Protective effect of the fruit of *Dialium guineense* Willd (Fabaceae) against oxidative stress

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ABSTRACT

Background: Plants with adequate antioxidant potential can be useful in combating cellular damage and diseases.

Objectives: The aim of the study is to determine the antioxidant capacity of the different parts (fruit coat, pulp and seed) of *Dialium guineenses* (Willd) *(Fabaceae)* fruit.

Methods: The extracts were screened for scavenging activity against 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, nitric oxide radicals, reducing power and metal chelating capacity. Protective effect of the seed extract was evaluated in ethanol-induced oxidative stress in rats at the dose of 50, 100 and 150 mg/kg and the levels of antioxidant markers; Lipid peroxidation (MDA) and catalase (CAT) were assayed using standard methods. The total polyphenolic content and GC-MS analysis of the seed extract were also done.

Results: The seed extract exhibited significant and higher antioxidant activity than the fruit coat and pulp extracts in three of the in vitro assays. The extract dose-dependently reduced lipid peroxidation and increased the activity of catalase at all the doses tested compared with the control. The total phenolics, flavonoids and proanthocyanidin content of the seed extract were 32.71 mg gallic acid equivalent/g, 2.41 mg quercetin equivalent /g and 130.21 mg catechin equivalent /g of the extract respectively. The major component in the seed oil was 10,13-Octadecadienoic acid methyl ester (52.38%) following GC-MS analysis. Our findings suggest that *D. guineense* seed showed high antioxidant potential with considerable content of total proanthocy anidins.

Conclusion: The seeds of *D. guineense w*hich are generally discarded as wastes may serve as viable functional food ingredients with protective antioxidant potential.

Key words: *Dialium guineense;* Antioxidant activity, Oxidative stress; Polyphenolic content; GC-MS analysis

Effet protecteur du fruit de *Dialium guineense* Willd (Fabaceae) contre le stress oxydatif

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RESUME

Contexte: Les plantes ayant un potentiel antioxydant adéquat peuvent être utiles pour combattre les dommages et les maladies cellulaires.

Objectifs: L'objectif de l'étude est de déterminer la capacité antioxydante des différentes parties (couche de fruits, pulpe et graines) du fruit *Dialium guineense* (Willd) (Fabaceae).

Méthodes: Les extraits ont été sélectionnés pour leur activité de piégeage contre le 1,1-diphényl-2-picrylhydrazyle (DPPH), les radicaux oxyde nitrique et hydroxyles, leur pouvoir réducteur et leur capacité de chélation des métaux. L'effet protecteur de l'extrait de graine a été évalué dans l'effort oxydant induit par l'éthanol chez les rats à la dose de 50, 100 et 150 mg/kg et les niveaux des marqueurs antioxydants; La peroxydation lipidique (MDA) et la catalase (CAT) ont été dosées à l'aide de méthodes standard. La teneur polyphénolique totale et l'analyse GC-MS de l'extrait de graine ont également été faites.

Résultats: L'extrait de graine a présenté une activité antioxydante significative et supérieure à celle des extraits de coucheet de pulpe de fruit dans trois des essais in vitro. L'extrait a réduit la peroxydation des lipides de manière liée à la dose et augmenté l'activité de la catalase à toutes les doses testées par rapport au contrôle. La teneur totale en phénoliques, en flavonoïdes et en proanthocyanidines de l'extrait de graine était respectivement de 32,71 mg d'équivalent acide gallique/g, de 2,41 mg d'équivalent quercétine/g et de 130,21 mg d'équivalent catéchine/g de l'extrait. La principale composante de l'huile de graine était l'ester méthylique d'acide 10,13-octadécadiénoïque (52,38%) suite à l'analyse GC-MS. Nos résultats suggèrent que le grain *D. guineense* a montré des potentiels antioxydants élevés avec une teneur considérable en proanthocyanidines totales.

Conclusion: Les graines de *D. guineense* qui sont généralement rejetées en tant que déchets peuvent servir d'ingrédients alimentaires fonctionnels viables avec un potentiel antioxydant protecteur.

Mots-clé: *Dialium guineense;* activité antioxydante, stress oxydatif, contenu polyphénolique; Analyse par GC-MS

INTRODUCTION

In the human system, there are antioxidant molecules capable of reducing the effects of reactive oxygen species accomplished by the endogenous enzymatic defense system such as superoxide dismutase, catalase, glutathione peroxidase and glutathione dismutase.[1] Fruits and vegetables have been identified as sources of antioxidants with positive effects against a number of diseases including ulcers.[2] Different parts of plants are known to contain antioxidants in varying degrees and these antioxidants have been reported to be phenolic in nature.

Dialium guineense (Willd) (Fabaceae) is commonly known as black velvet or velvet tamarind. The plant is valued for its fruits; the pulp of the fruit being licked for its sweetness.[3] The fruit, leaves and stembark of the plant are used as remedies for gastrointestinal ailments such as diarrhoea, stomach ache and ulcer.[4] The nutrient composition of the fruits has been extensively studied.[3,5-9] The fruit coat has been reported to possess wound healing and antimicrobial property[9] while the anti-hepatotoxic activity of the pulp was reported by Adeleye et al.[10] The present study investigates the *in vitro* antioxidant property of the different parts (fruit coat, pulp and seeds) of the fruits of *D. guineense* and determines the cyto protective effect of the seed extract on oxidative stress induced by ethanol in rat.

METHODS

Plant materials and preparation of extracts

The fruits of *D. guineense* were bought in May, 2017 from Yaba market (6˚ 29' 23" North, 3˚ 23 1"), Lagos State, Nigeria. The fruits were identified and authenticated at the Herbarium unit of the Department of Botany, University of Lagos and assigned voucher number 7730.

The fruits were sorted to remove all unwanted material sand separated into the different parts (fruit coat, seed and pulp). The different parts (100 g each) were pulverized using Christy 8" lab mill (serial no. 50158) and defatted with hexane using Soxhlet extractor. Subsequently, the marc was extracted with absolute ethanol for three hours. The ethanolic extracts from the different parts of the fruits were screened for free radical scavenging activities using various methods.

Determination of *in-vitro* antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated as described by.[11] 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (0.135Mm,1mL) was added to five different concentrations (20-100 $\mu g mL^{-1}$) of the extracts. The reaction mixture was thoroughly agitated and incubated at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Quercetin and α- tocopherol were used as references. The percentage of the DPPH radical scavenging was calculated.

Hydroxyl radical scavenging activity

The scavenging ability of hydroxyl radicals was measured by the method of Kunchandy and Rao.[12] The reaction mixture (1.0 mL) consisting of 100 µL of 2-deoxy- D-ribose (28mM in 20mM KH_2PO_4-KOH buffer, pH 7.4), 500 µL of the extract, 200 µL EDTA (1.04 mM) and 200 µM $FeCl_3$ (1:1 v/v), 100 µl of H_2O_2 (1.0 mM) and 100 µL ascorbic acid (1.0mM) which is incubated at 37°C for 1 h. 1.0 mL of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) were added and incubated at 100 °C for 20 min. After cooling, absorbance was measured at 532nm against a blank sample and was converted into percentage inhibition of deoxyribose degradation.

Nitric oxide (NO) scavenging assay

The effect of the extracts on NO radical inhibition was determined using Griess reagent.[13] A 2 mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of sample at various concentrations (20-100 $\mu g\ mL^{-1}$). The mixture was then incubated at 25 °C for 150 min. After incubation, 0.5 mL of the incubated solution was withdrawn and mixed with 1.0 mL sulfanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) at room temperature. The mixture was left to stand for 10 min for complete diazotization. Then 1 mL of naphthylethylenediamine dichloride (0.1% w/v) was added, mixed and allowed to stand for 30 min producing a pink coloured chromophore. Its absorbance was measured at 546 nm against the corresponding blank solution. Quercetin and α-tocopherol were used as standards.

Reducing power method

The assay was conducted according to the method described by Ordonez et al.[14] Phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and of $K_3Fe(CN)_6$ (1% w/v, 2.5 mL) were added to 1.0 mL of different concentrations (20-100 $\mu g\ mL^{-1}$) of the extracts. The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5

mL) and $FeCl_3$ (0.5 mL, 0.1%, w/v). The absorbance was then measured at 700 nm in a double beam spectrophotometer against blank sample. The reducing power of the extracts was compared with that of quercetin and α- tocopherol. Increase in absorbance of the reaction mixture indicates the reducing power of the sample.

Fe^{2+} chelating activity

The chelating activity of the extracts for Fe^{2+} ion was evaluated using the method of Dinis et al.[15] The extract (0.5 mL) was added to a solution of 0.5 mL ferrous chloride (2 mM) and 1.6 mL of deionized water. After 30 s, the reaction was started by the addition of 0.2 mL of ferrozine (5 mM) and incubated at room temperature for 10 min. After incubation, the absorbance of the Fe^{2+}-ferrozine complex formed was measured at 562 nm. EDTA was used as a positive control. The chelating activity of the extract for Fe^{2+} was calculated.

Experimental animals

Male Albino Wistar rats (150-200 g) were obtained from the Laboratory Animal Centre of the National Agency for Food and Drugs Administration and Control, Yaba, Lagos, Nigeria. Experimental animals were kept in well-ventilated, hygienic compartments maintained under standard environmental conditions (23-25ºC, 12 h/12 h light/dark cycle) and were acclimatized for two weeks before the start of experiments. The animals were fed with standard rodent diet (Livestock Feeds PLC, Lagos, Nigeria) and water ad libitum. The study was conducted following an approved animal use protocol from the Research Ethics Committee of the College of Medicine, University of Lagos, Lagos, Nigeria. (Ref no: CMUL/HREC/04/18/348).

Acute toxicity study

Acute toxicity evaluation was performed according to the Organization for Economic Cooperation and Development (OECD) guideline no. 423[16] using up to 2000 $mgkg^{-1}$ dose (p.o.) of the seed extract.

Ethanol-induced gastric ulcer in rats

In the *in vitro* assay, the seed extract had higher antioxidant potential and was therefore subjected to cytoprotective assay in rats. Thirty five (35) albino rats were randomly divided into five groups of five animals each. Group1 served as the control and received 1ml of distilled water. Group 2, 3 and 4 received 50, 100 and 150 $mgkg^{-1}$ of the *D. guineense* seed extract respectively, while group 5 received 100 μgkg^{-1} of misoprostol. After one-hour, absolute ethanol (1 mL) was administered orally to induce ulcer. The rats were sacrificed by cervical dislocation, one hour later. The stomachs were then removed, opened along the greater curvature and ulcer scores were determined.[17]

Measurement of antioxidant markers

Stomach homogenate (10%) was prepared in phosphate buffer (100 mM, pH 7.4) and used for the determination of antioxidant enzyme activities. Catalase (CAT)[18] and lipid peroxidation (MDA)[19] activities were determined using standard procedures.

Determination of polyphenolic content of the seed extract

The total phenol content in the active seed extract was determined by the modified Folin-Ciocalteu method. [11] The concentration of total phenolic compounds was expressed as milligram of gallic acid equivalent (GAE) per gram of dried extract using a standard calibration curve of gallic acid described by the equation: $y = 23.77x - 0.0174$, $R^2 = 0.9952$. Total flavonoids were estimated using the method of Ordonez et al[14] and calculated as quercetin equivalents (mg/g) based on the calibration curve: $y = 15.78x + 0.1767$, $R^2 = 0.9919$. Determination of proanthocyanidins was done as described by Sun et al [20] and the content expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: $y=1.7433x-0.005$, $R^2=0.9887$.

Preparation of fatty acid methyl esters (FAMEs) from the seed and GC-MS analysis

Seed oil (200 mg) was dissolved in 100 mL methanol, 10 mL benzene and 1 mL concentrated sulphuric acid. The mixture was heated under reflux in boiling water bath for 6 hours and then evaporated. Water (100 mL) was added to the residue.[21] The mixture was extracted three times with diethyl ether (3 x 75 mL). The combined ether extracts were washed with water, dried using anhydrous sodium sulphate and filtered, filtrate was evaporated. The produced fatty acid methyl esters were analyzed by GC-MS.

The GC-MS was done using a capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) on Agilent 6890N GC system furnished with an auto sampler (Agilent 7683 injector series) and coupled to a 5973 Network mass selective detector (GC-MS) (based on a quadruple mass separator). The carrier gas was helium at a constant flow rate of 1 mL min^{-1}. The oven temperature range was set at

70 °C and ramped at 4 °C/min to 250 °C. The injector temperature was set at 250 °C and detector temperature 280 °C. Mass spectra were obtained at 70 eV with a mass range of m/z 40–500. Retention indices were compared with the databank of the instrument, NIST 2005 library and the literature.[21]

Statistical analysis

Values are reported as *Mean*± SEM. Statistical analysis was done using One-way ANOVA followed by Dunnet's multiple comparison tests using GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Statistical significance was considered at $p < 0.05$.

RESULTS

***In- vitro* antioxidant assays**

Radical scavenging activity

The seed extract presented the highest scavenging activity against DPPH and hydroxyl radicals followed by the seed coat and pulp (Figure 1 A and B). The radical inhibition of the seed in DPPH assay at the concentration of 20, 40, 60, 80 and 100 µgmL^{-1} was 51.95%, 68.85%, 85.75%, 86.91% and 85.42% respectively. In the hydroxyl radical assay, the activity of the seed extract was higher than that of tocopherol but comparable to quercetin at all the concentrations tested. All the extracts showed moderate inhibition of nitric oxide radical (Figure 1C). The percentage inhibition of the fruit coat, pulp and seed at 100 µg mL^{-1} was 59.53%, 48.04% and 62.12%, respectively.

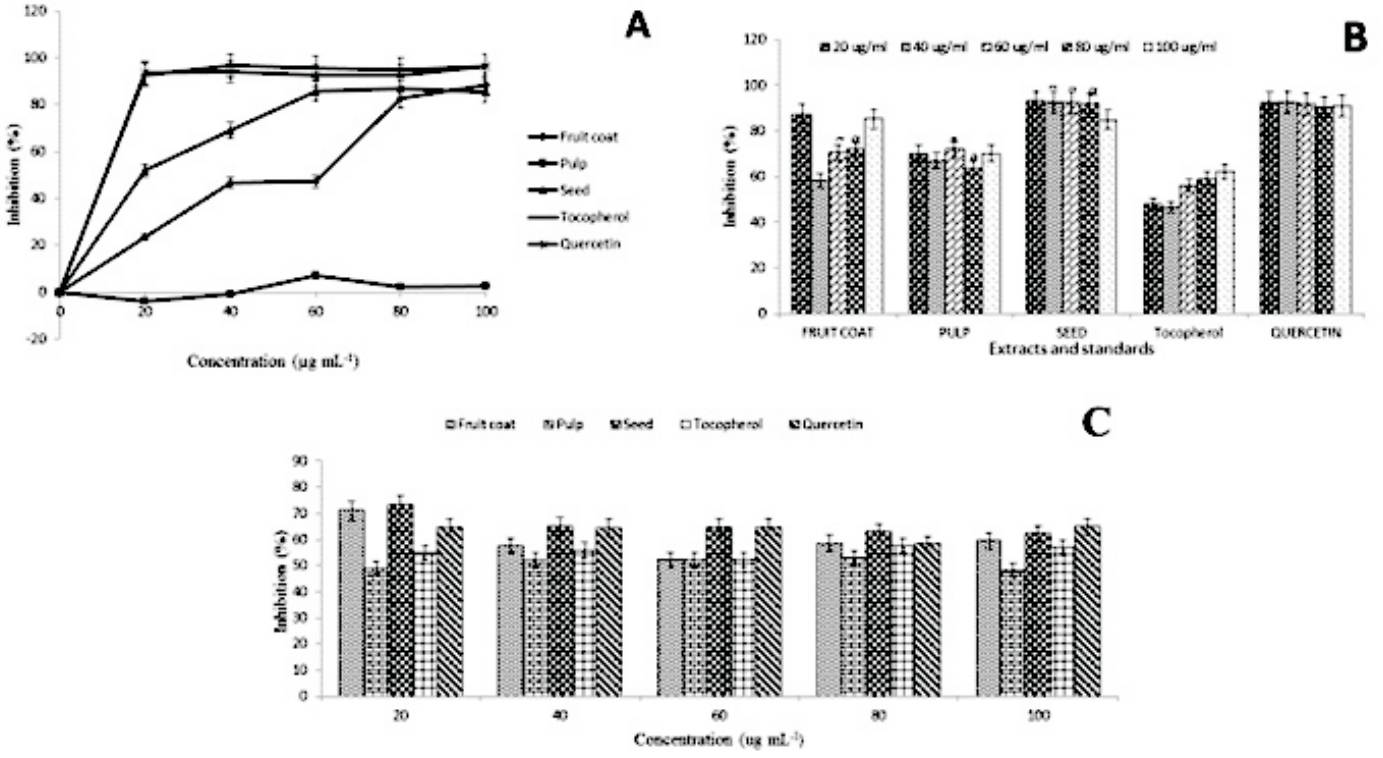

Figure 1. Radical scavenging activity of fruit coat, seed and pulp extracts of *Dialium guineense.*
A-DPPH radical scavenging, B-hydroxyl radical scavenging, C-Nitric oxide scavenging.
Values are means of triplicate determinations.

Reducing power

The reducing capacity of the extracts was significantly lower ($p< 0.05$) than that of quercetin (Figure 2). Among the extracts, the seed extract was more effective in reducing Fe^{3+} to Fe^{2+}.

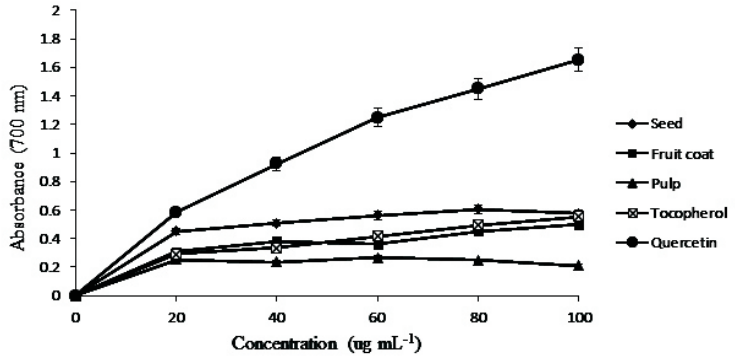

Figure 2. Reducing power of fruit coat, seed and pulp extracts of *Dialium guineense.* Values are means of triplicate determinations.

Fe^{2+} chelating activity

The chelating property of the extracts is shown in Figure 3. The activity of the extracts was low compared to EDTA. Amongst the extracts, the pulp displayed the highest chelating activity at the concentration range of 20-80 ug mL^{-1}. However, all the extracts showed negative effect at the highest concentration (100 ugmL^{-1}).

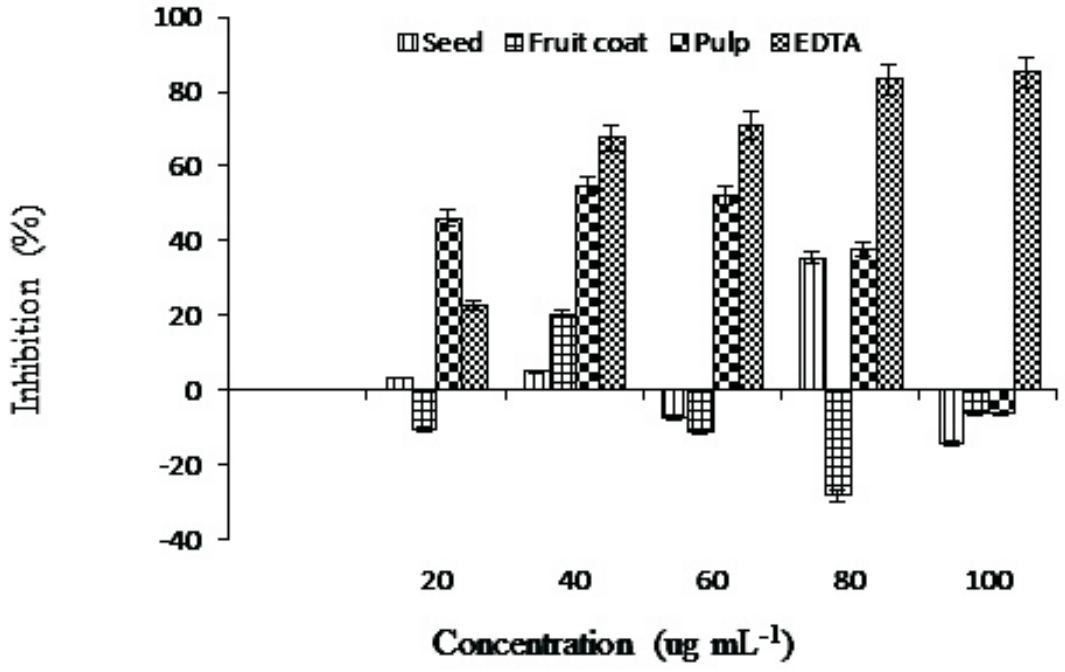

Figure 3. Fe^{2+} chelating effect of fruit coat, seed and pulp extracts of *Dialium guineense* in comparison with EDTA. Values are means of triplicate determinations.

Acute toxicity

Oral administration of the seed extract did not show any mortality of the animals up to the dose of 2000 mg kg^{-1} compared to control.

Effect of Dialiumguineense seed extract on ethanol-induced gastric ulcer

Administration of *D. guineense seed extract* produced a dose dependent protection against ethanol-induced gastric ulcers in the animals. A significant protection of 79.33% was recorded at the dose of 150 $mgkg^{-1}$ compared to control (Table 1)

Table 1: Effect of *Dialium guineense* seed extract on ethanol -induced gastric ulcer

Results are express as mean ± SEM, n=5.[a]*p*< 0.05 *vs* Control (One-way ANOVA followed by Dunnet's

Treatment	Dose(mg kg^{-1})	Mean ulcer score±SEM	Ulcer index	% Protection
Control (distilled water)	1 ml kg^{-1}	8.9±0.71	8.9	-
Misoprostol	0.1	2.8±1.38	2.8	68.54
Dialium guineense	50	5.3±0.73	5.3	40.45
	100	5.1±0.74	5.1	42.70
	150	1.8±0.72	1.8	79.33[a]

Results are express as mean ± SEM, n=5.[a]p< 0.05 vs Control
(One-way ANOVA followed by Dunnet's multiple comparison test).

Effect of *Dialium guineense* seed extract on antioxidant markers

The effect of the extract on antioxidant status in the stomach tissue of rats is reported in Table 2. The extract significantly reduced the level of TBARS in the stomach tissue of rats at the dose of 50, 100 and 150 mg kg^{-1} and increased the activity of catalase compared with the control.

Table 2: Effect of *Dialium guineense* seed extract on antioxidant markers in ethanol-induced ulcer

Treatment	Dose (mg kg^{-1})	MDA (U mol g^{-1} mg^{-1}protein)	CAT (U mg^{-1} protein)
Control	1 ml kg^{-1}	2.05 ± 0.83	1.54± 0.76
Misoprostol	0.1	0.61± 0.20	7.24± 1.82
D. guineense	50	0.25 ±0.13[a]	3.03 ± 0.44[a]
	100	0.33 ±0.04[a]	2.18 ± 0.57[a]
	150	0.42 ±0.14[a]	3.67 ± 1.05[a]

Results are expressed as mean± SEM, n= 5.[a]*p*< 0.05 *vs* control (One-way ANOVA followed by Tukey's multiple comparison).

Polyphenolic content of the seed extract of *Dialium guineense*

The total phenolics, flavonoids and proanthocyanidin content in the ethanolic seed extract *of D. guineense* were 32.71 mg gallic acid equivalent/g, 2.41 mg quercetin equivalent /g and 130.21 mg *catechin equivalent /g of the extract respectively.*

GC-MS analysis of the seed oil of the *Dialium guineense*

The seed oil of *D. guineense* contains predominantly unsaturated fatty acids. The most abundant unsaturated fatty acid was 10,13-Octadecadienoic acid methyl ester (52.38%). The other fatty acids present were Hexadecanoic acid methyl ester (19.16%), 9-Octadecenoic acid methyl ester (14.48%), 9,12-Octadecadienoic acid, methyl ester (6.39) and methyl stearate (3.44) (Table 3)

Table 3: GC-MS identification of methyl esters of fatty acids from the seed oil of *D. guineense*

S/no	RT (min)	Name of the compound (FAMEs)	Molecular formular	M.wt (g/mol)	Peak area %
1.	3.54	3-Buten-1-ol, 3-methyl-ester	$C_5H_{10}O$	86	3.76
2.	14.67	2,5-Cyclohexadiene-1,4-dione, 2,6- bis(1,1-dimethylethyl)-	$C_{14}H_{20}O_2$	220	0.11
3.	21.94	Methyl hexadec-11-enoate	$C_{17}H_{32}O_2$	268	0.27
4.	22.97	Hexadecanoic acid methyl ester	$C_{16}H_{32}O_2$	270	19.16
5.	28.37	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	1.11
6.	28.53	9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	1.31
7.	28.77	9,12-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	3.97
8.	28.88	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	2.88
9.	29.04	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	5.01
10.	29.14	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	4.10
11.	29.16	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	1.07
12.	29.19	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	1.17
13.	9.24	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	2.67
14.	29.34	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	5.28
15.	29.43	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	5.44
16.	29.56	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	8.34
17.	29.67	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	7.32
18.	29.76	10,13-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	9.10
19.	29.97	9-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	9.06
20.	30.00	9-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	3.91
21.	30.07	9-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	1.51
22.	30.59	Methyl stearate	$C_{19}H_{38}O_2$	298	3.44

DISCUSSION

Plants with adequate antioxidant potential can be useful in combating cellular damage and diseases. The antioxidant capacity of the different parts (fruit coat, pulp and seed) of *D. guineense* fruit was evaluated with five different *in vitro* assays including DPPH, hydroxyl, nitric oxide scavenging activities, reducing power and iron chelating capacity.

Proton radical scavenging capacity is considered as one of the mechanisms for antioxidation. The DPPH scavenging activity depends on the electron donation ability of natural products which reacts with the DPPH free radical.[22] In this assay, the seed demonstrated higher antiradical activity than the other extracts which suggests that the seeds extract of the fruit of *D. guineense* is nucleophilic thus readily donate electrons.

Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell.[22] The activity of the seed was higher than the other extracts and compared well with quercetin. This may suggest that the seed extract is an excellent hydroxyl radical scavenger.

Nitric oxide scavenging assay relies on the diazotization reaction which uses naphthylethylene diaminedihy drochloride under acidic conditions to compete for nitrite in Griess reagent.[23] Moderate scavenging activities against nitric oxide free radical were recorded for all the extracts.

In reducing power assay, the antioxidant potential of a compound is related to its ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion through the donation of an electron.[24] The reducing power of the extracts was lower than that of quercetin, However, among the extracts, the activity followed the order, seed > fruit coat > pulp. This simply implies that the seed extract has the potential to neutralize free radicals through the donation of electrons.

Metal ion chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid peroxidation. Thus, when complexes are formed

between the extract and ferrous (Fe^{2+}), the concentration of free ferrous is decreased, which invariably decreased ferrous induced lipid peroxidation.[25] Among the extracts, the pulp exhibited the highest metal chelating activity. However, its effect was low compared to the standard EDTA. The results suggest the pulp extract has ferrous chelating ability. The inhibition of lipid peroxidation may also be involved in the antioxidant property of the pulp extract.

Considering the result of the *in vitro* antioxidant assays, the seed extract demonstrated the highest activity; hence it was assessed for its inhibitory effect on lipid peroxidation and antioxidant status in ethanol-induced oxidative stress in rats. Reactive oxygen species have been identified to cause gastric ulcer development and the free radical build up can ultimately cause depletion in the tissue antioxidant status leading to lipid peroxidation. Superoxide dismutase, glutathione peroxidase, and catalase are the enzymes involved in protecting cells from the damaging effects of reactive oxygen species generated during oxidative stress.[26]

Dialium guineenseseed extract significantly and, in a dose-dependent manner reduced lipid peroxidation. Additionally, the extract increased the activity of catalase at the dose of 50, 100 and 150 mg/kg. The results suggest that the extract could positively regulate cellular antioxidative activities thereby preventing mucosa damage in rats.

The polyphenolic contents are reported to be responsible for overall antioxidant activity and several studies have shown a good correlation between the phenols and antioxidant activity.[27] The seed extract displayed considerable amount of phenolic, flavonoids and proanthocyanidin contents. These bioactive compounds could have impact on the high antioxidant activity observed for the seed extract, in this study. Many authors have recorded considerable levels of polyphenolic contents of non-edible portions of fruits with well correlated antioxidant activities.[28, 29]

Moreover, the seed oil contains majorly unsaturated fatty acids and, therefore, has potential nutritional value.

Further studies on the isolation and characterization of the active phyto constituents as well as determination of the precise mechanism(s) of action exhibited by *Dialium guineense* seed extract should be carried out.

CONCLUSION

The study showed that the seed extract of D. guineense exhibits the highest in vitro antioxidant activity compared to the other parts of the fruit. The seed extract also showed notable inhibition of lipid peroxidation and increase glutathione and catalase levels in ethanol-induced oxidative stress in rats. The seed and seed oil also contain considerable amount of polyphenolic contents and unsaturated fatty acids, respectively. Thus, the seeds which are generally discarded as waste may serve as viable functional food ingredients with protective antioxidant potential against diseases that are mediated by oxidative stress.

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