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In Vitro Free Radical Scavenging Activity and Total Phenolic Content of *Kigelia Africana* (LAM)

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Abstract: ***Recently, plants have received much attention as sources of biologically active substances including antioxidants. In the present study the total phenolic and flavonoid contents as well as the in-vitro antioxidant activity of petroleum ether, chloroform, methanol and aqueous extracts of Kigelia africana leaf were determined. The total phenolic and flavonoid contents were estimated using tannic acid and quercetin as standards respectively, while the in-vitro antioxidant activity was evaluated for all extracts using DPPH method. The results showed that methanol extract has the highest amount of phenolics and petroleum ether, the least. The flavonoid contents of the methanol and chloroform extracts are the same and are higher than those of petroleum ether and aqueous extracts. The methanol crude extract showed very promising antioxidant activity as compared with petroleum ether, chloroform and water extracts. The findings of the present study suggest that Kigelia africana leaf could be a potential natural source of antioxidants and thus play an important role as a therapeutic agent in preventing or retarding oxidative stress-related degenerative diseases.***

Keywords: oxidative stress, DPPH, *Kigelia africana*, antioxidant, degenerative diseases

1. Introduction

Highly reactive oxygen species and free radicals are generated in the body as a result of metabolic activities, stress, exposure to chemicals and other related factors. These free radicals include hydroxyl radicals ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot}$), lipoxyl radicals ($LOO^{\cdot}$) and so on [1].

Oxidative stress results if the availability of free radicals in the body greatly supersedes available antioxidants. This leads to a variety of biological and physiological lesions culminating in metabolic impairment, cell death, and degenerative diseases such as cancer, diabetes, obesity and neural disorder [1, 2]. *Kigelia africana* (Lam.) Benth. Syn. *K. pinnata* (Jacq.) belongs to the family Bignoniaceae, and is widely distributed in West and South Africa. The plant is commonly called Cucumber or Sausage tree, and different parts are used traditionally in the treatment of various ailments [3]. The fruits are used for the treatment of ulcer, as a purgative and to increase the flow of milk in lactating mothers [4]. The root and unripe fruits are employed as a vermifuge and aid in the treatment of rheumatism and hemorrhoids [5]. Natural antioxidants such as plant polyphenols play a vital role in scavenging and inhibiting free radicals, and are responsible for the antioxidant potentials of plants [6].

The purpose of this study was to evaluate the total *in vitro* antioxidant potential of the plant, as well as quantify the total phenolics and flavonoid content of *Kigelia africana* leaf.

2. Materials and Method

2.1 Collection and Extraction of the Plant Material

The Kigelia africana leaves used for the research were collected from the wild in Ewu community of Esan-Central Local Government Area of Edo state, Nigeria. They were duly identified and authenticated by Mr. Emmanuel of Professor J.C Okafor's herbarium, Paxherbals, Ewu, Nigeria, where voucher specimens were deposited.

2.2 Pre-Extraction of Plant

Foreign matters and elements in the collected *Kigelia africana* were removed, the plant rinsed twice with large quantity of de-ionized water, spread on a clean sack and placed under shade to air dry at ambient temperature. The dried material was ground into coarse powder using a modern laboratory electric milling machine (Chris Norris, England), and stored in a plastic container with tight fitting lid.

2.3 Extraction of Plant

The leaf powder was extracted successfully with petroleum ether, chloroform, methanol and water (Laboratory graded solvents) in a soxhlet apparatus. The crude extracts were reduced in vacuo; the obtained extracts were weighed and stored in the refrigerator at 4^{o}C.

2.4 Phytochemical Screening of Extracts

Preliminary phytochemical screening was performed on the extracts using the standard methods described in [10, 11].

2.4 Estimation of Total Phenolic Content

1 mL of the extract (containing 100mg) was mixed with 2.0 mL of 7.5% sodiumtrioxo carbonate(IV) solution (Na_2CO_3), and 2ml of Folin-Ciocalteau reagent. After incubation on a water bath at 40^{o}C for 45 min, the absorbance of the reaction mixture was measured at 765 nm on a UV-visible spectrophotometer. Tannic acid was used as a standard. The calculation of total phenol content was based on the calibration curve of the tannic acid standard and the data was expressed as milligram tannic acid equivalents (TAE) per 100 mg plant extract.

2.6 Estimation of Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method (Lin and Tang, 2007). From the homogenate, 0.5 mL of extract was mixed with 0.5 mL of methanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCL_3.6H_2O$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of deionized water reagents. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm on a UV-visible spectrophotometer (model SM23A; Microfield®, England) and compared with the absorbance of deionized water as the control. Flavonoid contents were calculated on the basis of the calibration curve of quercetin standard (2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one with 98% purity;

Table 1: Phytochemical screening of *Kigelia africana* leaf

Classes of phytochemicals	*Pet. Ether extract*	*Chloroform extract*	*Methanol extract*	*Aqueous extract.*
Glycoside	++	+	++	+
Cardiac glycoside	-	+	-	-
Saponin	-	++	++	+
Phenolic compound	+	+	+	++
Hydrolysable tannin	+	+	+	++
Condensed tannin	-	-	-	-
Alkaloid	+	++	++	++
Phlobatannin	-	-	-	-
Terpenoids	-	-	-	++
Flavonoid	+	+	+	+
Reducing sugar	++	+	++	+
Polysaccharide/starch.	-	-	-	-

Reg. 317313 Sigma, St. Louis, Missouri, USA). Data was expressed as milligrams quercetin equivalents (QE) per 100 mg plant extract.

2.7 Determination of Antioxidant activity

The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. Radical scavenging activity was measured using a slightly modified method previously described 8, 9. 0-100mg/ml of crude extract and Vitamin C were prepared in methanol (Analar grade). I ml of the extract was placed in a test tube, 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \{[A_b - A_a]/A_b\} \times 100 \ldots\ldots\ldots (1)$$

Where A_b and Aa represent the absorbances of the blank and extracts repsectively.

2.8 Statistical Analysis

Data from three replicate determinations were used for statistical analysis. Statistical processing of the original data was performed using one-way analysis of variance (ANOVA) and differences at $P < 0.05$ were considered significant.

3. Results & Discussion

Phytochemical screening results of all the extracts of *Kigelia africana* are shown in Table 1. The presence of glycosides, phenolic compounds (hydrolysable tannins), alkaloids, flavonoid and reducing sugar were noted in all the extracts.

Table 2: Total phenolic and flavonoid content of *Kigelia africana*

Sample	*Phenolics (mg/100mg)*	*Flavonoid (Mg/100mg)*
Petroleum ether extract	6.69±0.04	0.51±0.01
Chloroform extract	9.13±0.05	0.49±0.01
Methanol extract	9.23±0.07	0.49±0.00
Aqueous extract	9.17±0.04	0.51±0.01

Values are expressed as mean ± SEM (n = 3).

Research shows that phenolic compounds of plants are responsible for its antioxidant potential [7, 8]. Total phenolic and flavonoid content of the various extracts of *Kigelia africana* are shown in Table 2. Flavonoid content was expressed as mg/100mg quercetin equivalent, while total phenolic content was expressed as mg/100mg tannic acid equivalent.

Table 3: The IC_{50} value of different extracts of *Kigelia africana*

Extract	IC_{50}.
Chloroform extract	0.62±0.01
Methanol extract	0.32±0.02
Aqueous extract	0.43±0.03
Petroleum extract	2.34±0.01
Vitamin C	0.27±0.03

Values are expressed as mean ± SEM (n = 3).

Methanol extract had the highest amount of phenolics while petroleum ether extracts, the least (Table 2). Flavonoid content of petroleum ether and aqueous extracts are the same and were slightly higher than those of chloroform and methanol extracts. Although there was no significant difference in the amount of flavonoids in all the extracts of *K. africana* (Table 2). A positive concentration-dependent activity was noted in all extracts except the methanol extract, which showed a higher activity at 250 mg/100ml as shown in Table 3.

4. Conclusion

This study suggests that the methanolic extract of *Kigelia africana* possess an average free radical scavenging activity (comparable to that of Vitamin C) and phytochemical constituents which might be useful for further studies to unravel novel treatment strategies for diseases associated with free radical induced tissue damage.

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