Research Article

Phytochemical Screening, Flavonoid Content and In-Vitro Antioxidant Activity of Methanolic Extract of *Kigelia Africana*

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ABSTRACT

Methanolic extract of leaves of *kigelia africana* were screened for flavonoids content and in-vitro antioxidant activity. Phytochemical screening was carried out according to standard procedures. Total Flavonoids content was determined by spectrophotometric methods and that was found to be 2.56 g & 2.20g quercetin equivalent per 100 g of methanolic & water extract respectively. *In-vitro* antioxidant activity of methanolic leaves extract of *kigelia africana* was determined by DPPH free radical scavenging assay and reducing power method. All the analysis was made with the use of UV-Visible Spectrophotometer (Jasco V-530). The methanolic leaves extracts of *kigelia africana* had shown very significant DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity and reducing power of the extract was increased with the increasing concentration. In DPPH free radical scavanging assay IC_{50} value of methanolic leaves extracts of *kigelia africana* was found to be 36.48±0.09 as compared with standard ascorbic acid (43.79±0.10).

Keywords: *kigelia africana*, DPPH, free radical scavenging activity, Flavonoids content.

INTRODUCTION

Reactive free radicals, including superoxide, hydroxyl radical, and peroxyl radical, generally result in degradation of protein, lipid peroxidation, and oxidation of DNA, which have been considered to be linked with many chronic diseases, such as diabetes, cancer, and atherosclerosis[1]. Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[2,3].

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 candelebra. 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. Kigelia in a native of W. Africa[4]. The *K.africana* plant has many medicinal properties due to the presence of numerous secondary metabolites. These compounds include irridoids, flavonoids, naphthoquinones, and volatile constituents etc[5,6]. 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*[7, 8]. The aim of this experiment is to investigate free radical scavenging activity of the leaves aqueous and methanolic extracts of *kigelia africana* using DPPH method, reducing power method and to quantitative total flavonoids content using a spectrophotometric method.

MATERIALS AND METHOD

Plant Material

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

Chemicals and Instruments

Quercetin, DPPH and ascorbic acid were obtained from Hi Media Labs, Mumbai. Aluminium chloride was purchased from Research lab Mumbai. All organic solvents were of analytical grade and supplied from Research Lab, Mumbai. UV-Visible Spectrophotometer (JascoV-530) was used for antioxidant activity determination by DPPH method and for total flavonoid content determination.

Preparation of extract

Dried powder of leaves sample were extracted by using petroleum ether, chloroform and methanol as a solvent by Soxhlet Extractor. Also the water extract obtained by maceration method.

Phytochemical procedure

The preliminary phytochemical screening of the methanolic extract of *Kigelia africana* was carried out in order to ascertain the presence of its constituents by utilizing standard conventional protocols[8,9].

Total Flavonoid Content Determination

5 ml of 2% aluminium chloride ($AlCl_3$) in methanol was mixed with the same volume of *Kigelia africana* leaves extracts (0.02 mg/ml). Absorption readings at 415 nm were taken after 10 minutes against a blank sample without $AlCl_3$. The total flavonoid content was determined using a standard curve of quercetin (0.01-0.1 mg/ml). The mean of three readings was used and expressed as mg quercetin equivalent (QE)/100 g extract[10].

Antioxidant activity

DPPH method

Free radical scavenging activity of compounds was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Briefly, 2 ml extract and standard of various concentrations (10-100μg/ml) were added to 2 ml of 100 μm DPPH solution[11]. After 20 minute incubation at room temperature, the absorbance was read against a blank at 517nm. The change in absorbance with respect to the control (containing DPPH only without sample, expressed as 100% free radicals) was calculated as percentage scavenging using following the equation:

$(A517_{blank} - A517_{sample}) \div A517_{blank} \times 100\%$.

The reading was taken in triplicate and mean used for calculation of IC_{50}. The IC_{50} (mean ± SEM) stand for the concentration required for 50% inhibition of DPPH radicals.

Assay of Reducing power

This was carried out as per the method of Yildrim *et al*. and Lu and Foo[12,13]. 1 ml of plant extract solution (final concentration 100-500 mg/l) was mixed with 2.5 ml phosphate buffer(0.2M, pH 6.6) and 2.5 ml potassium ferricyanide $[K_3Fe(CN)_6]$ (10g/l), then mixture was incubated at 50^0C for 20 minutes. Two and one-half, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Jasco V-530 UV-Visible Spectrophotometer 117, INDIA). Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power[14].

RESULT AND DISCUSSION

Phytochemical screening

Petroleum ether, chloroform, methanol and aqueous extracts of *Kigelia africana* were subjected to Preliminary phytochemical studies. This study reveals the presence of flavonoids, glycosides, steroids, alkaloids and Saponins.

Total Flavonoid content

Total Flavonoid content was determined by spectrophotometric method. The obtained observations are mentioned in table I and plotting graph absorbance vs concentration (in fig I). Total flavonoid content was found to be 2.56 g & 2.20g quercetin equivalent per 100 g methanolic & water extract resp. by using the equation (y = 9.837x + 0.031).

Antioxidant activity

DPPH method

In order to determine the extent of scavenging effect, methanolic extract of the leaves of *Kigelia africana* was tested for antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Extract has showed antioxidant activity (Table 2 & 3, Fig.-II). Extract showed (36.48±0.09)µg/ml IC_{50} as compared with standard ascorbic acid (43.79±0.10)µg/ml.

Assay of reducing power

The reductive capabilities of the *Kigelia Africana* leaves extract was compared to ascorbic acid. The reducing power of *Kigelia Africana* leaves extracts was very potent and the power of the extract was increased with quantity of sample (Table 4 & Fig.-III).

CONCLUSION

In the present work, it was confirmed that leaves of *Kigelia africana* contains high amount of flavonoids and the plant bears good antioxidant activity. This study suggest that it might play a pivatol role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. 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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Table 1: Absorbance by sample for total flavonoid content

S.No.	Sample	Concentration (mg/ml)	Absorbance
1	Standard	0.01	0.1060
2	Standard	0.02	0.2172
3	Standard	0.04	0.4306
4	Standard	0.06	0.7028
5	Standard	0.08	0.7906
6	Standard	0.1	0.9912
7	Test (alc. Extract)	1	0.6107
8	Test (water Extract)	1	0.4245

Table 2: % Radical Scavenging activity of alcoholic extract

S.No.	Conc. µg/ml	Standard (Ascorbic acid)		Test (Methanolic extract)	
		Absorbance±SEM	% RSA±SEM	Absorbance±SEM	% RSA±SEM
1	10	0.983±0.010	21.91±4.14	0.833±0.142	33.83±11.71
2	20	0.915±0.007	27.32±3.90	0.884±0.007	29.79±3.31
3	30	0.855±0.010	32.11±3.48	0.813±0.006	35.44±3.11
4	40	0.805±0.012	36.08±3.34	0.754±0.010	40.13±2.56
5	50	0.764±0.009	39.30±2.77	0.704±0.009	44.13±2.38
6	60	0.709±0.007	43.69±2.87	0.637±0.009	49.41±2.14
7	70	0.632±0.022	49.81±2.95	0.607±0.006	51.81±2.19
8	80	0.606±0.008	51.85±2.47	0.542±0.008	56.95±1.70
9	90	0.547±0.012	56.54±2.06	0.483±0.010	61.63±1.29
10	100	0.494±0.005	60.72±2.13	0.412±0.008	67.30±1.16
11	Blank	1.2631±0.070		1.2631±0.070	

Table 3: Antioxidant acitivity expressed in IC_{50}

Sample	DPPH scavenging activity IC_{50}(± SEM) (µg/ml)[a]
Standard (Ascorbic acid)	43.79±0.10
Test (Methanolic extract)	36.48±0.09

The results are expressed as IC_{50}±SEM (n=3) (µg/ml), the concentration of the test compound that provides 50% scavenging of the DPPH radicals already available in the solution.

Table 4: Assay of reducing power

S.No	Concentration (µg/ml)	Absorbance of standard	Absorbance of test
1	100	0.1251	0.0904
2	200	0.1664	0.1390
3	300	0.1953	0.1603
4	400	0.2102	0.1673
5	500	0.2420	0.1863

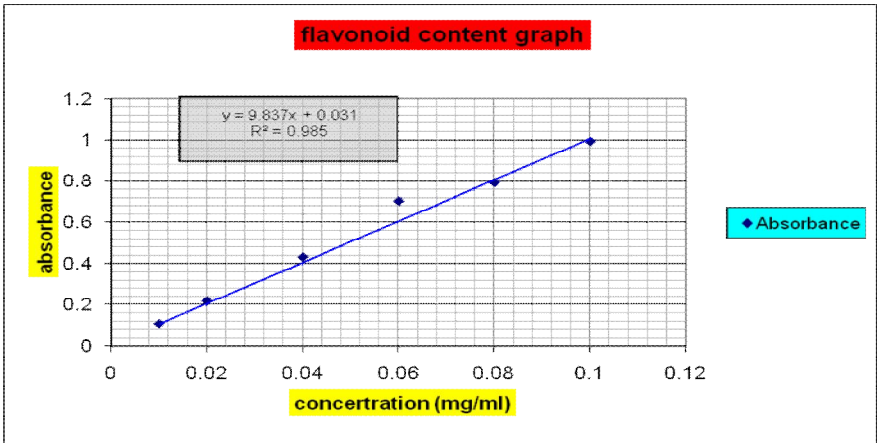

Fig. 1: Graph of absorbance against concentration for total Flavonoids content

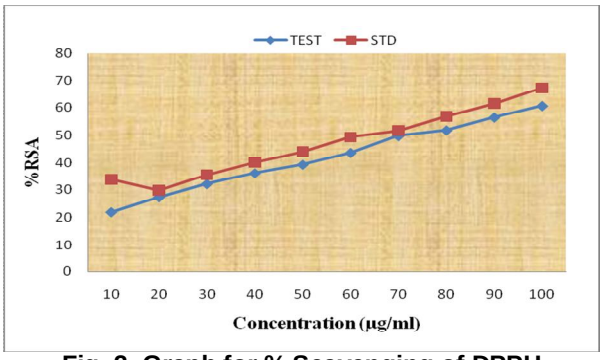

Fig. 2: Graph for % Scavenging of DPPH against Concentration

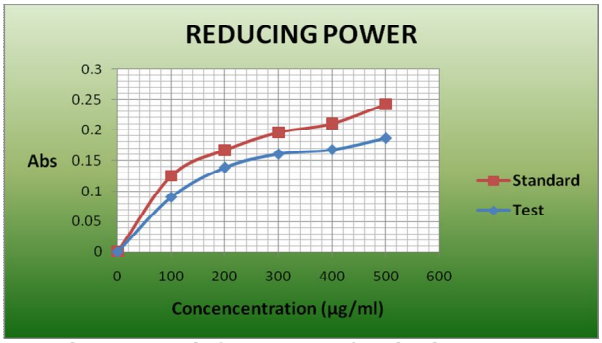

Fig. 3: Graph for assay of reducing power

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