VOL. 4, NO. 2, February 2014 ISSN 2225-7217
ARPN Journal of Science and Technology

http://www.ejournalofscience.org

Phytochemical Screening and Free Radical Scavenging Activities of the Fruit and Leaves of Kigelia Africana (Bignoniaciae)

[*1] Ipav Selumun Solomon, [2] Ibok Nne Umoh, [3] Dasofunjo Kayode
[1] Department of Chemical Sciences, University of Mkar, Mkar, Benue State, Nigeria
[2] Department of Chemical Science and Technology, Federal Polytechnic, Mubi, Adamawa State
[3] Department of Medical Biochemistry, Cross River University of Technology, Okuku, Cross River State
[*1] selumunipav@gmail.com

ABSTRACT

This study set out to compare the phytochemical constituents in the leaves and fruits of Kigelia Africana, and determine their free radical scavenging activities. The fruit and leaves of KA collected from Okeigbo, Ondo State, Nigeria, were dried and extracted with methanol. Phytochemical screening was carried out according to standard procedures. The decrease in the visible absorbance of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) on plant extract was used to determine the free radical scavenging activity. The mean inhibitory concentration (IC_{50}), that is, the concentration of extract needed to reduce the initial absorbance of DPPH by 50% was determined statistically. Spectrophotometric methods were used to determine proanthocyanidin, flavonoids and total phenolic contents. The Phytochemical results indicated the presence of alkaloids, tannins, steroids, flavonoids and cardiac glycosides in both the fruit and leaves, while anthraquinones, saponins, phlobatannins and terpenoids were absent in both plant parts of K. Africana. IC_{50} values of vitamin E and KA fruit were found to be 0.01 and 0.08 respectively while IC_{50} value of KA Leaves was not traceable. Total phenolic, total flavonoids and proanthocyanidin contents were 2.28, 2.1 and 0.57mg/g of powdered plant material for KA fruit, and 1.104, 0.147 and 0.022mg/g of powdered plant material for KA leaves as gallic acid, rutin and catechin equivalents respectively. KA fruits are far more potent as a free radical scavenger compared to the leaves.

Keywords: *DPPH, flavonoids, Free radical scavenger, Kigelia Africana, Phytochemicals*

1. INTRODUCTION

Nature has contributed immensely in the development of modern drugs. Many of these drugs have been isolated based on their uses as medicinal agents in traditional medicines **[1]**. The lack of western medical facilities (that is, in terms of the development of drugs, problems of cost and transportation of drugs) in Africa has been a blessing in disguise as it has encouraged research into the bioactive properties of the tropical plants of Africa. **[2]**. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. These free radicals have a tendency of oxidizing nucleic acids, proteins, lipids or DNA and can initiate a variety of disease processes such as cancer, cardiovascular diseases, cataracts, diabetes, asthma, macular degeneration and inflammatory diseases **[3]**. Typical reducing agents such as thiols or polyphenols are antioxidants which aid in the slowing down or inhibition of the oxidation of other molecules. These antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Phenolic compounds such as flavonoids, phenolic acids, phenolic diterpenes and tannins have been fingered in antioxidant activity **[4]**. Recent studies have revealed that phenolic molecules have anticancer and antimutagenic activities **[5]**.

Kigelia Africana, an evergreen tree may be found at altitudes as high as 1830m (6000ft). it is native to warm, wet grasslands of tropical West Africa. It is an ornamental plant which can grow as tall as 4.5 to 7.5m (12 to 25ft) tall and sometimes attain heights of between 15 and 23m. It has fruits that resemble giant sausages and leaves which are densely covered like a spreading crown especially in the summer with attractive flowers. **[6][7]**. In Tanganyika, the heated bark is applied to women's breast to reduce swellings. In Nigeria and Ghana, the bark is pounded and swallowed for dysentery while the bark and fruits are powdered and dust is applied to sores and oily ointments made from it, are used to rub on rheumatic parts and on malignant tumors. The bark is used to treat syphilis and gonorrhea in Nigeria. A bark fruit concoction is taken in Congo to relieve asthma and in Tanganyika, the inner bark is soaked in water which is then used for washing in treating ringworm and constipation **[6]**. In Ghana, the decoction of the root is used to treat tapeworm and constipation **[7]**.

In East Africa, the root is used as a remedy for boils and sore throats and in Tanganyika, a medicine is made of them for infertility in women. The root mixed with other roots is drunk to treat syphilis **[6]**. In West Cameroons, sap expressed from the buds is used for sore eyes. The fruit is used to treat scrotal elephantiasis and oedema of the legs in Ivory Coast. The fruit and roots along with the 'male' tassel of the plantain inflorescence are boiled together to make a woman's nostrum in Ghana and the bark and fruit are used to treat sores and to restore taste. The unripe fruit is not edible, nor is the ripe fruit. It is a purgative and toxic in Nigeria, Gabon, Tanganyika and

Malawi. In Uganda, Tanganyika and Kenya, the fruit is an additive to beer preparation as ferment. The fruit is used as a stimulant and intoxicant in Tanganyika. In W. Cameroons, a paste is made from the fruits for treating boils. The Jebel Marra of S. Africa prepares powdered fruit as a poultice for treatment of complaints in breasts, possibly for mastitis or cancer **[6].**

In South eastern Nigeria, the fruits and flowers are mixed with alcohol or water and used by traditional healers for fertility treatment among women and men of child bearing age **[8]**. Recent antimicrobial studies show their potency as antimicrobials and antifungal against Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans and Escherichia coli**[7]** . Previous studies revealed the fruits have hepatoprotective effect **[9][10]**, anti-inflammatory effects **[11][12]**, and anticancer activity **[11][13][14].** This study is aimed at determining the correlation between the free radical scavenging activity of the methanolic extract of KA fruit and leaves and the phenolic, total flavonoids and proanthocyanidin contents of the plant. To the best of our knowledge such comprehensive studies on samples collected from this source and phytochemical constituent contents have not been done yet.

2. MATERIALS AND METHOD

2.1 Collection and Identification of Plants

2.1.1 Preparation of Plant Material.

The plant materials were collected fresh from forest resources in South-West Nigeria and were identified at the Forestry Research Institute of Nigeria (FRIN), Ibadan and given a voucher number. The plant materials were chopped into bits in the case of the fruits and dried in the oven at a temperature of 40^{o}C for about three days. The leaves were air dried in the oven for three days at a temperature of about 40^{o}C. The dry plant materials were blended using a kitchen blender after which the powdered sample was weighed and recorded.

2.1.2 Extraction of Plant Material and Phytochemical Screening

The powdered samples were extracted as follows; Kigelia Africana fruits; 300g in 1500ml methanol (Sigma-Aldrich, UK). Kigelia Africana_leaves; 60g of sample in 600ml methanol.

The individual extracts were concentrated in an oven at 40^{o}C.

Chemical tests were carried out on the aqueous extracts and on the powdered specimens using standard procedures to identify the constituents as described by Sofowora **[15]**, Trease and Evans **[16]** and Harbone **[17]**.

2.1.3 Determination of Antioxidant Activity of Extract Using DPPH.

Triplicate portions 1ml each of concentrations of 0.02mg/ml, 0.04mg/ml, 0.06mg/ml, 0.08mg/ml and 1.0mg/ml for each of the sample extract and vitamin E were pipette into test tubes labeled and incubated for about 40mins with 1ml DPPH (0.0394g) solutions and the absorbance read at a wavelength of 517nm. Absorbance of three 1ml portions of blanks were also taken (1ml methanol and 1ml DPPH solutions). The radical scavenging activity of each sample was calculated using the formula below;

$\%\ \text{Inhibition} = [(A_B - A_A)/A_B] \times 100$
Where A_B = Absorbance of blank sample.
A_A = Absorbance of sample extract.

2.1.4 Determination of Total Phenolic Content

Total phenolic content was determined according to the Folin-ciocalteau's method [18](1927). Gallic acid was used as a standard. Concentrations of 0.01mg/ml, 0.02mg/ml, 0.03mg/ml, 0.04mg/ml and 0.05mg/ml of Gallic acid were prepared in methanol. Concentrations of 0.1mg/ml and 1.0mg/ml of each of the plant extracts were also prepared in methanol. 0.5ml of the sample was mixed with 2.5ml of a ten-fold diluted Folin-ciocalteau's reagent and 2ml of 7.5% sodium carbonate. The mixture was allowed to stand for 30mins at room temperature before the absorbance was read at 760nm, spectrophotometrically. All determinations were performed in triplicates. The total phenolic contents were expressed as Gallic acid equivalents (GAE).

2.1.5 Determination of Total Flavonoid Content

Total flavonoid was determined using a method of Miliauskas, Venskutonis, & Van Beck **[19]**. 2ml of 2% $AlCl_3$ in ethanol was added to 2ml of sample. The UV absorption was measured at 420nm after 1hr at room temperature. Concentrations of 0.1mg/ml and 1.0mg/ml were used for the sample while, rutin concentrations of 0.01mg/ml, 0.02mg/ml, 0.04mg/ml, 0.08mg/ml and 0.1mg/ml were used to obtain a calibration curve. Total flavonoids contents were calculated as rutin equivalents (RE) from the concentration of rutin obtained from the calibration curve.

2.1.6 Proanthocyanidin Test

Concentrations of 0.1mg/ml and 1.0mg/ml of the sample extract were prepared and 0.025mg/ml, 0.05mg/ml, 0.1mg/ml, 0.2mg/ml and 0.4mg/ml of catechin equivalent were prepared as the standard solutions. 0.5ml of HCl was added and allowed to stand for 15mins. The absorbance at 500nm was taken and the final result was expressed as catechin equivalent.

Table 1: Result of phytochemical screening of K. Africana leaves and fruits.

Phytochemical	K. Africana leaves	K. Africana Fruit
Anthraquinones	_	_
Alkaloids	+	+
Tannins	+	+
Saponins	_	_
Steroids	+	+
Phlobatannins	_	_
Terpenoids	_	_
Flavonoids	+	+
Cardiac glycosides	+	+

Table 2: IC_{50} values, total phenolic, total flavonoids, and Proanthocyanidin contents methanolic extract of K. Africana fruit and leaves

.

Plant part	IC_{50} (DPPH Inhibition) mg/ml	Total Phenolic Content (GAE) mg/g of plant material	Total flavonoids content (RE) mg/g of plant material	Total Proanthocyannidin content (CE) mg/g plant material
K. Africana fruits	0.08	2.28	2.1	0.57
K. Africana leaves	-	1.104	0.147	0.02

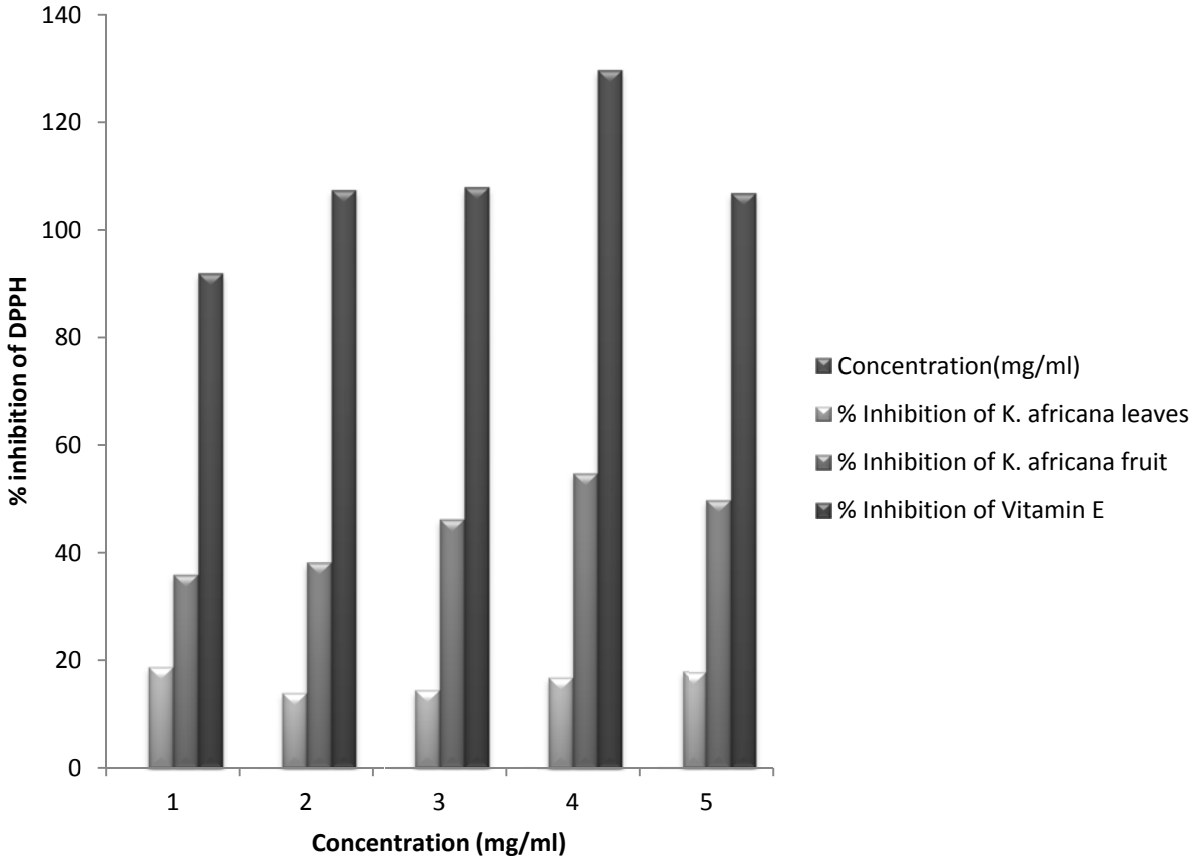
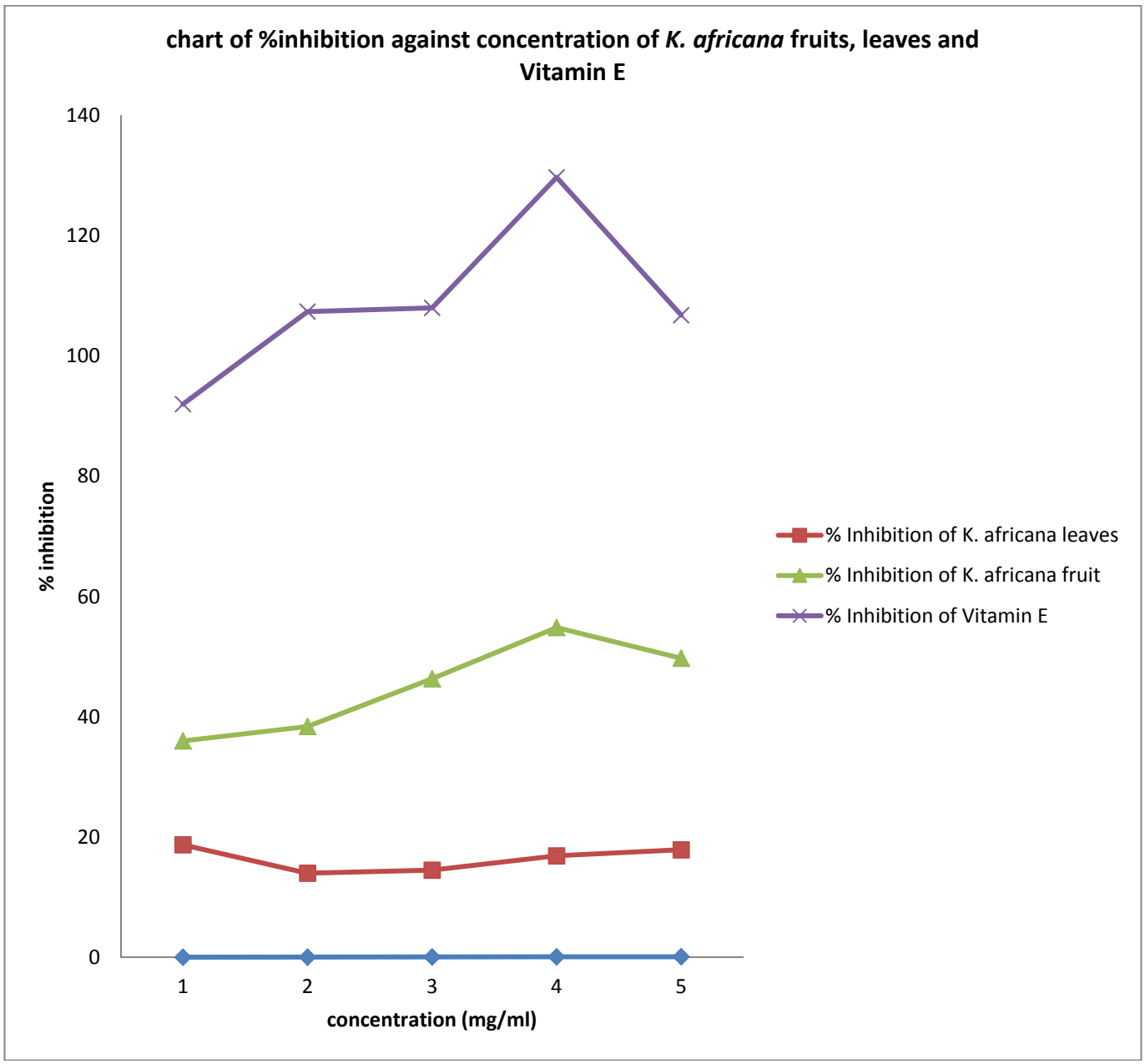


Fig 1: Inhibition (%) of DPPH against concentration of extracts of K. Africana fruit and leaves and Vitamin E



After extraction, 53.61g (17.87%w/w) and 3.21g (5.35%w/w) of methanol extract was obtained from the fruits and leaves respectively of Kigelia Africana. The Phytochemical results indicated the presence of alkaloids, tannins, steroids, flavonoids and cardiac glycosides in both the fruit and leaves, while anthraquinones, saponins, phlobatannins and terpenoids were absent in both plant parts of K. Africana.

The IC_{50} result of DPPH inhibition of K. Africana fruit was 0.08mg/ml while K. Africana leaves had very little activity such that the IC_{50} value could not be

determined. Percentage inhibition of DPPH and IC_{50} are widely used as parameters in measuring antioxidant activity **[20][21][22][23]**. Since plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts. The results show that the phenolic content of KA fruit is 2.28mg/g while KA leaves is 1.104mg/g. According to Ayoola **[24]**, Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Hence, the usefulness of these plants under study cannot be overemphasized due to the heavy presence of flavonoids in them.

The content of phenolic compounds (mg/g) in methanolic extracts, determined from regression equation of calibration curve (y=10.738x + 0.061, R =0.98) and expressed in Gallic acid equivalents (GAE), shows that highest amounts were found in the extracts of K. Africana fruits. It was observed that the contents of phenolics in the extracts correlates with their anti radical activity (e.g. correlation coefficient between data of DPPH assay and total phenolic compounds is 0.89), confirming that phenolic compounds are likely to contribute to the radical scavenging activity of these plant extracts.

The content of flavonoids (mg/g), in Rutin equivalents is 0.147mg/g and 2.1mg/g for KA leaves and KA fruits respectively. Relatively low amounts of flavonoids were found present in K. Africana fruit which in contrast has a reasonable amount of phenolics present. It can be observed that the amount of flavonoids in the analyzed plant extracts showed good correlation with phenolic content (R=0.90). It is known that only flavonoids of a certain structure and particularly hydroxyl position in the molecule determine antioxidant properties; in general, these properties depend on the ability to donate hydrogen or electron to a free radical.

The concentration of proanthocyanidin expressed in catechin equivalents (regression equation of calibration curve y=5.242x + 0.222, R=0.93) in mg/g of plant extract showed amounts of 0.022mg/g and 0.57mg/g for KA leaves and fruits respectively and are present in very low amounts compared to total phenolic and flavonoids. The correlation between total phenolics and proanthocyanidin is 0.87 while the correlation between proanthocyanidin and flavonoids is 0.99. This shows a good correlation between both phytochemicals. This study shows a better correlation between these phytochemicals than with antiradical activity which is 0.79.

Flavonoids have been reported to have antibacterial, antiviral, antineoplastic, anti-inflammatory, anti-allergic, anti-thrombotic and vasodilatory activities **[25]**. The potent antioxidant activities of flavonoids have been suggested to be responsible for many of the above actions as oxidative damage is implicated in most disease processes **[24]**. These results show that the plant extracts of KA can be exploited for anti-inflammatory and analgesic activities since these two have a relationship with free radical scavenging activity.

3. CONCLUSION

Methanolic extracts of K. Africana fruits particularly showed potent free radical scavenging activity against DPPH. KA fruit was 8 times less potent compared to vitamin E while the IC_{50} value of KA leaves could not be identified. The phenolic content, Proanthocyannidin and flavonoids contents of KA fruit were found to be more than in the KA leaves. Further studies are currently being carried out to isolate and characterize the phytochemicals of K. Africana to enhance a detailed knowledge of their free radical scavenging properties.

REFERENCES

[1] Cordell GA. Biodiversity and Drug Discovery; A Symbiotic Relationship. Phytochemistry. 2000; 56:463-480.

[2] Elkin NL. A Hausa Herbal pharmacopoeia; Biomedical evaluation of commonly used Plant Medicines. J. Ethnopharmacol.198;4:75-98.
[3]Atawodi SE. Antioxidant potentials of African medicinal plants. African Journal of Biotechnology. 2005; 4(2):128-133.

[3] Miller AL. Antioxidant Flavonoids: Structure, Function and Clinical Usage. Alt. Med. Rev. 1996; 1(2): 103-111.

[4] Polterait O. Antioxidants and free-radical scavengers of Natural Origin. Current Org. Chem. 1997;1: 415-440.

[5] Ginter E. The Role of Antioxidants in the Prevention of Tumours. Bratisl Lek Listy.1995; 96:195-209.

[6] Burkill HM. The useful plants of West Tropical Africa. Royal Botanical Garden Kew. 1985;4: 385-386.

[7] Saini S, Kaur H, Verma B, Ripudaman, Singh SK. Kigelia Africana (Lam.) Benth. An overview. Natural Product Radiance. 2009; 8(2): 190-197.

[8] Ogbeche KA, Ogunbiyi YO, Duru FIO. Effect of methanol extract of Kigelia Africana on sperm motility and fertility in rats. Niger. J. Health and Biomedical Sci. 2002; 2: 113-116.

[9] Olaleye MT, Rocha JB. Commonly used tropical medicinal plants exhibit distinct in vitro antioxidant activities against hepatotoxins in rat liver. Exp. Toxicol. Pathol. 2007;58: 433-8.

[10] Olaleye MT, Rocha BT. Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defence system. Exp. Toxicol. Pathol. 2008;59: 319-327.

[11] Picerno P, Autore G, Marzocco S, Meloni M, Sanogo R, Aquino RP. Anti-inflammatory activity of verminoside from Kigelia africanaand evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. J. Nat. Prod. 2005; 68: 1610-4.

[12] Owolabi OJ, Omogbai EKI. Analgesic and anti-inflammatory activities of the ethanolic stem bark extract of Kigelia Africana(Bignoniaceae). Afr. J. Biotechnol.2007; 6: 582-585.

[13] Houghton PJ, Photiou A, Uddin S, Shah P, Browning M, Jackson SJ, Retsas S. Activity of Kigelia pinnata against melanoma and renal carcinoma cell lines. Planta Med 1994; 60: 430-3.

[14] Jackson SJ, Houghton PJ, Restsas S and Photiou A. In vitro cytotoxicity of norvibutinal and isopinnatal from Kigelia pinnata against cancer cell lines. Planta Med 2000; 66: 758-61.

[15] Sofowora A. Medicinal plants and Traditional Medicine in Africa. Ibadan; Spectrum books; 1993: 150.

[16] Trease GE, Evans WC. Pharmacognosy. 11th edn. London: Bailliere Tindall. 1978:176-180.

[17] Harbone JB. Phytochemical Methods. (1st edn.). London, UK: Chapman and Hall Ltd, 1973: 49-188.

[18] Folin O, Ciocalteau V. On tyrosine and tryptophane determination in proteins. Journal of Biological Chemistry. 1927; 27: 627-650.

[19] Miliauskas G, Venskutonis PR, Van Beck TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry.2004; 85: 231-237.

[20] Qian H, Nihorimbere V. Antioxidant power of phytochemicals from Psidium guajava leaf. Journal of Zheijiang University Science. 2004; 5(6): 676-683.

[21] Yoshida T, Mori K, Hatano T, Okumura T. Studies on inhibition mechanism of autoxidation by tannins and flavonoids. Radical-scavenging effects on tannins and related polyphenols DPPH radical. Chemical and Pharmaceutical Bulletin, 1989;37(7):1919-1921.

[22] Vinson JA, Dabbagh YA, Serry MM, Jang, J. Plant flavonoids especially tea flavonols are powerful antioxidant using in vitro oxidation model for heart disease. Journal of Agricultural and Food Chemistry. 1995; 43(11): 2800-2802.

[23] Ayoola GA, Sofidiya T, Odukoya O, Coker HAB. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. J. Pharm. Sci. & Pharm. Pract. 2006; 8:133-136.

[24] Ayoola GA, Ipav SS, Sofidiya MO, Adepoju-Bello AA, Coker HAB, Odugbemi TO. Phytochemical Screening and Free radical scavenging activities of Fruits and Leaves of Allanblackia floribunda Oliv (Guttiferae). Int. Journal of Health Research. 2008; 1(2):87-93