



Preliminary phytochemical detection and in vitro antioxidant activity of *Kigelia africana* (Lam.) Benth leaves

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Abstract: This study intended to evaluate free radical scavenging capacity to screen the presence of phytochemical compounds and quantitative estimation of total flavonoid and phenol contents of the *Kigelia africana* (Lam.) Benth Leaves. Preliminary phytochemical screening was performed by chemical testing of the components. The antioxidant activity was assessed through DPPH, ABTS, FRAP, and hydroxyl radical scavenging assays. Total flavonoid contents were determined by using quercetin as reference standard and total phenols determined by Folin Ciocalteu method with gallic acid as reference standard. Phytochemical screening indicates the presence of alkaloids, glycosides, sterols, tannins, flavonoids and saponins. The total flavonoid and phenolic content found to be higher in methanolic extract i.e. 5.8415 ± 0.0015 mg QE/g and 92.0809 ± 0.0045 mg GAE/g respectively. Antioxidant assays revealed that the methanolic extract exhibited the strongest activity among all tested extracts. The IC₅₀ values were recorded as 28.38 µg/mL for DPPH, 26.41 µg/mL for ABTS, 135.40 µg/mL for FRAP, and 156.70 µg/mL for hydroxyl radical scavenging. In comparison, the standard ascorbic acid showed IC₅₀ values of 23.55, 17.86, 82.29, and 92.52 µg/mL for the respective assays. The findings justified plants potential and *Kigelia africana* (Lam.) Benth could be represents as good candidate for further chemical and biological activity. These findings suggest that *Kigelia africana* leaves possess significant antioxidant potential, supporting their use as a promising candidate for further phytochemical isolation and biological investigations.

Introduction

Free radicals from metabolism or from environmental sources continuously interact in biological systems and their uncontrolled generation is directly related to the molecular level of many diseases¹. Much research has clearly shown that free radicals would damage nearby structures, including DNA, proteins or lipids. Anti-radical antioxidants are particularly important in the antioxidant defense in the protection of free radical cells². Plants are a good source of biologically active compounds known as phytochemicals. Phytochemicals have been found to act as antioxidants by scavenging free radicals and may have therapeutic potential for ailments associated with free radicals³. Free radicals are known to be the leading cause of numerous chronic and degenerative diseases, including coronary heart disease, inflammation, strokes, diabetes mellitus, and cancer. Therefore, it is important to evaluate the antioxidant activity of the plants used in herbal medicine or to clarify the mechanism of their pharmacological action or to provide information on the antioxidant activity of these herbaceous plants^{4,5}.

Kigelia Africana (Bignoniaceae), commonly known as the sausage tree because of the huge fruits, which hang from long fibrous stems. It is also known as Balamkheera in Hindi and distributed throughout India but found abundantly in West Bengal⁶. It is a medicinal plant traditionally used for the treatment of numerous diseases such as wound healing, rheumatism, psoriasis, diarrhea and stomach ailments⁷. It is a small, sprawling tree with



hanging clusters of dull liver-colored flowers and long, squash-shaped fruit. It is widely cultivated in the tropics and was introduced from India to tropical Africa⁸. The *Kigelia* plant has a long history of use by rural communities, particularly for its medicinal properties. These properties are found in all parts of the tree, including the fruits, bark, roots, and leaves, which are used for medical purposes⁹. This plant has a traditional use that includes anticancer, antimicrobial, antioxidant, anti-inflammatory and antimalarial properties. It is also widely applied in treating genital infections, gynecological disorders, kidney disorders, fainting spells, epilepsy, rheumatism, sickle cell anemia, psoriasis, respiratory disorders, skin disorders, body weakness, leprosy, worm infestation, and kidney stones¹⁰. Due to the potential as traditional medicinal plant, attempt had been made to screen leaves of the plant to determine presence of phytoconstituents with free radical scavenging effect.

Materials And Methods

Plant material: Leaves of *Kigelia africana* were collected from Indira nagar, Nashik-422009, Maharashtra. The plant species was identified and authenticated (BSI/WRC/IDEN.CER./2017/579KRC 01) by J. Jayanthi, Scientist 'D' at botanical survey of India, Western regional center, Pune-411001, Maharashtra, India.

Preparation of extract: The air-dried leaves were coarsely powdered using a grinder and 500 g of powdered leaves are used for the preparation of extract. Powdered leaves were extracted by Soxhlet extraction method. The extraction was carried out successively using petroleum ether, chloroform and methanol. The extract obtained was then concentrated under reduced pressure to dryness in a rotary vacuum evaporator. The dried dark brown color extract was stored in a desiccator until further use¹¹.

Phytochemical screening: A preliminary phytochemical screening^{11,12,13} of the methanolic extract was carried out to detect the presence of various phyto-constituents such as alkaloids, glycosides, flavonoids, phenolic, tannins, saponins, proteins, and steroids.

Estimation of loss on drying: 2 g of the sample leaf powder was weighed and kept in hot air oven at 105⁰ C. The sample dried until constant weight obtained. Weight of the sample drying noted and difference in weight provides the loss on drying^{14, 15}.

Estimation of total ash value: 2 g of the sample leaf powder was weighed and placed in a silica crucible. The crucible was incinerated using muffle furnace. After incineration of sample the silica crucible was cooled, weighed and total ash calculated with reference to the air dried sample ^{14, 15}.

Estimation of water soluble ash value: The total ash which was obtained boiled with 25 ml of water for 5 min. The insoluble matter collected and washed using hot water. The insoluble ash transferred into silica crucible, again ignited for 15 min, after incineration of sample the silica crucible was cooled, weighed and water soluble ash value calculated ^{14,15}.

Estimation of Acid insoluble ash value: The total ash boiled gently with 25 ml of 2N hydrochloric acid for 5 min. The insoluble ash ignited using crucible for 15 min. After incineration of sample the silica crucible was cooled, weighed and acid insoluble ash value calculated ^{14, 15}.

Determination of water-soluble extractive value: 5 g of air dried leaf powder was macerated with 100 ml of chloroform water in a closed flask for 24 hours. Then it was filtered, 25 ml of the filtrate was evaporated and dried at 105⁰c and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs ^{14, 15}.

Determination of alcohol-soluble extractive value: 5 g of air dried leaf powder was macerated with 100 ml of ethanol in a closed flask for 24 hours. Then it was filtered, 25 ml of the filtrate was evaporated and dried at 105⁰c and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs^{14, 15}.

Estimation of phenolic and flavonoid contents: The total phenolic content of the extract was evaluated following the Folin-Ciocalteu colorimetric method¹⁶ and the results were expressed in mg of gallic acid equivalent (GAE) per g of dry weight of the extract. The total flavonoid content was estimated using the aluminum chloride colorimetric method¹⁷ and the results were expressed in mg of quercetin equivalent (QE) per g of dry weight of the extract.

Estimation of total phenolic content (TPC): 1 ml of extract made up to 3 ml with distilled water, thoroughly mixed with 0.5 ml of Folin-Ciocalteu reagent for 5 min. followed by the addition of 2 ml of 20% (w / v) aqueous sodium carbonate. The mixture was allowed to stand for another 60 minutes in the dark and the absorbance at 765 nm was measured. The total phenolic content was calculated from the gallic acid calibration curve (Figure 1). Results were obtained as the mean $\pm$ SEM of three replicate studies.

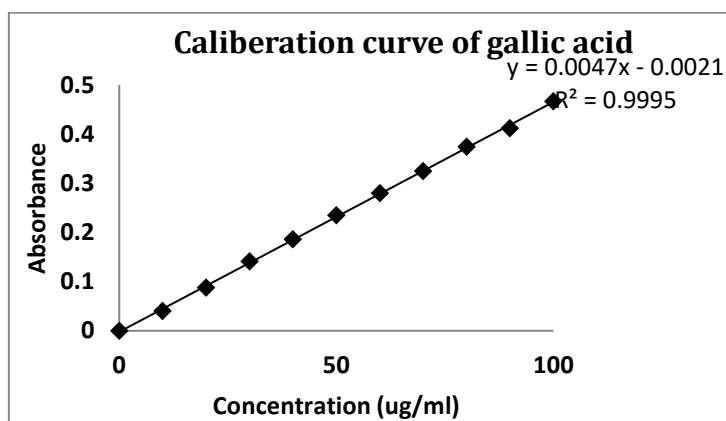


Figure 1: Gallic acid calibration curve for assessment of total flavonoid content

Estimation of the total flavonoid content (TFC): 0.5 ml of extract made up to 1 ml with ethanol were prepared, mixed with 4 ml of distilled water and then with 0.3 ml of 5% NaNO_2 . Solution, 0.5 ml of 10% AlCl_3 solution was added after 5 minutes of incubation and the mixture was left to stand for 5 minutes. To the above solution 2 ml of 1 mol / L NaOH solution were added and the final volume of the reaction mixture was brought to 10 ml with distilled water. The mixture was allowed to stand for 15 minutes and the absorbance was measured at 415 nm. The total flavonoid content was calculated from a quercetin calibration curve (Figure 2). Results were obtained as the mean $\pm$ SEM of three replicate studies.

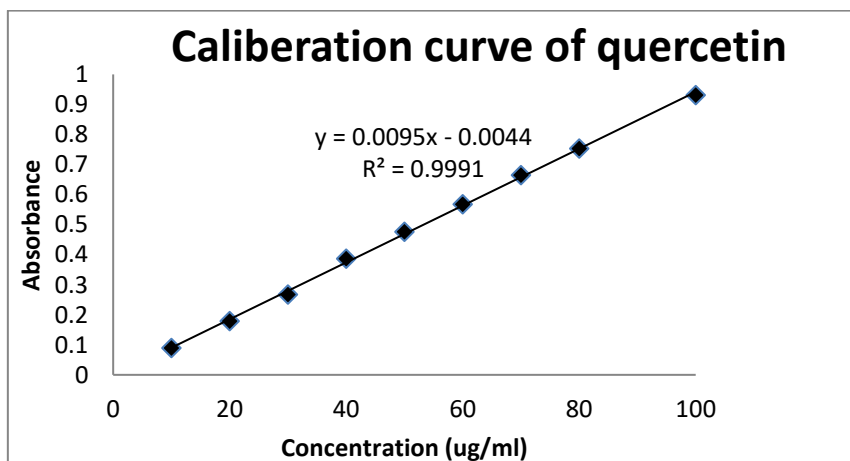


Figure 2: Quercetin calibration curve for assessment of total flavonoid content

In vitro antioxidant activity

DPPH radical scavenging activity: 1 ml of a solution of DPPH in methanol (0.1 mmol / L) was mixed with 3.0 ml of extract in various concentrations for 30 minutes at room temperature in the dark and the absorbance at 517 nm¹⁸. The scavenging activity of the extracts was estimated based on the percentage of scavenging radicals of DPPH (I%) using the equation $I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$ Where A_{sample} is the absorbance of a sample solution and A_{control} is the absorbance of the control solution (which contains all reagents except the test sample).

ABTS Radical Scavenging Assay¹⁹: The ABTS•+ radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and incubating in the dark for 16 h at room temperature. The solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 1.0 mL of ABTS•+ solution was mixed with 10 µL of plant extract (5–30 µg/mL). After 1 min, absorbance was recorded at 734 nm. Ascorbic acid and Trolox were used as standards. % Inhibition was calculated as:

$$\% \text{Inhibition} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100$$

IC₅₀ values were determined by interpolation from the inhibition curve.

FRAP Assay²⁰: The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃·6H₂O (10:1:1, v/v/v). 900 µL of FRAP reagent was added to 90 µL distilled water and 30 µL extract (5–30 µg/mL). After incubation at 37 °C for 30 min, absorbance was measured at 593 nm. A standard curve of FeSO₄·7H₂O was used to express results as µmol Fe²⁺ equivalents per g extract.

Hydroxyl Radical Scavenging Assay²¹: The hydroxyl radical scavenging activity of the extracts was determined using the deoxyribose degradation method. The reaction mixture (1.0 mL) contained 0.10 mL of 10 mM 2-deoxy-D-ribose, 0.10 mL of 10 mM FeSO₄, 0.10 mL of 10 mM H₂O₂, 0.50 mL of 20 mM phosphate buffer (pH 7.4), and 0.10 mL of test extract at different concentrations. The mixture was incubated at 37 °C for 60 min. After incubation, 1.0 mL of 2.8% trichloroacetic acid and 1.0 mL of 1% thiobarbituric acid were added, followed by heating at 95 °C for 15 min. The solutions were cooled, centrifuged, and the absorbance of the supernatant was measured at 532 nm against a reagent blank. Ascorbic acid was used as the standard antioxidant. The hydroxyl radical scavenging activity was expressed as percent inhibition relative to control, and IC₅₀ values were calculated from concentration–response curves.

RESULTS & DISCUSSION:

Phytochemical screening

The results of preliminary phytochemical detection had shown the presence of steroids, alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds (Table 1). The results of physicochemical parameters of *Kigelia africana* leaves presented in (Table 2).

Table 1: Phytochemical screening of *Kigelia africana* leaves.

Constituents	Petroleum ether extract	Chloroform extract	Methanolic extract
Steroids	+	-	-
Alkaloids	-	+	++
Glycosides	+	++	++
Saponins	-	+	++

Tannins	+	++	++
Flavonoids	+	+	++
Terpenoids	-	-	-
Phenolic Compounds	+	+	++
Proteins	-	-	-

(++) indicates strong presence, (+) moderate presence, (-) absence.

Table 2: Physicochemical parameters of *Kigelia africana* leaves.

Sr. no	Physico-chemical Parameters	<i>Kigelia pinnata</i>
1	Loss on drying	04.01% w/w
2	Total ash	10.10% w/w
3	Water-soluble ash	04.58% w/w
4	Acid-insoluble ash	08.32% w/w
5	Water soluble extractive	14.71% w/w
6	Alcohol soluble extractive	17.00% w/w

Total phenolic and flavonoid contents

Total phenolic content and total flavonoid content were determined from the calibration curves of gallic acid ($Y = 0.0047x + 0.0021$, $R^2 = 0.9995$), and quercetin ($Y = 0.0095x + 0.0044$, $R^2 = 0.9991$), respectively. The total phenolic and total flavonoid contents among the petroleum ether, chloroform and methanol extracts are presented in Table 3. The results showed that methanol extract possessed the highest phenolic [(92.0809 ± 0.0045) mg GAE/g of dry material] and flavonoid components [(5.8415 ± 0.0015) mg QE/g of dry material], followed by the chloroform extract, while the petroleum ether extract contained less polyphenolic compounds.

Table 3: Total phenolic and flavonoid contents of *Kigelia africana* leaves.

Sr. no.	Extracts	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
1	Petroleum ether extract	46.7475 ± 0.0066	5.4915 ± 0.0026
2	Chloroform extract	57.3809 ± 0.0085	5.0145 ± 0.0117
3	Methanolic extract	92.0809 ± 0.0045	5.8415 ± 0.0015

In vitro antioxidant activity

The antioxidant activity of *Kigelia africana* leaf extracts was evaluated using multiple in vitro assays, namely DPPH, ABTS, FRAP, and hydroxyl radical scavenging. The IC_{50} values obtained are summarized in Table 4. Among the different extracts, the methanol extract consistently exhibited the strongest free radical scavenging

activity, with IC_{50} values of 28.38 $\mu\text{g/ml}$ (DPPH) and 26.41 $\mu\text{g/ml}$ (ABTS), which were significantly lower than those of the chloroform and petroleum ether extracts. In the FRAP and hydroxyl radical assays, the methanol extract also demonstrated higher reducing power and hydroxyl radical scavenging capacity ($EC_{50} = 135.4 \mu\text{g/ml}$ and $IC_{50} = 156.7 \mu\text{g/ml}$, respectively) compared to the non-polar extracts.

The superior performance of the methanol extract may be attributed to its higher content of polar phenolics and flavonoids, which are known to contribute to antioxidant activity through hydrogen atom transfer and single electron transfer mechanisms. Although the standard ascorbic acid showed the most potent activity across all assays (DPPH $IC_{50} = 23.55 \mu\text{g/ml}$, ABTS $IC_{50} = 17.86 \mu\text{g/ml}$), the methanol extract approached comparable efficacy in certain assays, particularly ABTS. The relatively weaker activity observed in petroleum ether and chloroform fractions suggests a lower abundance of antioxidant phytochemicals in the lipophilic fraction.

The complementary use of different antioxidant assays highlights the broad radical scavenging spectrum of *Kigelia africana* extracts. These findings are consistent with earlier reports indicating that the antioxidant potential of plant extracts is strongly correlated with their phytochemical composition, particularly polyphenols. Thus, the methanol extract of *Kigelia africana* leaves demonstrates promising antioxidant potential and warrants further investigation for therapeutic applications.

Table 4: Comparative IC_{50} values of *Kigelia africana* leaf extracts in different antioxidant assays

Assay	Ascorbic acid IC_{50}	Petroleum ether IC_{50}	Chloroform IC_{50}	Methanol IC_{50}
DPPH	23.55	40.97	36.87	28.38
ABTS	17.86	43.73	39.82	26.41
FRAP	82.29	221.4	184.36	135.4
Hydroxyl	92.52	248.3	205.1	156.7

Studies suggest that high molecular weight phenolic compounds, including plant flavonoids, including the hydroxyl group and the aromatic ring, serve as powerful free radical scavengers²². Phenolic compounds can suppress lipid peroxidation through various chemical mechanisms, including free radical quenching, electron transfer, radical addition, or radical recombination²³. Plants rich in phenolic compounds, flavonoids and related substances, have antioxidant activity thanks to their redox properties and because their ability to eliminate free radicals is facilitated by hydroxyl groups²⁴. Therefore, the determination of the total content of phenols and flavonoids could be used as a basis for evaluating the antioxidant potential of plant extracts. The high content of phenols and flavonoids is believed to be responsible for the bioactivity of plants. The antioxidant properties of phenolic compounds of plant origin have been reported to be primarily determined by the scavenging of superoxide anionic radicals²⁵. The results of the study indicated that presence of phenolic and flavonoid compounds are responsible for the scavenging activity of the methanolic extract of plant.

Conclusion:

The study reported the quantitative estimation of phenolic and flavonoid compounds present in *Kigelia africana* (Lam.) Benth leaves along with the in vitro free radical scavenging effect of extracts obtained. In order to apprehend the health benefits from potential medicinal plant sources, it is very important to determine the antioxidant activity of the plants, as due to which plants can be useful in the treatment of various ailments such as atherosclerosis, cancer, inflammatory disease, asthma and diabetes caused by free radical damage. The results of



the present study suggested that *Kigelia africana* (Lam.) Benth leaves could be utilized for its antioxidants due to the presence of phenolic and flavonoid content and free radical scavenging activity.

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