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FREE RADICAL SCAVENGING AND ANTI-DIABETIC ACTIVITY OF *KIGELIA PINNATA*

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ABSTRACT

Medicinal plant plays an essential role in the management of various diseases. *Kigelia pinnata* commonly known as the Sausage tree, it is a multipurpose medicinal plant with many characteristics and considerable potentials. The plant has traditional uses which consist of anticancer, antiulcer, anti-aging, antioxidant, and anti malarial, in the treatment of genital infections, gynaecological disorders, renal ailments, fainting etc., this work represents the recent profile of applications of *Kigelia pinnata* as examined by different contemporary scientific researches. In this study, the *in vitro* anti-oxidant activity, anti-diabetic activity and phytochemical evaluation of four different solvent extracts, from the leaves of *Kigelia pinnata* were determined. Phytochemical screening of the extracts of the leaves showed the presence of flavanoids, alkaloids, etc. Anti-oxidant activities of these extracts were evaluated through DPPH assay, reducing power assay and phospho-molybednum assay. The anti-diabetic activities of these extracts were determined by amylase inhibition assay. The anti oxidant and anti-diabetic activity of extracts was found to be promising camparble with standard. **IC_{50}** was calculated for both antioxidant and antidiabetic activity.The results obtained showed that the leaves of *Kigelia pinnata* has anti-oxidant and anti-diabetic properties, which helps in providing a basis for the traditional use of medicinal plant.

Key words: *Bignoniaceae, Kigelia pinnata,* anticancer, anti-oxidant, anti-diabetic, DPPH.

INTRODUCTION

Human use of plants as medicinal purpose predates recorded history. Medicinal plant use data in many forms has been heavily utilized in the development of pharmacopoeias and

formularies, providing a major focus in global healthcare, as well as contributing substantially to the drug development process for alternate of synthetic drug [1]. Generally, natural drug substances often form vital and appreciable roles in the modern system of medicine thereby justifying their presence in the prevailing therapeutic arsenal, namely- serve as extremely useful natural drugs, provide basic compounds affording less toxic and more effective drug molecule, modification of inactive natural products by suitable biological and chemical means into powerful drugs [2]. Infectious diseases are important in public health concern for communities in African countries and the developing world like India [3]. These diseases and subsequent deaths have devastating consequences for developing economies. Lack of health budgets and inadequate medical facilities hinder efforts by developing countries to match the over-whelming treatment and prevention burden presented by these diseases [4]. Western or modern medicine has for many years been used, with varying degrees of success, in the treatment of infectious disease. Traditional knowledge to solve health problems of mankind and animals exists in all countries of the world [5]. In most of the traditional medicine, the medicinal plant include the fresh or dried part, whole, chopped, powdered or an advanced form of the herb usually made via extraction by a solvent such as water, ethanol or an organic solvent play a major role and constitute the backbone of traditional medicine [6]. The exploration of the chemical constituent of the plants and pharmaceutical screening may provide us the basis for developing the lead for development of novel agents. Herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. Among the estimated 400,000 plant species, only 6% have been studied for biological activity and about 15% have been investigated phytochemically [7]. This inadvertedly shows a dare need for the in-depth dissertation of various chemical constituents, medicinal viability, pharmacological evaluation and biological activities of herbal medicine such as the *k. Africana* (Lam) Benth (or *K. pinnata*) of the family Bignoniaceae an exceptional indigenous medicinal plant in Africa.

K. pinnata (Lam) Benth, (*K. pinnata*) belongs to the family Bignoniaceae. Its common names include sausage tree (Eng.); mranaa (sw) [8]. It is a tree growing up to 20 m tall or more. The bark is grey and smooth at first, peeling on older trees. It can be as thick as 6 mm on a 15 cm branch. The wood is pale brown or yellowish, undifferentiated and not prone to cracking [9]. The tree is evergreen where rainfall occurs throughout the year, but deciduous where there is a long dry season. The leaves are opposite or in whorls of three, 30 - 50 cm long, pinnate, with six to ten oval leaflets up to 20 cm long and 6 cm broad; the terminal leaflet can be

either present or absent. The flowers (and later the fruit) hang down from branches on long flexible stems (2 - 6 m long). Flowers are produced in panicles; they are bell shaped (similar to those of the African tulip tree but darker and more waxy), orange to reddish or purplish green and about 10 cm wide. Individual flowers do not hang down but are oriented horizontally [10]. Some birds are attracted to these flowers and the strong stems of each flower make idea footholds. Their scent is most notable at night indicating their reliance on pollination by bats, which visit them for pollen and nectar Flowers are bisexual, very large; pedicel up to 11 (-13.5) cm long up curved at tip; calyx shortly tubular to campanulate, 2 - 4.5 cm long, suddenly widening and incurving upwards, limp 2-lipped, with the super or lip 2- lobed, the lower one 3-lobed and recurred The fruit is a woody berry from 30 - 100 cm long and up to 18 cm broad; weighs between 5 - 10 kg hangs down on a long rope-like peduncles the fruit is indehiscent, with woody wall and heavily marked with lenticels at the surface. It is grey-brown and many seeded when matured. Seeds are obovoid, ca.10 mm x 7 mm with leathery testa, embedded in a fibrous pulp [11-12].

Fruit, Leaves and Flowers of *Kigelia pinnata* .

ANTI-OXIDANT ACTIVITY

Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50

diseases [13-18] fortunately; free radical formation is controlled naturally by various beneficial compounds known as antioxidants. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, so that they can act as primary and secondary antioxidants. In reducing power assay, the antioxidant compounds convert the oxidation form of iron from ferric chloride (Fe^{+3}) to ferrous (Fe^{+2}). The reducing power increased with increasing amount of the extract.

The phosphor molybdenum assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyze and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Increase in the absorbance shows higher reducing property.

DPPH assay is based on the reduction of DPPH in presence of methanol due to the hydrogen–donating antioxidant leads to the formation of the non radical form of DPPH. This transformation results in a color change from purple to yellow, which is measured spectro-photometrically. DPPH radicals react with suitable reducing agent, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. The result of the different tests carried on the plant material is compared with standard result of ascorbic acid [19-20].

The extract of the plant has been shown to possess anti-oxidative property which apparently makes it useful in the treatment of diseases especially the liver-borne disease [21]. The important compound which is responsible for this activity is “Kigelinone and Kigelinol”.

ANTI-DIABETIC ACTIVITY

Diabetes is a defect in the body’s ability to convert glucose (sugar) to energy. Carbohydrates, when digested, change to glucose. In order for glucose to be transferred from the blood into the cells, the hormone - insulin is needed. Insulin is produced by the beta cells in the pancreas (the organ that produces insulin).In individuals with diabetes, this process is impaired. Diabetes develops when the pancreas fails to produce sufficient quantities of insulin Type 1 diabetes or the insulin produced is defective and cannot move glucose into the cells. In Type 2 diabetes. Either insulin is not produced in sufficient quantities or the insulin produced is defective and cannot move the glucose into the cells. In vitro screening of anti-diabetic drug is carried out by estimating the levels of PPARγ and the two principle enzymes which involve in the carbohydrate digestion and glucose absorption process. Various methods are reported for antidiabetic activity. In the work that is described here, inhibition of α-Amylase

assay method has been adopted for our study [22-23].

MATERIALS AND METHODS

COLLECTION OF SAMPLE

The leaves of the plant *Kigelia pinnata* were collected from Nursery of Lalbhag Botanical garden, Bangalore. The plant was authenticated by Botanist from Lalbhag. Karnataka, India.

CHEMICALS AND REAGENTS

All the chemicals and reagents used were of analytical grade and are purchased from Lancaster Research Lab, Chennai, India and Himedia Lab, Mumbai, India.

PREPARATION OF EXTRACT

Ethanolic extract: The leaves of the plant collected were dried under low sunlight for 7-8 days and then grinded to a fine powders in a grinder. The powdered plant material (15g) was subjected to maceration using ethanol for 4 days, then filtered with muslin cloth and evaporated to dryness. Extract was kept in desiccator.

PHYTOCHEMICAL ANALYSIS

The leave extract was used for preliminary screening of phytochemicals such as alkaloids (Wagner's and Meyer's tests), saponins (foam test), tannins (gelatin test), and flavonoids (Alkaline reagent and Lead acetate tests), the screening was done as per the standard method.

Test for alkaloids

Dragendroff's test: 2mg of the test extract and 5ml of distilled water was added, 2M hydrochloric acid was added until an acid reaction occurs. To this 1ml of Dragendroff's reagent was added. Formation of orange red precipitate indicated the presence of alkaloids.

Hager's test: 2mg of the test extract was taken in a test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.

Wagner's test: 2mg of extract was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids.

Mayer's test: few drops of Mayer's reagent, 2mg of extract were added formation of white or pale yellow precipitation indicated the presence of alkaloids.

Test for flavonoids

Ferric chloride test: test solution with few drops of ferric chloride solution shows intense green color.

Zinc hydrochloride acid reduction test: test solution with zinc dust and few drops of hydrochloric acid shows magenta red color.

Lead acetate solution test: test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

Test for terpenoids: taken 0.5 gm of plant extract in a 2ml of chloroform. Add concentrated sulphuric acid carefully to form a layer. Observe for the presence of reddish brown color interface to show positive result for the presence of terpenoids.

Test for saponins

Foam test: to the extract solution, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. The formation of honeycomb like froth indicates the presence of saponins.

Test for tannins

Ferric chloride test: 1-2ml of the extract, few drops of 5%w/v FeCl3 solution was added. A green color indicated the presence of gallotannins; while brown color indicates the Presence of pseudotanins.

Gelatine test: test solution when treated with gelatine solution gives white precipitate.

Test of Iridoids

A very important compound which is found in this plant is Iridoids and for analyse this Trim-Hills reagent is used which is 10ml Acetic acid, 1ml 0.2% $CuSO_4$, 5ml H_2O and 0.5ml HCL. The extract is heated on flame and a blue or violet coloured product is formed [24].

ANTIOXIDANT ACTIVITY

Reducing Power Assay (Iron (III) to iron (II) reduction) 2.5 mL of extract solution of different concentrations (100 to 500 µg) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 0.1%). This was incubated at 500 C for 20 min. After the incubation, 2.5 mL of 10% trichloroacetic acid was added.2.5 mL of the reaction mixture was mixed with distilled water (2.5mL) and ferric chloride (0.5 mL, 0.1%). The solution absorbance was measured at 700 nM. Increasing absorbance of the reaction mixture

indicates increasing reducing power. The same procedure was applied for ascorbic acid which acts as the standard. Increase in the absorbance indicates increase in reducing power.

PHOSPHO MOLYBDENUM ASSAY

The antioxidant activity of all the extract was determined by the phospho-molybdenum Method as described by Prieto et al [25]. 0.3 ml of extract of different concentrations (100 to 500µg) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodiumphosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95^0C for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer against blank. Distilled water (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid.

DPPH FREE RADICAL SCAVENGING ASSAY

Plant extract of different concentration ranging from 100-500µg was taken in test tubes and made the volume to 1 ml with methanol. 4ml of DPPH solution was added to all the tubes. The reaction mixture was kept 30 min at room temperature. Measure the Optical density at 517 nm against Blank containing 5 ml of methanol, and control is containing 1 ml methanol and 4 ml of DPPH.

The % of inhibition is calculated by using formula

$$\% \text{ of Inhibition} = \frac{AC - AS}{AC} \times 100$$

Where, AC is absorbance of control

AS, is absorbance of sample.

ANTIDIABETIC ACTIVITY

INHIBITION OF ALPHA AMYLASE ENZYME

STANDARD MALTOSE CURVE: 0.2-1ml of standard maltose (1mg/ml) was taken into different test tubes. Make the volume to 1.0 ml in each case with distilled water. Added 1.0 ml of DNS reagent to the each tube & then placed all the tubes in boiling water bath for 15 minutes. Added 8.0 ml of distilled water in each test tube & Mix the contents of the tube thoroughly. Then read the absorbance of the solution in calorimeter at 570 nM against blank solution.

ALPHA AMYLASE INHIBITION ASSAY

100-500µl (100-500µg) of extract was taken into different test tubes. Made the volume to 0.5ml with phosphate buffer of pH 6.9, Blank was measured by taking 1 ml of phosphate buffer. Control was measured by taking 0.5ml of phosphate buffer. The solution was then treated with 0.5ml of alpha amylase (0.5mg/ml). The solution was incubated at 25^{o}C for 10 minutes. Added 0.5ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25^{o}C for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled water. Read the absorbance of the solution in calorimeter at 570 nM against blank solution.

Amount of maltose produced is calculated using standard maltose curve, and Enzyme activity is calculated by using formula

$$\text{Enzyme Activity} = \frac{\text{Amount of Maltose formed x 2}}{10 \text{x} 342}$$

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS

In this study *In vitro* antioxidant activity, antidiabetic activity of leaves extracts of *Kigelia pinnata* was evaluated by different *In vitro* screening methods. Preliminary phytochemical screening of Ethanolic extract of the dried leaves revealed the presence of secondary metabolites like alkaloids, tannins, flavonoids and iridoids. The results are tabulated in Table 1.

The free radical scavenging activity of extract tested by reducing power assay method, phosphor-molybdenum method and DPPH method, the results are summarized in table 2-4 and fig 1-3.

The phosphor-molybdenum method is quantitative since the total antioxidant capacity is express as ascorbic acid equivalents. The percentage of antioxidant activity was found 62%. In reducing power assay extracts of *Kigelia pinnata* seemed to have quite significant antioxidant properties i.e. 61% compared to ascorbic acid. The results are summarized in table 2 & 3 and fig 1-2.

In DPPH assay extracts of *Kigelia pinnata* shows the (minimum 12% and maximum of 65%)

free radical scavenging activity compared to standard. The results are compared with standard ascorbic acid. The results are summarized in table 4 and fig-3. From the results revealed that extracts of *Kigelia pinnata* has antioxidant activity.

In vitro antidiabetic activity was done by alpha amylase inhibition assay method, the inhibitory effect on amylase by extracts of *Kigelia pinnata* was shown in table 5 and fig-4. Results show that extracts of *Kigelia pinnata* has Antidiabetic activity which is compared with maltose standard.

Table 1: Screening of secondary metabolites.

Test for alkaloids:	
Dragendroff's test	++
Hager's test	++
Wagner's test	++
Mayer's test	++
Test for flavonoids:	
Ferric chloride test	++
Zinc test	++
Lead acetate test	++
Test for terpenoids	++
Test for saponins:	
Foam test	--
Test for tannins	--
Test for pseudo-tannins	++
Gelatine test	--
Test of iridoids	++

ANTIOXIDANT ACTIVITY

Table 2: Reducing power assay of different extracts at different concentrations

Extract (µg/ml)	Acetone	Ethanol	Chloroform	Water	Ascorbic Acid
100	24	26	17	19	19
200	33	37	22	26	46
300	38	43	30	37	59
400	44	54	39	46	79
500	49	62	43	58	84

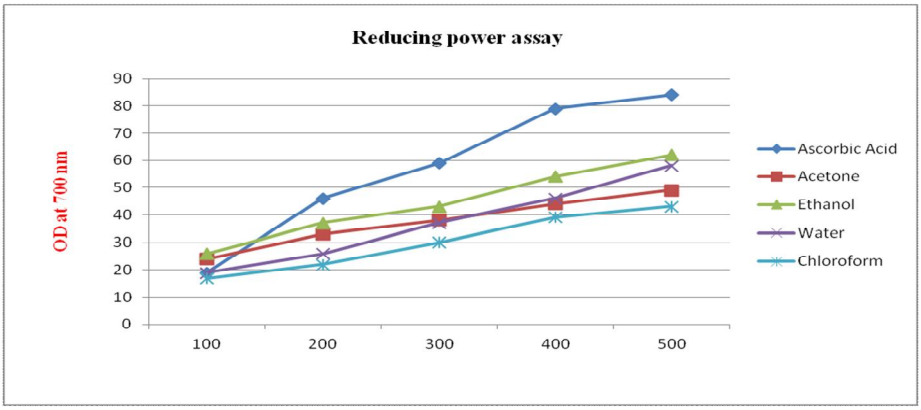

Figure1. Reducing Power Assay

Table 3: antioxidant activity of different extracts at different concentrations by phospho-molybdenum method

Extract (µg/ml)	Acetone	Ethanol	Chloroform	Water	Ascorbic Acid
100	27	26	19	20	18
200	39	34	22	25	47
300	43	40	26	34	60
400	53	47	33	42	78
500	59	61	43	55	85

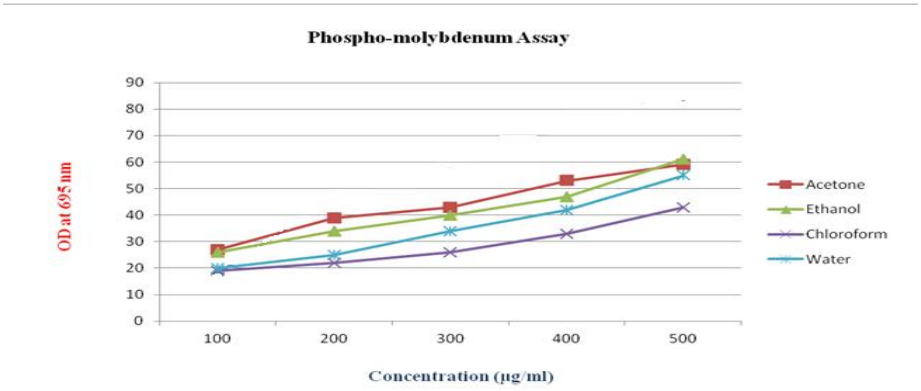

Figure2. Phospho-molybdenum Assay

Table 4 Free radical scavenging activity of different fractions at different concentrations by DPPH free radical scavenging assay

Extract (µg/ml)	Acetone	Ethanol	Chloroform	Water
100	25	21	17	12
200	39	29	24	16
300	41	40	31	17
400	54	48	45	22
500	65	63	56	45

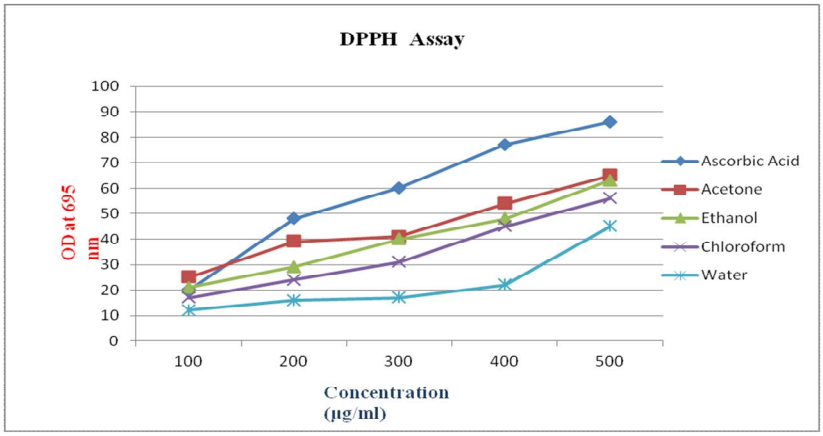

Figure3. DPPH free radical scavenging assay

ANTI-DIABETIC ACTIVITY

Table 5: Inhibition of alpha amylase enzyme

Extract (µg/ml)	Acetone	Ethanol	chloroform	Water	Standard
	Amylase activity (µmol/ml/min)	Amylase activity (µmol /ml/min)	Amylase activity (µmol /ml/min)	Amylase activity (µmol /ml/min	Amylase activity (µmol /ml/min)
100	0.016	0.019	0.012	0.015	0.024
200	0.038	0.040	0.028	0.032	0.068
300	0.059	0.075	0.049	0.056	0.165
400	0.096	0.124	0.066	0.135	0.224
500	0.107	0.151	0.099	0.147	0.278

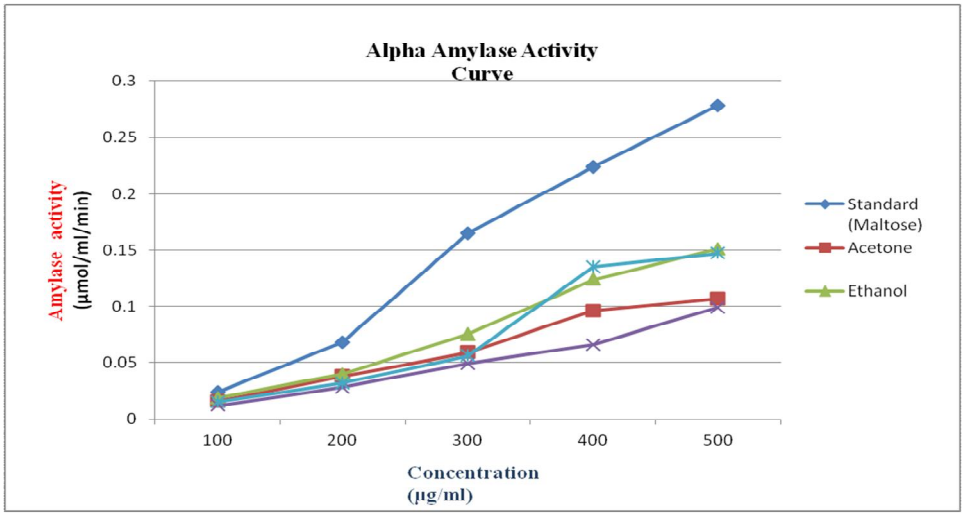

Figure 4. Alpha amylase Activity Curve

CONCLUSION

Kigelia pinnata leaves were collected, extracted and screened for phytochemical analysis. Phytochemical analysis of the extract revealed the presence of alkaloids, tannins, flavonoids and iridoids. The extract of *Kigelia pinnata* was also subjected to antioxidant and antidiabetic activity. From research investigations we observed that the increase in the % of Antioxidant activity. Maximum activity was seen in the order acetone > ethanol>chloroform>water. IC_{50} 250µg/ml was found in DPPH free radical scavenging activity compared to the standard Ascorbic acid. *In vitro* antidiabetic activity was done by alpha amylase inhibition assay method, the inhibitory effect on amylase by extracts of *Kigelia pinnata*. Results show that extracts of *Kigelia pinnata* has Antidiabetic activity which is compared with acarbose standard. The comparative results of extract of *Kigelia pinnata* leaves shows more anti-oxidant activity as compared to anti-diabetic activity and it proven that this medicinal plant could be futuristic alternative for other medicines for anti-oxidant and anti-diabetic properties.

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