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Phytochemical, Pharmacognostic, Anti-Radical, Anti-Arthritic and Antibacterial Potential of the Leaf Extract of *Dialium guineense*

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Abstract: This research evaluates the extract of the leaf of *Dialium guineense* for its phytochemical and pharmacognostic potential. GC-MS, UV-Visible Spectrophotometer and other established biochemical methods assays were used for the study. GC-MS analysis showed the presence of 37 phytochemicals. The main component was: 4-O-methylmannose (49.0%). The β-carotene, lycopene, total carotenoid, chlorophyll a, b, total chlorophyll, TPC, TFC, TTC, TAA values were 0.20 mgg^{-1}, 0.10 mgg^{-1}, 12.68 mgg^{-1}, 4.40 mgg^{-1}, 4.70 mgg^{-1}, 7.80 mgg^{-1}, 2,668.17±0.01 μgmg^{-1}, 17.80±0.00 μgmg^{-1}, 152.65±0.01 μgmg^{-1}, 43.75±0.04, μgmg^{-1}, respectively while Galvinoxyl and DPPH antioxidant IC_{50} were 125.0 and 6.0 μgmL^{-1}, respectively. The TAC value was 279.49±0.01 μgmg^{-1}. The highest anti-arthritic value was 96.00%. The extract inhibited the growth of all the screener bacteria. These results showed that the leafcan be used in the treatment of various human and animal's related aliment or diseases and more importantly in formulation food additives and preservative.

INTRODUCTION

Africa nations are naturally blessed with enormous biodiversity of edible plants which are used in consumption as food supplements and therapeutic purposes. Globally, medicinal plants including vegetables, herbs and trees; have been serve as a major and natural sources for human being to meet their daily health care and nutritional needs[1-5].Natural products especially plants represent an inexhaustible reservoir of novel molecules for new drug discovery. In many parts of rural African rural community, medicinal plants are the most easily accessible and affordable health resource available to the local community and at times the only therapy that subsists. Nonetheless, there is still a paucity of updated comprehensive compilation of promising medicinal plants from the African continent[3, 6-7]. Medicinal plants and vegetables grown in Africa are used for therapeutic purposes and as precursors for chemo pharmaceutical semi-synthesis[9-12]. The phytochemicalspresentin the plant with pharmacologicalpotentials could serve as lead sources for development of novel drugs for human and

animal uses. There has been tremendous increasein the interest of scientists in the field of pharmacognostic, food chemistry, pharmacy etc in tracking the health benefits of vegetables and other medicinal plants for their useful secondary metabolites and treatment of various diseases[3, 4, 14]. Green vegetables contain varietiesof metabolitesthat may act singly or synergistically to improve health of man and animal[3, 15, 6, 16]. Many plants contain diverse of polyphenols that are excellent antioxidants to scavenge free radicals and inhibit lipid peroxidation[18, 19, 6, 20]. Nowadays, many people prefer uses of natural antioxidants due to low resistance to high temperatures and the side effects such as carcinogenicity of some of the synthetic compounds[21-23].

Dialium guineense (Velvet tamarind)is an evergreen fruit bearing tree in the family of *Fabaceae*. It belongs to the subfamily of *Dialioideae*[24-25]. The plant is rich in micronutrients. It is a potential food supplement, rich sources of secondary metabolites used in traditional medicine. It has sweet edible pulp can be eaten raw or can be soaked in clean water for the production of fresh local juices[24-31].The plant exhibits natural pharmacological response against infectious and non-infectious diseases. It is used in the treatment of diseases such as heart disease, diarrhea, severe cough, bronchitis, stomach aches, malaria, fever, jaundice, hypertension and haemorrhoids, tooth decay and dental plaque[32]. It has also been observed to possess some analgesic, wound healing, antioxidant, antiulcer and anti-vibrio properties[31-35].

To the best of our knowledge, the phytochemistry, pharmacognostic and pharmacognostical activities of the leaf of this plant grown in Nigeria have not been extensively investigated despite the traditional uses of the green herb. Therefore, the present studywas undertaken with the aim of looking into the phytochemical, carotenoid,chlorophyll, ascorbic acid, phenolic, flavonoid, tannin, contents, antioxidant, anti-arthritic and antibacterial potential of the leaf of the plant investigated.

Practical applications: This study revealed that the leaves of *D. guineense* have abundant bioactive secondary metabolites that may be applied in the formulation food or drug supplement and preservative. With its high medicinal potential and it could be alternative sources of natural therapeutic agents. The high antioxidant capability of the leaf extract shows that it can be used to prevent diverse forms of diseases such as:microbial, respiratory digestive, various chronic infectious, degenerative, respiratory, foodborne, deficiency, Reactive Oxygen Species (ROS) related diseases. Moreover, the antibacterial and anti-arthritic activities indicate the capability of the leaf as a source of natural anti-radical, antibiotic or anti-inflammatory agent with possible brilliant health benefits for human and animals. Therefore, the results showcase the possible application in the food, pharmaceutical and veterinary industries/sectors.

MATERIALS AND METHODS

Identification of the plant: The medicinal plant investigated was collected Atowode, Ota, Ogun State, Nigeria and it was identified as *Dialium guineense* Wild (*Fabaceae*) at the Department of Biological Science, University of Medical Sciences, Ondo, Nigeria by Dr. Oghale Ovuakporie-Ova.

Preparation of extract: The solvent extraction of the air died leaves was carried out according to method described by Azwanida[36] with slight modification. The pulverized sample materialwasextracted for at least 3 daysby maceration using methanol and ethyl acetate (2:1) at room temperature with periodical shaking. After filtration, the solvent was evaporated using Uniscope water bath. The concentrated extract was refrigerated until used. The extraction and dilution procedures were protected from contamination.

Phytochemical analysis using gas chromatography-mass spectrometry: The qualitative and quantitative analysis of the extract was carried out by using GC-MS QP2010 Plus (Shimadzu, Kyoto, Japan) systemat the Shimadzu Training Centre for Analytical Instruments (STC) Lagos, Nigeria. The analytical specifications of the GC-MS was used as in our previously study[37].

Determination of lycopene and β-carotene contents: Evaluation of the lycopene and β-Carotene were quantitatively determined according to the method previously used by Ogunmola *et al*.[37] and Lakra *et al*.[38]. It is a simultaneous assay for determination of plant pigments quantitatively. 0.2 g of the fresh leaves sample was homogenized and extracted with 10.0 mL of mixture of 40% acetone and 60%hexane solution. The liquid phase was centrifuged at 4000 x g for 15 min using K-1000 Laboratory Centrifuge. The aliquot was taken from the upper solution (supernatant) and the absorbance were measured at 663, 645, 505 and 453 nm using UniscopeSM 7504 UV Spectrophotometer. The results were expressed as mean±SD for triplicate values. Lycopene and β-carotene contents in mgg^{-1}fwwere determined using the following expression:

$$\text{Lycopene} = -0.0458A_{663}+0.204A_{645}+0.372A_{505}-0.0806A_{453}$$

$$\beta\text{-Carotene} = 0.216A_{663}-1.22A_{645}-0.304A_{505}+0.452A_{453}$$

where, A absorbance recorded at specific wavelengths.

Total chlorophyll and carotenoid: Total chlorophyll and carotenoid were quantitatively determined according to the assay previously used by Silva-Beltran *et al*.[39]. Briefly, 0.20 g of the fresh leavessample was extracted thriceusing 80% acetone:water (ratio 4:1) till the green pigments absolutely extracted and then filtered. The upper layer of the aliquot for the three extractions up wereadded together and the total volume was increased to 25.0 mL. The absorbance was measured at 663, 652, 645 and 470 nm using UniscopeSM 7504 UV Spectrophotometer. The chlorophyll contents were determined as mgg^{-1} of extract (ge) basedon the following expression:

$$\text{Chlorophll a} = \left(12.7A_{663}\text{-}2.7A_{645}\right)$$

$$\text{Chlorophll b} = \left(22.9A_{645}\text{-}2.7A_{663}\right)$$

$$\text{Total Chlorophll a} = \left(27.8A_{652}\right)$$

$$\text{Total chlorophll} = \frac{\left(\begin{matrix}1000A_{470} - 1.63\,\text{Chlorophyll a}\\ \times 104.96\,\text{Chlorophll b}\end{matrix}\right)}{221}$$

Evaluation of Polyphenol Content (PC): The quantitative amount of phenolic compounds was evaluated using Folin-Ciocalteau reagent as described by Nahla *et al*.[40] using Gallic acid was used as standard for the preparation the calibration curve. 1000 μgmL^{-1} of the extract solution was addedto 1.0 mL Folin-Ciocalteu reagent (1:10 v/v in distilled H_2O), 4 mL of 7.5% Na_2CO_3 was added to the mixture after 3 min in order to neutralize the solution. The mixture was allowed to incubate at room temperature for 3 hrs with periodical shaking; the absorbance at 760 nm was measured using UniscopeSM 7504 UV Spectrophotometer. The readings were done in triplicate. Theamount of phenolic compounds was expressed in term of Gallic Acid Equivalent (GAE) in µg per mg of extract[41].

Evaluation of total flavonoid content: The quantitative amount of flavonoid compounds of the extract was evaluated using $AlCl_3$ solution as described in the previous method used by Shafii*etal*.[42]. Briefly, 0.10 mL $AlCl_3$(1:10 v/v in distilled H_2O), 0.10 mL of CH_3COONa (1 M) and 2.80 mL of distilled H_2O was added to 1.0 mL of the extract. The absorbance was read at 415 nm using UniscopeSM 7504 UV Spectrophotometerafter 40 min of incubation at room temperature. To evaluate the total amount of flavonoids in the extract, a calibrated curve was prepared using quercetin as standard. The readings were done in triplicate. The total amount of flavonoid compounds was expressed in µg Quercetin Equivalent (QE) per mg of extract[43].

Evaluation of tannin content: The tannin contentof the sample was evaluatedusing the iron(III) chloride/gelatine test described by Alsiede *et al*.[44].0.10 g of the air dried leaveswas boiled in 50 mL distilled H_2O for 30 min. 500 mL of distilled H_2O was then added to the filtrate of the solution. 0.5 mL of the aliquot was added to 1 mL 1% $K_3Fe(CN)_6$ along with 1 mL of 1% $FeCl_3$ and volume of the mixture was adjusted to 10 mL by adding deionized water. The absorbance of solutionwas measured at 720 nmusing UniscopeSM 7504 UV Spectrophotometer after 5 mina calibrated curve was prepared using tannic acid as standardin order to evaluate the amount of tannin in the extract.The total amount of tannin compounds in the leaves was expressed in µg Tannic Acid Equivalent (TAE) per mg[45].

Evaluation of total ascorbic acid: 1.0 mL 2,4-dinitrophenylhydrazine solution was added to 0.1 mL of the leaf extract (1000 μgm^{-1}). The absorbance of the solution was taken at 515 nm using UniscopeSM 7504 UV Spectrophotometer afterthe solution was incubated in the dark for 30 min. Ascorbic acid was used as a standardfor calibration curve; result was expressed in mg per g of ascorbic acid equivalent[46].

Antioxidant activities; evaluation of *invitro* galvinoxyl antioxidant potential: The *in vitro* Galvinoxyl antioxidant activity was carried out according to the method previously described by Amira *et al*.[47] with slight modification. Briefly, 1.0 mL of various concentrations (62.5-1000 μgmL^{-1}) of the extract was added to 1.0 mL of 0.1 mM Galvinoxyl in methanol. The absorbance was of the solutions was measured using UniscopeSM 7504 UV Spectrophotometer at 429 nm after 3 h of incubation in the dark. Ascorbic acid was used as positive control. The percentage of radical scavenging was calculated using the expression:

$$^{1\%}\text{Galvinoxyll} = \frac{A_{blank}\text{-}A_{ext}}{A_{blank}} x100$$

Where:

A_{blank} = Equal the absorbency of blank solution (Galvinoxyl in methanol)

A_{ext} = Equal the absorbance of the extract solution

The dose-response curve was plotted and IC_{50} value for the leaf extract and the reference compound were determined.

Evaluation of *in vitro* DPPH antioxidant activity: DPPH antiradical potential of the extract was determined as described by Foe *et al*.[48] with slight modification; this was based on the ability of the leaf extractsolution to neutralize DPPH˙radical. Different concentrations of the test sample (1000-62.5 μgmL^{-1})were allowed to react with 0.004% DPPH˙in methanol for 30 minfor proper incubation in the dark. The absorbance was measured at 517 nm using UniscopeSM 7504 UV Spectrophotometer. Percentage radical scavenging activity of the sample was

determined using ascorbic acid as positive control. The antioxidant ability of the extract was calculated using the following expression:

$$^{I\%}\,DPPH = \frac{A_{blank} - A_{ext}}{A_{blank}} x100$$

Where:

A_{blank} = Equal the absorbance of blank solutionie (DPPH in methanol)

A_{ext} = Equal the absorbance of the extract solution

The dose-response curve was plotted and IC_{50} value for the leaf extract and the reference compound were determined.

Antioxidant activity index: The Antioxidant Activity Index (AAI) was calculated as:

$$AAI = \frac{DPPH^{\square}\ Initial\ Conccentration}{IC_{50}}$$

AAI was classified as weak when AAI<0.5, moderate, when AAI ranged between 0.5-1.0, strong, when AAI ranged between 1.0-2.0 and very strong when AAI>2.0[48].

Phosphomolybdate total antioxidant capacity: The total antioxidant of the extract solution was investigated by phosphomolybdate method described by Afsar *et al*.[49] with slight modification. 1 mL of theextract solution(1000 μgmL^{-1}) in methanol was combined a with mixture of 1.0 mL of reagent solution (0.6 M Sulfuric acid, 0.028 M sodium phosphate and 0.004 M ammonium molybdate). The mixture was incubated for 1½ hr at 95 °C in a water bath. The absorbance of the solution was measured at 695 nm using UniscopeSM 7504 UV Spectrophotometerafter coolingat room temperature. The antioxidant capacity was expressed mg per g of ascorbic acidequivalent.

Anti-arthritic protein denaturation: *In vitro* anti-arthritic inhibition of protein denaturation was evaluated by the method previously used by Alrasheid *et al*.[50] with slight modificationusing fresh egg albumin. 5.0 mL of reaction mixture comprised of egg albumin (0.2 mL) plus phosphate buffer at pH of 6.4 (2.8 mL) and varying concentration of extract (2.0 mL each). Distilled H_2O of the same volume served as control. The solution was incubated at 37°C in a Bio-Oxygen Demand (BOD) laboratory incubator for about 15 min and then heated in water bath at 70°C for 5 min. The absorbance was measured at 660 nm after cooling at room temperature. Diclofenac sodium (100 mg) was used as reference drug. The percentage anti-arthriticpotential was evaluated using the following expression:

$$I\% = 100 \times \frac{V_e}{V_c} - 1$$

Where:

V_e = The absorbance of leaf extract

V_c = The absorbance of the control

Evaluation of *in vitro* antibacterial properties: The antibacterial property of varying concentrations of theleaf extract was investigated by Agar-well diffusion method as previously described by Ogunmola *et al*.[37]; Yunana *et al*.[5] with slight modification.Fifteen clinical isolates made up of six Gram positive and nine Gram negative bacteria were employed in the antibacterial test. The Gram-positive bacteria were: *Bacillus spp*, *Enterococcus faecalis*, *Micrococcus varians*, *Streptococcus agalactiae* and *Staphylococcus aureus* and *Staphylococcus saprophyticus* while the Gram-negative bacteria were: *Acinetobacter* spp, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens* and *Shigella dysenteriae*.

Antibacterial Activity Index (AI): The activity index ofthe extract was determined using the formula below:

$$Antibacterial\ Index\ (AI) = \frac{Zone\ of\ inhibition\ of\ Extract\ against\ each\ Bacteria}{Zone\ of\ the\ Reference\ Drug\ against\ each\ Bacteria}$$

The Relative Percentage Inhibition (RPI): The Relative Percentage Inhibition (RPI) of the test extract with respect to the positive control was calculated by using the method described by Hepsibah and Jothi[52]:

$$RPI\% = \frac{I_{ext} - I_{solv}}{I_{std} - I_{solv}} \times 100$$

Where:

I_{ext} = Total area of inhibition of the test extract

I_{solv} = Total area of inhibition of the solvent

I_{std} = Total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area = πr^2. r = radius of the zone of inhibition.

RESULTS AND DISCUSSION

Chemical constituent of the leaf extract of *D. guineense:* From the GC-MS phytochemical analysis, (37) pharmacologically active secondary metabolites were identified in the leaf methanolic/ethylacetate extract

of *D. guineense*, accounting for 99.2% of the total chemical compositionof the leaf extract (Table 1) and the most abundant constituents identified were: 4-O-methylmannose (49.0%), D-(-)-quinic acid (7.0%), methyl 8-[2-((2-[(2-ethylcyclopropyl)-methyl]-cyclopropyl)methyl)- cyclopropyl]-octanoate (6.7%), 3-O-methyl-d-glucose (6.0%), methyl-α-d-mannofuranoside (6.0%) and palmitic acid (5.8%). Most of the metabolites in the leaf extract belong to the family of saccharides. Some phenolic compounds such as p-vinylguaiacol, 3',5'-dimethoxyacetophenone and methyl salicylate were also present in a trace amounts in the leaf extract of the plant studied. Comparatively, the previously study done by Osman *et al*.[53] on the characterization of the seed and endocarp of the fruit of *D. indum*from Malaysiafor their chemical composition using GC-MS showed that a total of thirty-eight (38) metabolites were detected in derivatizedexocarp dichloromethane(DCM) fraction and seed methanol fraction (SMF).The principal secondary metabolites identified in the seed methanol fraction (SMF) were: sucrose (18.86%), β-D-glucopyranose (3.89%) and β-D-galactofuranose (2.43%) while the most abundant phytochemical identified in the *D. indum* derivatized exocarp dichloromethane (DCM) fraction were: palmitic acid (9.71%), oleic acid (7.17%) and vanillin (3.48%) (Osman *et al*., 2018). It was also reported that some other classes of phytochemicals such as derivatives of phenolic compounds and amino acids were also detected in the analysis. Additionally, the Seed Methanol Fraction (SMF) still contained some other derivatives of amino acids, saccharides, polyol and sesquiterpene which were not present in in the derivatized exocarp dichloromethane (DCM) fraction. Surprisingly, some of the therapeutically active organic compounds detected in our work on *D. guineense* grown in Nigeria were also present in the sample of *D. indum*from Malaysiaas reported by Osman *et al*.[53].

β-carotene and lycopene contents: The quantitative amount of β-carotene and lycopene contents of the leaf extract were 0.2 and 0.1mgg^{-1}, respectively (Table 2).

Table 1: Phytohemical composition of leaf extract of *D. guineense*

Compound	Retention index	Composition (%)
Ethyl-2,3-dimethylbutanoate	856	0.3
1-ethynyl-4-fluorobenzene	866	0.1
Coumaran	1036	0.1
E-3-methyl-2-hexenoic acid	1059	0.1
Thymine	1118	0.1
5-hydroxymethylfurfuran	1163	0.2
Ethyl linalool	1181	0.1
N-(3-butenyl)-N-methylcyclohexanamine	1217	0.1
1-(octylsulfanyl)ethylene	1256	0.3
3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	1269	0.6
Dimethyl d-tartrate	1277	0.7
Methyl salicylate	1281	0.2
p-vinylguaiacol	1293	0.7
n-capric acid	1372	0.1
3',5'-dimethoxyacetophenone (phenolic)	1407	1.0
4-methylpentylcyclohexanecarboxylate	1480	0.2
3,4-diketo-a-methylglutaric acid	1538	0.1
3-O-methyl-d-glucose	1647	6.0
Methyl-a-d-mannofuranoside	1667	6.0
5-dimethylsilyloxytetradecane	1677	0.2
4-O-methylmannose	1714	49.0
D-(-)-quinic acid	1852	7.0
n-hexadecan-1-ol	1854	0.1
Palmitic acid, methyl ester	1878	3.6
Methyl-(7E)-7-hexadecenoate	1886	0.1
n-heptadecanol-1	1954	0.1
Palmitic acid	1968	5.8
Trans-phytol	2045	0.2
Methyl stearate	2077	1.0
Oleic acid, methyl ester	2085	0.1
Methyl linolenate	2101	4.5
Methyl petroselinate	2104	0.1
14-hydroxy-15-methyl-15-hexadecenoic acid	2197	0.1
Methyl 8-[2-((2-[(2-ethylcyclopropyl)methyl]cyclopropyl)methyl) cyclopropyl]octanoate	2266	6.7
Z,Z,Z-9,12,15-octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	2804	1.0
Farnesyl-ß-D-mannofuranoside	3014	1.6
Adenosine, N6-phenylacetic acid	3731	0.3
Percentage total		98.5

Table 2: Pigment and their contents in the leaf extract of *D. guineense*

Pigment	Content (mgg^{-1})
Lycopene	0.1
ß-Carotene	0.2
Total carotenoid	12.68
Chlorophyll a	4.4
Chlorophyll b	4.7
Total chlorophyll	7.8

This showed that the β-carotene content is twice the lycopene content in the leaf of *D. guineense*. Lycopene has several health benefits such as antioxidative, anti-inflammatory, anti-atherogenic, anti-atherosclerotic, cardioprotectiveand blood pressure control[54-60].

Total pigment: Chlorophyll a, Chlorophyll b, Total Chlorophyll and caroteinod contents. Quantitatively, the amount of chlorophyll a, chlorophyll b, and total chlorophyll content in the leaf extract investigated were 4.4, 4.7 and 7.8, respectively (Table 2). It was reported that chlorophyll and its derivatives as fat-soluble compounds play many therapeutic roles such as anti-mutagenic and anti-carcinogenic properties[61-63]. Plant pigments play important roles in human and animal health. According to Lehto *et al*.[64]; Sen *et al*.[65] carotenoids such as lycopene, β-carotene, xanthophylls, lutein, capsanthin, astaxanthin, cantaxanthin, cryptoxanthinand zeaxanthinetc found in natural green vegetables are natural food additives, colouring agents and preservatives. They have key roles playing in maintaining growth, good health, prevention and cure of diseases. They are good sources of naturalprovitamin A and non-provitamin A[66-68].

Evaluation of polyphenol content; total phenolic content: The total amount polyphenolsin the leaf extract investigated was 2,668.17 μgmg^{-1} GAE (Table 2). The presence of some active phenolic secondary metabolite such as p-vinylguaiacol, 3',5'-dimethoxyacetophenone and methyl salicylate in the leaf extract. Comparatively, the phenolic content evaluated in the leaf extract studied was approximately 14 times higher than those reported in other *Fabaceae* family such as the methanolic extract of *D. ovoideum* grown in Sri Lanka with TPC of 192.90 mg PGE/g[70]. Likewise, Muthukumaran *et al*.[71] reported that the phenolic content of the leaf and flower ethanolic extracts of *Peltophorum pterocarpum* (*Fabaceae*) from India were 33.17 mg and 24.91 mg of GAE/g, respectively, this is a bout 89% lower than the phenolic proportion in the leaf extract investigated in this study.

Flavonoid content: The quantitative estimate of the flavonoid contentin the leaf extract was 17.80 μgmg^{-1} (Table 3), this is comparable to the amount of flavonoid in the ethanolic leaf extract of *P. pterocarpum* (*Fabaceae*) with the value of 1.44±0.01 mgQE/g[71].

Table 3: Polyphenol and TAA of the leaf extract of *D. guineense*

TPC	TFC	TTC	TAA
2,668.17±0.01 μgmg^{-1} GAE	17.80±0.00 μgmg^{-1} QE	152.65±0.01 μgmg^{-1} TAE	43.75±0.04 μgmg^{-1} AAE

Data are shown as the mean value±S.D of triplicate

Tannin content: The leaf extract of *D. guineense* studied had high amount of tannin 152.65 μgmg^{-1} TAE and its derivatives (Table 2). Comparatively, another report showed that the tannin content of ethanolic extract of flower and aqueous methanolic extracts of leaves of *P. pterocarpum* (*Fabaceae*) from India have highest content of tannin of 844±10.38 mg TAE/g and 776.32±35.01 mg TAE/g, respectively[71]. These classes of polyphenols considered in this study have a lot of potential health benefits; they are good antioxidants that protect human body from heart diseases and cancer by preventing cellular damage[72]. Lin *et al*.[73]; Panche *et al*.[74]; De-Silva and Alcorn[75] stated that polyphenols are an important class of naturalproducts widely available in green vegetables, fruitsand some natural juices. Vinha *et al*.[76]; Panche *et al*.[74]; Kozłowska and Szostak-Węgierek[77]; Forni *et al*.[78]; Liu *et al*.[79] reported that polyphenols have the ability to interfere and combat the pathogenesis of degenerative and neurodegenerative diseases such as cancer, cystic fibrosis, parkinson's and huntington's, Alzheimer's Disease (AD), atherosclerosis etc. Some polyphenolhaveability to inhibit the replication of HIV while some also lower blood glucose levels and have insulin-like problems[74, 80, 81].

Ascorbic acid (vitamin c) content: The amount of ascorbic acid in the leaf extract was evaluated as 43.75 μgmg^{-1} AAE (Table 3). Ascorbic acid is an essential dietary nutrient both at infant, adolescence and adult stages of life. It has series of metabolic, physiological and therapeutic functions. Aghajanian *et al*.[82] reported that vitamin C is an important phytochemical which is good antioxidant and cofactor playing a key role in the development, regulation, function and maintenance of several cells in human and animals. Ascorbic acid and its derivatives enhance the absorption of iron in the gut by reducing ferric to ferrous state and also aid the conversion of cholesterol into bile acids, in order to reduce the blood cholesterol. It also facilitates tissue and wound healing. Moreover, it is a fundamental in the lipid metabolism, atherogenesis and management of neurodegenerative disorders, cancer, diabetes and male infertility problems[83]. Moreover, Young *et al*.[84]; Tan *et al*.[60] also reported that vitamin C plays a serious role in biosynthesis of collagen by facilitating the hydroxylation of proline and lysine residues, therefore, helps in proper intracellular folding of pro-collagen for export and deposition as mature collagen. Grosso *et al*.[85]; Aghajanian *et al*.[82] stated that deficiencies in vitamin Cin human and animal lead to symptoms such as scurvy, Gingivia, bone pain, impaired wound healing etc.

Table 4: Galvinoxyl, DPPH and TAC antioxidant properties of the leaf extract of *D. guineense*

Extract	IC_{50} μgmL^{-1}	AAE μgmg^{-1}
GALV	125.0	-
DPPH•	6.0	-
TAC	-	279.49±0.01

Anti-radical and antioxidant potential; galvinoxyl activities: The galvinoxyl percentage radical scavenging of by the extract at varying concentrations (1000, 500, 250, 125 and 62.5 μgmL^{-1}) were 77.92, 60.73, 59.13, 25.35 and 20.40%, respectively. The leaf extract of *D. guineense* having IC_{50} value of 125.0 μgmL^{-1}. This waslower than that of ascorbic acid which had an IC_{50} value of 15.0 μgmL^{-1} (Table 4).

DPPH activity: The radical inhibitions of the extract at varying concentrations (1000, 750, 500, 250 and 62.5 μgmL^{-1}) were 91.92, 88.53, 86.22, 85.48 and 85.25% respectively. The methanolic leaf extract of *D. guineense* with IC_{50} and AAI values of 6.0 μgmL^{-1}and 7.0, respectively (Table 4). This is similartothe IC_{50} and AAI of ascorbic acid with value of 9.0 μgmL^{-1} and 4.4, respectively. Thisstudy showed that the leaf extract investigated in this study had more anti-radical potential than other plant sample in the family such as the seed crude methanolic extract of *D. indium* from Malaysia with DPPH percentage free radical scavenging and IC_{50} values of 90.99±0.03% and 99.95±0.98 μgmL^{-1}, respectively[53]. In the same manner, methanolic extract of *D. ovoideum* from Sri Lanka had DPPH percentage antiradical activity of 92.90±0.4%[70] .

Total antioxidant capacity: The evaluated amount of total antiradical capacity of leaf extract of *D. guineense* grown in Nigeria was 279.49±0.00 μgmg^{-1} AAE (Table 4). The result shows that the leaf extract used in this study had more free radical scavenging potential compared to other related species such as the seed crude methanolic extract of *D. indium* from Malaysia with TAC value of 222.72±16.03[53]. Medicinal plants have significant high phenolic contents and other phytochemicals with high antioxidant properties compared to the conventional antioxidants that in some cases have side effects[86]. Moreover, medicinal plants present abundant sources of natural antioxidants and find enormous application in human nutrition, not only as flavouring spices but also as natural remedy. Datta *et al*.[87]; Da-Oliveira *et al*.[88] stated that food enrichment and fortification with natural products such as polyphenols from naturally grown vegetables is a new trend to improve the nutritional and therapeutic values of food at home and industries. Plants are rich in minerals, vitamins, flavoring agents and natural antioxidants. It was reported that natural product from medicinal plants added to food substances increased the total phenolic and antioxidant values of raw material and final food product[89, 90]. Some of these medicinal plants have been shown to have both chemo-preventive or therapeutic potential on breast and other form of cancer[91-92].

Table 5: Anti-arthritic activity (Egg albumin assay) of the leaf extract of *D. guineense*

Concentration μgmL^{-1}	Inhibition (%)	EC_{50} μgmL^{-1}
1000	96.00±0.00	
500	94.19±0.01	
250	90.05±0.00	5.00
125	88.76±0.00	
62.5	83.33±0.00	

Data are presented as the mean value±S.D. of triplicate

Anti-arthritic and anti-inflammatory potential: The anti-arthritic/anti-inflammatory efficacy of the investigated leaf extract was studied using egg albumin protein denaturation method. The extract of *D. guineense* showed high percentage inhibition of anti-arthritic activities ranging between 83.33-96.00% as compared to the Diclofenac sodium (reference drug) with percentage inhibition of 80.00% (Table 5). In support of the research on the mechanism of the anti-arthritic activity of natural products from plants, ability of theleaf sampleused in this study to inhibit arthritic and inflammatory was investigated. Rheumatoid arthritis is joint-related, immune-based inflammatory disarray that affects almost 1% of the world's population[93-96]. Fu *et al*.[97]; Rahman *et al*.[98]; Walling and Kim[99] reported that pathogenesis is observed in the T-cell activation that leads to clusters of differentiation in the T-cells and concurrently increases various inflammatory indicators. These include matrix metalloproteinase, cytokines, chemokines, NF-kβ, adhesion molecules, lipoxygenase, nitric oxide and arachidonic acid. They destroy bone and cartilage by changing the structure and shape which lead to degradation. Based on this, incorporation of phytochemicals such as polyphenols, terpenoids and other secondary metabolites have high efficacy against arthritis via targeted action on inflammatory biomarkers[100-102]. Obaseki*etal*.[103]; Sangeetha and Vidhya[104] stated that different anti-arthritic and anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation. In this study, the extract of *D. guineense* was able to significantly inhibited heat induced protein denaturation and therefore, shows strong anti-arthritic activity.

Antibacterial activities: The effects of the leaf extract of *D. guineense* at a 1000-250 μgmL^{-1} concentration on the growth of the 15 clinically isolated bacteria strains tested are presented in Table 6. The zones of inhibition of the leaf extract (20.0-08.0 mm) showed high antimicrobial activities as compared to gentamicin antibiotic (10 μg per disc). Greater or similar activities were observed as concentration of the extract 1000-250 μgmL^{-1} by the

Table 6: Antibacterial potential (Zones of Inhibition in mm) of the leaf extract of *D. guineense*

Conc. (µgmL^{-1})/	ZI of the leaf extract			Gentamicin		
Organisms	1000	500	250	10 µg	AI	RPI
Acinetobacter spp (-)	10	10	10	-	10	10.00
Bacillus spp (+)	15	10	10	20	0.8	56.23
E. coli (-)	20	20	08	15	1.3	177.85
E. faecalis (+)	15	11	10	14	1.1	114.80
K. pneumoniae (-)	16	12	10	15	1.1	113.80
M. varians (+)	15	-	-	19	0.8	63.33
P. aeruginosa (-)	10	08	08	20	0.5	25.00
P. mirabilis (-)	16	16	10	18	0.9	79.02
P. stuartii (-)	14	14	-	16	0.9	76.55
S. agalactiae (+)	10	10	10	20	0.5	25.00
S. aureus (+)	20	20	10	20	1.0	100.00
S. dysenteriae (-)	10	10	10	20	0.5	25.00
S. marcescens (-)	15	15	14	17	0.9	77.85
S. typhimurium (-)	15	-	-	20	0.8	56.23
S. saprophyticus (+)	20	08	08	17	1.2	138.50

Resistant (--), not sensitive (<8 mm), sensitive (9-14 mm), very sensitive (15-19 mm) and ultrasensitive (>20 mm)

extract than gentamicin (standard antibiotic). The Activity Index (A.I) was greater than or equal to1.0 against the bacteria isolates. No significant reduction in activities was observed as the extract concentrations were reduced gradually from 1000-250 µgmL^{-1}. Comparatively, the extract investigated in this study had a similar antibacterial potential as reported by Ijoma *et al*.[105] on their study on *Dialium indum* leaves fractions with average diameter zones of inhibition ranging between 14-36 mm on the following bacteria *B. typhi*, *E. aerogenes*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. vulgaris*, *S. typhi*, *S. albus*, *S. aureus*, *S. muteus*. Moreover, previous work on the extract of a related species such as the ethanol extract of the aerial (leaf and bark) of *Butea monosperma* (*Fabaceae*) grown in India was found to be only effective against *B.* subtilis and *S. aureus* (13 mm at 100 mgmL^{-1}) among the bacteria isolates used, the petroleum ether extract did not inhibit *P. aeruginosa* while *E. coli* was not inhibited by any type of extract[106]. From the results obtained in this study, the leaf extract exhibited a notable and promising antimicrobial inhibitory activity against all the tested pathogenic bacteria. In fact, the presence of various polyphenols such as p-vinylguaiacol, 3',5'-dimethoxyacetophenone, methyl salicylate etcin the leaf studied contributed tremendously to the antibacterial activities. In addition, synergistic activity of all secondary metabolite in the extract also played central roles to the antibacterial activities observed in this study[107, 108]. Therefore, the leaves of the plant can be used in developing antibacterial drug in combating multidrug resistant bacteria.

CONCLUSION

The study demonstrated the therapeutic potential of *D. guineense* grown in Nigeria. The medicinal properties of the leaf may be related to its polyphenols and other active metabolites. This study indicated that the leaf of the plant can be used for development of important drugs for human and animals. This observation is very significant because of the possibility of developing therapeutic drug that will be active against multi-drug resistant organisms and easily accessible natural antioxidant substances that may help to modulate oxidative stress related disorders. Further study is therefore recommended on the pharmacology properties of the leaves of the plant which are not covered by this study.

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