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Original article:

CHEMICAL COMPOSITION AND ANTIOXIDANT POTENTIALS OF *KIGELIA PINNATA* ROOT OIL AND EXTRACTS

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ABSTRACT

The chemical composition of *Kigelia pinnata* root oil extracted with n-hexane was analyzed by GC/GCMS. The antioxidant potential of the oil was compared to that of ethyl acetate and methanol extracts of the root. UV and IR spectroscopic techniques were used to carry out partial characterization of the oil and extracts. The free radical scavenging activity by spectrophotometric assay on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was examined while the total antioxidant activity (TAA) and relative antioxidant activity (RAA) were compared with standard antioxidant, α-tocopherol. The antioxidant activity (which correlated with the total phenolic content of the extracts) was assumed to be from the total phenolic content of the extracts. TAA was found to be higher in methanol extract (at 0.25 mg/mL). We hereby report for the first time the major component of the oil from the root of *Kigelia pinnata* to be elaidic acid (56.12 %). It is a reported toxicant which thereby underscores the risk in the use of the plant in traditional therapies.

Keywords: Antioxidant, *Kigelia pinnata*, GC-MS, Free radical, α-tocopherol

INTRODUCTION

Antioxidant compounds are abundantly available in plants and play an important role in scavenging free radicals, thus providing protection to humans against oxidative DNA damage (Ponnan et al., 2006). Although an excess of Reactive Oxygen Species, ROS (oxidative stress) can result in non-controlled oxidation and damage of cellular structures such as DNA, protein and membrane lipids. It is believed that the presence of ROS is essential in cells as they can act as key signaling molecules for the activation of the stress-responses and defense pathways (Halliwell, 2006; Foyer and Noctor, 2005). In humans, the plant polyphenols consumed through the diet are considered as effective protective agents against the ROS, which are known to be involved in the pathogenesis of aging and many degenerative diseases such as cardiovascular diseases and cancers (Virgili and Scaccini, 2003; Kris-Etherton et al., 2004). Numerous epidemiological studies have suggested a protective role of food polyphenols on human health (Arts and Hollman, 2005). Recent studies have, however, stressed that the mechanisms of biological actions of polyphenols go beyond their ROS scavenging and metal chelating properties (Halliwell et al., 2005) but may also offer indirect protection by activating endogenous defense systems and by modulating cellular signaling processes (Yang et al., 2001; Feng et al., 2005).

Kigelia africana (Lam.) Benth. belongs to the family of *Bignoniaceae* and has a wide geographical distribution in west and

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central Africa. The tree grows on river-banks, wet areas along streams and on flood plains of Nigeria, Cameroon, Kenya, Guinea and Senegal. It can also be found in open woodland from KwaZulu-Natal to Tanzania, Chad, Eritrea, South Africa and Namibia (Ogbeche et al., 2002; Abioye et al., 2003). The tree is widely grown as an ornamental plant in tropical regions for its decorative flowers and unusual fruit that conceived the name 'sausage tree' (Roodt, 1992).

The *Bignoniaceae* family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, coumarins, saponins and caffeic acid in the fruits, stem, leaves and roots (Akunyili and Houghton, 1993; Houghton et al., 1994; Moiden et al., 1999; Weiss et al., 2000; Picerno et al., 2005; Bharti et al., 2006; Asekun et al., 2007; Owolabi and Omogbai, 2007).

Though a large number of plants worldwide show strong antioxidant activities (Baratto et al., 2003; Katalynic et al., 2006), there is no report to our knowledge on the antioxidant properties of the root of this plant in any experimental protocol. In view of this, we have investigated the in vitro antioxidant effect of these extracts by DPPH assay and examined the phytochemicals in each extract. The plant root was selected for the study because of the reported phytochemicals which include iridoids, naphthoquinones and coumarins among others. The present study provides basic data on the natural antioxidant potential of *Kigelia pinnata* root for the food, pharmaceutical or cosmetic industries, and also offers scientific reference for the large scale usage and exploitation of *Kigelia pinnata* as a vital resource.

EXPERIMENTAL

Material and methods

Root of mature growing *Kigelia pinnata* tree was obtained from Abeokuta metropolis in Ogun state Nigeria during the dry season and was taxonomically authenticated and documented with the Voucher number LUT/3525 at the Herbarium of Botany Department at the University of Lagos, Lagos, Nigeria. The root material was air dried and pulverized.

Chemicals

Gallic acid, α-Tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Germany), Folin-Ciocalteu, reagent, Na_2CO_3, aluminium chloride, potassium acetate, phosphate buffer, $K_3Fe(CN)_6$, trichloroacetic, acid (TCA), ferric chloride, HCl, Dragendorff's reagent, potassium persulphate were obtained from the chemical store of the Chemical Sciences Department of the Redeemer's University, Nigeria, while bismuth nitrate, hexane, ethyl acetate and methanol were obtained from the Chemistry Department of the University of Ilorin, Ilorin, Nigeria. Solvents were re-distilled before use.

Instruments

A Gas Chromatography-Mass Spectroscopy, GC-MS system, GCMS-QP 2010 PLUS (Shimadzu Japan) interfaced with a finigan MAT ion trap detector ion source Temperature, was used with the following settings; 200 °C, interfaced Temp., 250 °C, solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time – end time; 3.00 min – 46.00 min, event time, 0.50 sec; scan speed, 1428. Identification of the volatile component was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS, their spectral data were compared with those of NIST library mass spectra. The infra red spectrum was recorded on a Shimadzu (8400s) Fourier Transform-Infrared Spectroscopy (FT-IR) Spectrum spectrophotometer using KBr pellets; UV spectra were recorded using Shimadzu (1600s) Spectrophotometer.

Preparation of extract

The scheme for the extraction is shown in Figure 1. The pulverized plant material weighing (420 g) was extracted exhaustively with n-hexane at room temperature

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for five days. The extract was decanted, filtered and concentrated under reduced pressure using rotary evaporator to afford 344 mg of a yellow oil which was coded KPRH. The remaining plant material was subsequently extracted with ethyl acetate for five days. The ethyl acetate-extract was decanted, filtered using a Whatman No.1 filter paper and concentrated in vacuo to yield 1.55 g of a reddish-brown extract coded KPRE. Finally, the remaining extracted plant material was extracted again for five days with methanol. The methanol-extract was decanted, filtered and concentrated in vacuo to yield 20.50 g thick blackish syrup coded KPRM. The extracts were stored in a cool dark place until further analysis.

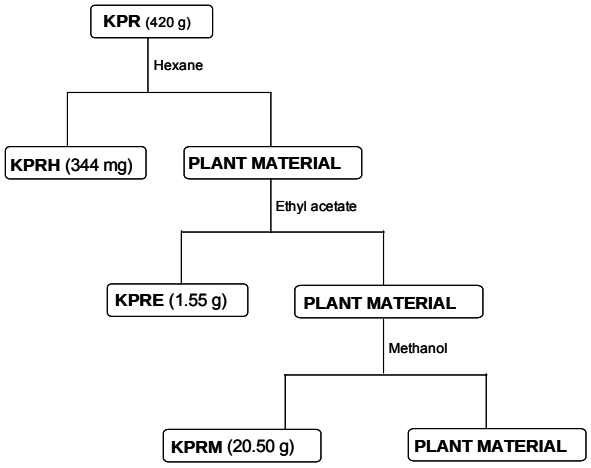

Figure 1: Extraction schematics

Phytochemical screening of the plant extracts

A small portion of the dry extract was used for the phytochemical screening for compounds including tannins, phlobatannins, flavonoids, terpenoids, alkaloids, cardiac glycosides, anthraquinone, saponins, and steroids in accordance with methods described by Harborne (1973), Trease and Evans (1989) and Sofowora (1993) with minor modifications.

Determination of total phenolic composition

The amount of phenolic compound in the root extracts of *Kigelia pinnata* was determined with Folin Ciocalteu reagent using the method of (Ebrahimzadeh et al., 2008a, b). To 0.5 mL of each sample (two replicates) of plant extract methanol solution (1 mg/mL) was added 2.5 mL of 10 % Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (2 % w/v). The resulting mixture was incubated at 50 °C for 30 minutes. The absorbance of the samples was measured at 765 nm using UV/visible spectrophotometer. Concentrations for the extracts were extrapolated from a calibration curve of gallic acid using the formula y = 0.646x. Results were expressed as milligrams of gallic acid equivalent/gram of powder dissolved in methanol.

Determination of reducing power

The reducing powers of the extracts were evaluated according to the method of Oyaizu (1986). The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1 % w/v) was added to 1.0 ml of the extract dissolved in distilled water. The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 ml of TCA (10 % w/v). The mixture was centrifuged at 3000 g for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of $FeCl_3$ (0.1 %, w/v). The absorbance was then measured at 700 nm against reference blank. Higher absorbance of the reaction mixture indicates higher reductive potential.

Estimation of antioxidant activity

The antioxidant acivity was measured using DPPH assay. This spectrophotometric assay uses the stable radical 1,1-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Amarowicz et al., 2004). The DPPH free radical is commercially available and it was prepared at a 0.1 mM concentration (25 mg/L) in methanol, following the procedure described by Sánchez-Moreno et al. (1998) and Larrauri et al. (1999). The radical was protected from light. The absorbance at 518 nm was monitored in presence of different concentrations of extracts. Blank experiment was also carried out to determine the absorbance of DPPH before interacting

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with the extract. Absorbance was recorded to check the stability of the radical throughout the time of analysis. The total antioxidant activity (TAAs) and relative antioxidant activity (RAA) were calculated using the following equations (Arnao et al., 1998).

$TAA = 100x[(Abs_{control}-Abs_{sample})]/(Abs_{control})$ and RAA = (TAA of test compound)/(TAA Standard compound)

Qualitative and quantitative analysis of Kigelia pinnata root-oil

The afore-mentioned GC/GCMS program was used. The compounds were identified on the basis of their retention times and mass-spectral fragmentation patterns compared with those of reference compounds stored on the spectrometer database and the NIST library. Quantification of identified constituents was performed by injecting 1 µl of the samples (on-column injector; hydrogen as carrier gas) and calculations from the electronic integration of the FID peak areas.

Statistical analysis

The group mean ± S.E.M. was calculated for each analyte and significant difference between means evaluated by analysis of variance (ANOVA). Post-hoc test analysis was done using the Duncan multiple comparison test. Values at $p < 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analysis conducted on the *K. pinnata* extracts revealed the presence of tannins, flavonoids, steroids phlobatannins, cardiac glycoside, terpenoids and saponins. The result is as shown in Table 1. These phytochemicals are known to support bioactive activities in medicinal plants and may therefore be responsible for the antioxidant activities of the plant extracts. Tannins are generally known to be useful in the treatment of inflamed or ulcerated tissues and have remarkable activity in cancer prevention (Ruch et al., 1989; Motar et al., 1985). Thus, the presence of these constituents in *Kigelia pinnata* partly supports the common traditional use of plant in the treatment of cancer. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the AT-Pase and phospholipase A2 (Li et al., 2003), and this property may possibly explain the mechanisms of antioxidative action of *K. pinnata* root extract. Flavonoids serve as health promoting compound as a results of its anion radicals (Havsteen, 1983). Alkaloid was conscupiously absent in the root of the study plant. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994).

Table 1: The phytochemical components of *Kigelia pinnata* based on the preliminary extract screening

Phytochemical compounds	KPRE	KPRM
Tannins	+++	+++
Flavonoids	++	++
Steroids	+	-
Alkaloids	-	-
Saponins	++	++
Alkaloid	-	-
Phlobatannin	+	-
Anthraquinone	-	+
Cardiac glycoside	++	++
Terpenpoids	+++	++

+++ = high amount; ++ = moderate amount;
+ = trace amount; - = Not detected

Kigelia pinnata root-oil composition

KPRH; UV (Hexane) λ_{max} (log ε) 426 (3.4), 419.5 (3.4), 348.5 (4.0), 305.5 (4.0), 250.5 (2.3) nm; IR υ_{max} 3429, 3007, 2955, 2854, 1743, 1710, 1465, 1379, 1166, 1100-721 cm^{-1}; In the GC-MS analysis, 19 bioactive phytochemicals were identified in the root oil of *Kigelia pinnata* as shown in Table 2. Elaidic acid, ($C_{18}H_{34}O_2$), Figure 2a, with RT 31.915 with peak area 56.12 % was the major compound identified in the oil.

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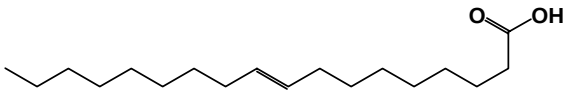
Table 2: *Kigelia pinnata* root-oil profile obtained from the GC/GCMS

Peak	Compounds	Molecular Formula	Retention Time (min)	% Yield	Peak Area	Base Peak	KRI
1.	Undecane	$C_{11}H_{24}$	11.145	0.24	422353	57	1115
2.	3,3-Dimethyl-hepta-4,5-dien-2-one	$C_9H_{14}O$	12.772	0.49	876643	95	NA
3.	Tridecane	$C_{13}H_{28}$	13.647	0.25	452824	57	1313
4.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	25.140	1.52	2712109	81	1907
5.	6-Methoxymellein	$C_{11}H_{12}O_4$	27.919	0.46	820185	208	1863
6.	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	29.540	0.48	853696	74	1878
7.	Palmitic acid	$C_{16}H_{32}O_2$	30.067	18.02	32178578	43	1968
8.	Lapachol	$C_{15}H_{14}O_3$	30.434	1.67	2979619	27	2093
9.	Margaric acid	$C_{17}H_{34}O_2$	31.045	0.84	1503572	43	2067
10.	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	31.399	0.52	937173	55	2085
11.	Elaidic acid	$C_{18}H_{34}O_2$	31.915	56.12	100175674	55	2175
12.	Stearic acid	$C_{18}H_{36}O_2$	32.105	12.80	22851575	43	2167
13.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	33.150	0.44	782647	57	4395
14.	6(Z) -Octadecenoic acid	$C_{18}H_{34}O_2$	33.712	1.00	1785306	67	2175
15.	Stearic acid, butyl ester	$C_{22}H_{44}O_2$	34.009	0.40	717835	56	2375
16.	cis-9-Hexadecenal	$C_{16}H_{30}O$	34.631	0.91	1626121	55	1808
17.	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	35.160	0.37	653579	57	2832
18.	Heneicosane	$C_{21}H_{44}$	36.675	0.52	930579	57	2109
19.	Squalene	$C_{30}H_{50}$	37.848	2.95	5273329	69	2914

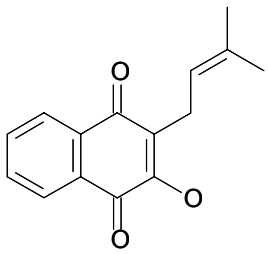
KI: Kovats indices, NA: Not Available

The biological importance and toxicity of elaidic acid, a trans fatty acid have remained controversial. Some were of the opinion that trans fatty acids increased fragility of red blood cells, changed the aggregation of thrombocytes (Ascherio et al., 1994, 1999; Ascherio, 2002) and evidenced their negative effects on the metabolism of linolenic acid and arachidonic acid (Larque et al., 2000). It was established that they caused lack of essential fatty acids (Kummerow et al., 2004), inhibited synthesis of prostaglandin (Kushi and Giovannucci, 2002) and increased the risk of certain cancers. An increased risk of breast cancer has been associated with increasing levels of the *trans*-monounsaturated fatty acids palmitoleic acid and elaidic acid (Chajès et al., 2008). Lately, it has been reported that incorporation of trans fatty acids into the phospholipids of the membranes affected its properties and mainly the activity of enzymes attached to the membrane, in fact, in recent times a positive relation has been established between allergic diseases and trans fatty acid consumption (Kritchevsky, 1997; Stender and Dyerberg, 2004). Elaidic acid has also been shown to have both 5α-reductase inhibitory activity and hair regrowth stimulation effects (Kuniyoshi et al., 2000).

In view of the various reports on the toxicity of elaidic acid, the therapeutic effect of the plant should be weighed along side its toxicity when administered in folk medicine. Special attention may have to be paid to the extraction method.



a. Elaidic acid



b. Lapachol

Figure 2: Structure of Elaidic acid and Lapachol

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Other notable compounds that are present include palmitic acid (18.02 %), stearic acid (12.08 %), squalene (2.95 %) and lapachol, Figure 2b, (1.67 %), (R)-(-)-14-methyl-8-hexadecyn-1-ol (1.52 %) and 6(Z) -octadecenoic acid (1 %). Lapachol has been reportedly isolated from the root of the plant previously (Govindachari et al., 1971). Lapachol and derivatives as constituents of plant extracts are well documented for anti-inflammatory, antimicrobial, and antineoplasic activities (Miranda et al., 2001). The aqueous and methanol extracts of *T. avellanedae* for instance also showed antifungal, antinociceptive and antiedematogenic activities (Miranda et al., 2001). Species that contain lapachol and several biogenetically related naphthoquinones (e.g., *tahaebo, pau d'arco and lapacho roxo*) are widely used in American folk medicine for the treatment of cancer, lupus, infections, wounds, and many other diseases (Sacau et al., 2003). Other activities of lapachol and its derivatives include the prevention against *Schistosoma mansoni* cercarial skin penetration, *Biomphalaria glabrata* infection as well as forestalling embryo alteration in rats (Maganha et al., 2006). It is also reported for its antioxidant activities (Wenceslau et al., 2006), cytotoxicity in human Promyelocytic Leukemia HL-60 cell line (Perez-Sacau et al., 2007), analgesic and antipsoriatic activities (Felicioa et al., 2002).

Total phenolic content and reducing power potentials of the extract

The total phenolic content was also found to be higher in KPRE at 0.5 mg/mL when compared to that of KPRM (Figure 3). It is possible that the extraction solvent contributed to the difference observed in the total phenolic contents for KPRE and KPRM. Figure 4 shows the reducing power potentials of the extracts in comparison with a standard, gallic acid at 700 nm. The reducing capacity of the extracts, another significant indicator of antioxidant activity was also found to be appreciable. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex was then monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability. The results show that there was increase in the reductive capability of KPRH which peaked at around 0.4 mg/ml. This activity may be connected with the antioxidant capability of the extract.

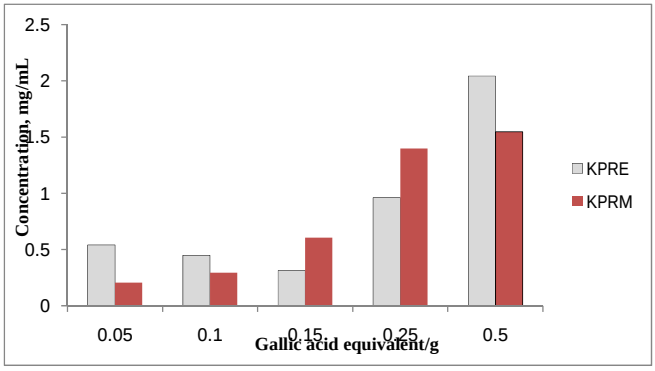

Figure 3: Phenolic content of KPRE and KPRM as gallic acid equivalent/g of powder

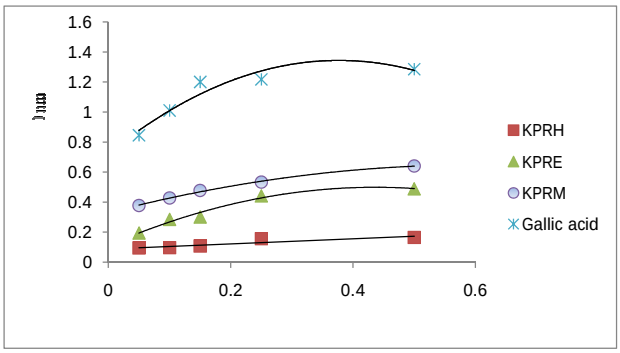

Figure 4: Reducing power activities of the extracts of *Kigelia pinnata* in comparison with a standard (Gallic acid) at λ, 700 nm

Free radical scavenging activities total and relative antioxidant activities

The *in vitro* antioxidant activities of the plant extracts (Figure 5) suggest synergistic antioxidant potentials though significantly lower compared to the standard, α-tocopherol. Generally, KPRE showed a higher antioxidant value and the TAA peaked at 0.25 mg/mL as the activity declined toward 0.5 mg/mL and further decline at higher concentration. KPRH showed an increase in TAA as concentration increases, which shows that it is dose dependent. KPRM

showed the peak activity at 0.2 mg/mL. The trend line shows a decline at increase concentration in the RAA (Figure 6) except for KPRH. This implies that lower concentration of the KPRE and KPRM extracts may be required for effective antioxidant activities. The dose dependent trend observed for the KPRH could be as a result of synergistic free radical scavenging ability of compounds in the oil which apparently dampens the effect of the erucic acid.

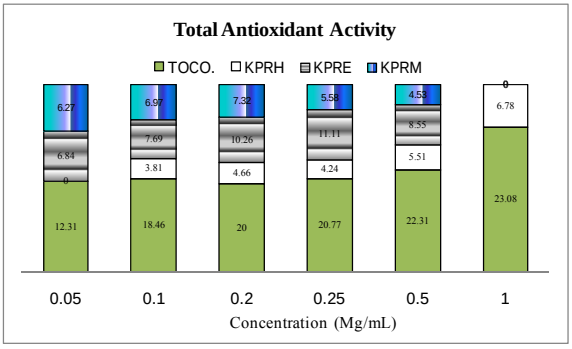

Figure 5: Total antioxidant activities of KPRH, KPRE, KPRM and α-Tocopherol

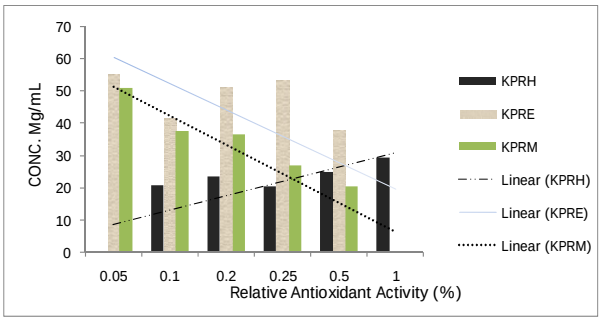

Figure 6: Relative antioxidant activities of KPRH, KPRE and KPRM

CONCLUSIONS

Kigelia pinnata root is rich in phytochemicals with proven antioxidant activities. The phytochemical analysis conducted on *Kigelia pinnata* extracts revealed the presence of tannins, flavonoids, steroids, phlobatannins, phenolics, anthraquinones, terpenoids and saponins. This study indicates that the ethyl acetate fraction of the plant root has high antioxidant activity against DPPH than the hexane and methanol extract. It is due to the presence of high content of phenolics, which could be the most effective in protecting the body against various oxidative stressors. The structures of phenolic compounds which exhibited antioxidant activity in the ethyl acetate fraction would need to be elucidated. Though the plant is highly regarded for its unique medicinal potencies due to the presence of lapachol, squalene, naphthoquinones etc, the content of elaidic acid content in the oil calls for caution in the use of the plant in traditional medical therapy.

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