefore, it is suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

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