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Antioxidant and antimicrobial activities of *Dialium guineense* (Willd) leaf extract

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ABSTRACT

The *in vitro* antioxidant and antimicrobial activities of methanolic leaf extract of *Dialium guineense* were studied in order to provide a pharmacological basis for their ethomedicinal applications. Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power assay. In addition, the total phenolic content was also analyzed. Antimicrobial activity was tested against clinical isolates of *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Candida albicans*, *Microsporium gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum* using agar well diffusion method. The results of the DPPH scavenging activity of the extract showed a concentration dependent antioxidant activity with maximum scavenging activity (85.35%) observed at 250 µg/ml concentration and comparable to those of ascorbic (95.75%) and gallic acids (93.67%). The reducing potential of the extract (0.069 ± 0.003 nm) was also comparable to that of gallic acid (0.078 ± 0.022 nm), while the total phenolic content was 69.45 ± 0.002 mg/g gallic acid equivalent. The antimicrobial inhibition zone and minimum inhibitory concentration values of the extract ranged between 10.2 to 25.9 mm and 7.81 to 62.5 µg/ml respectively, with the Gram positive bacteria generally being most sensitive, followed by the fungi and the Gram negative bacteria. This study indicates that *D. guineense* leaf extract has significant antioxidant and antimicrobial properties, and thus substantiates its popular and wide traditional applications in diverse ailments**.** The plant may therefore be exploited as a potential preservative in the pharmaceutical and food industries.

Keywords: *Dialium guineense,* antioxidant properties, DPPH assay, total phenolics, gallic acid.

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INTRODUCTION

Antioxidants have been found to play a major role in protecting the human body against damage induced by reactive free radicals (Halliwell and Gutteridge, 1990; Mates et al., 1999; Omale and Omajali, 2010) by reacting with free radicals, chelating and also by acting as oxygen scavenger (Shahidi and Wanasundara, 1992; Buyukokuroglu et al., 2001). Some of the established diseases of free radicals, resulting from products of oxidative stress, included rheumatoid arthritis, atherosclerosis, skin-aging, nephritis, reperfusion injury, asthma, diabetes mellitus, carcinogenesis, neurodegenerative diseases (Alzheimer's and Parkinson's diseases) and AIDS (Stadtman and Oliver, 1991; Cerutti, 1994; Feig et al., 1994; Florence, 1995; Dandona et al., 1996; Alho and Leinonen, 1999; Kremsner et al., 2000; Olukemi et al., 2005). Naturally, there is a dynamic equilibrium between the quantity of free radicals generated in the body and antioxidants to scavenge or quench them in order to protect the body against pathological effects. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated under pathological conditions. It is, therefore, pertinent to fortify our diet with antioxidants to protect against harmful diseases. Hence, there has been an increased interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials (Amaeze et al., 2011). Several researchers have reported

that numerous tropical plant possess potential antioxidant properties (Miller, 1996; Akinmoladun et al., 2007; Atrooz, 2009; Aliyu et al., 2009; Amaeze et al., 2011; Okoko and Ere, 2012; Ibeh et al., 2013).

Dialium guineense Wild (Fabaceae), is commonly called black velvet or velvet tarimand (English), Icheku (Ibo, Eastern Nigeria), Awin (Yoruba, Western Nigeria), Tamarinier noir (French) (Ezeja et al., 2011). It is a woody plant that occurs in the rain forest region of West Africa and can grow up to 10 to 20 m. The tree possess densely, hairy leafy crown, smooth greyish bark and whitish flowers which bears densely velvet black fruits that are more or less circular and flattened enclosing dry, brownish, sweet acidic edible pulp (Hutchinson and Daniel, 1958). In Nigeria, the tree flowers from September to October, and fruits from October to January (Keay, 1989).

The ripe fruits of the plant are chewed among some women in southeast Nigeria to improve lactation and check genital infection (Nwosu, 2000). Among the Esan tribe of Edo State in Nigeria, the twig or bark is chewed for oral hygiene and stomach ache (Idu et al., 2009). The genus *Dialium* comprises five species in West Tropical Africa but *D. guineense, D. dinklagel* and *D. packyphyllum*are represented in Nigeria (Omotayo, 1999). Traditionally, *D. guineense* leaves and stem bark are used as remedies for diarrhoea, severe cough, bronchitis, wound, stomachaches, malaria fever, jaundice, antiulcer and haemorrhoids (Ogu and Amiebenomo, 2012).

Scientifically, the molluscicidal activity of the fruits and leaves of *D. guineense* have been reported (Odukoya et al., 1996). The antimalarial properties of the aerial parts have also been reported (Bero et al., 2009). Recently, the methanolic leaf and stem bark extracts were reported to possess analgesic, anti-vibrio potentials and anti-diarrhoeal potentials (David et al., 2011; Ezeja et al., 2011; Ogu and Amiebenomo, 2012). The phytochemicals identified in the leaf and stem bark extracts of the plant were tannins, alkaloids, flavonoids, saponins, steroids and cardiac glycosides (David et al., 2011; Ogu and Amiebenomo, 2012). Earlier researchers have linked the antioxidant and antimicrobial potentials to the content and richness of some phytochemicals such as alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952; Aliyu et al., 2009; Amaeze et al., 2011; Omoregie and Osagie, 2012).

In this study, the antioxidant and antimicrobial activities of methanolic leaf extract of *D. guineense* were investigated so as to provide a pharmacological basis for their ethomedicinal applications.

MATERIALS AND METHODS

Source of chemicals

All other chemicals and reagents used were of analytical grade. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, gallic acid, ascorbic acid and Folin-ciocalteau reagent were obtained from Sigma- Aldrich, USA.

Collection and Identification of plant material

Fresh leaves of *D. guineense* were collected from forest along Amai-Obiaruku in January, 2013 and the taxonomic identity of the plant was confirmed at the Department of Biological Sciences, Novena University Ogume (Amai Campus), Delta State, Nigeria, where voucher specimens were deposited for future reference.

Processing and extraction of plant material

The leaves collected were and air-dried to crispiness on the laboratory workbench (prevailing room temperature of 30 ± 2°C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles with an electric blender (Super Search Model 2815). 200 g of the powdered leaves was subjected to soxhlet extraction and exhaustively extracted with 2.5 L of methanol for about 48 h. The extracts were filtered and concentrated under reduced pressure using rotatory evaporator to a slurry sticky mass with yield of 19.93% w/w. Prior to antimicrobial assay the extracts were dissolved in 20% Dimethyl-Sulfoxide (DMSO) to obtain the working concentration of 250 µg/ml

Source of test microorganisms

The pure clinical isolates of *Staphylococcus aureus*, *Streptococcus mutans, Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhi,* and *Candida albicans* were collected from Lahor Research Diagnostic and Environmental Consortium (LRDEC) in Benin City, Nigeria. The cultures were maintain at 35°C on Nutrient agar (bacteria) and Sabouraud Dextrose Agar (fungi) and used for the study. The dermatophytes (*Microsporium gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum*) were stock culture maintained in the microbiology laboratory of Novena University, Amai Campus, Delta State.

Antimicrobial activity of plant extract

Antibacterial assay

The antibacterial activity of the extract was determined using agar well diffusion method of Rath et al. (2002), with slight modification. An overnight culture of each organism was prepared by taken two wire loop of the organism from the stock and inoculated each into the sterile nutrient broth of 5 ml, each incubated for 18 to 24 h at 37^{o}C. The overnight cultures was then diluted to 10^{-2} (by adding 0.1 ml of each organism into 9.9 ml of sterile distilled water) from which 0.2 ml was aseptically inoculated into prepared sterile molten Mueller Hinton Agar (Hi-media India) Petri dishes, mixed properly and allowed to solidify. With a sterile cork-borer (8 mm diameter), four (4) wells were made on each solidified plate and the bottom of each hole was covered with molten agar. 100 µl of each extract dilution (250, 125 and 62.5 µg/ml) was added into each well on different plates. 100 µl of Ciprofloxacin (20 µg/ml) was used as positive control while organism seeded-plates without antibacterial agents were used as negative control. The Petri dishes were left on the bench for about 2 h to allow the extract diffuse properly into the

agar. They were then incubated at 37°C for 24 h and zone of inhibition were observed and measured using transparent ruler in millimeters. The procedure was carried out in triplicate.

Antifungal assay

The method formerly described by Ogu et al. (2011) was adopted. Fungal spores were harvested after 7 days old SDA slant culture was washed with 10 ml normal saline in 2% Tween 80 with the aid of glass beads to help in dispersing the spores. The spore suspensions were standardized to 10^5 spores/ml. Sabouraud Dextrose Agar (SDA) (Lab M, India) was prepared according to specifications, autoclaved (121°C for 15 min), supplemented with 0.05% chloramphenicol and dispensed into 11 cm diameter Petri dishes. 1 ml of each standardized spore suspension (10^5 spores/ml) was evenly spread on the surface of the gelled SDA plates. Then, sterile cork a borer (8 mm in diameter) was used to make well at the center of each seeded plates. Thereafter, 100 µl of the reconstituted extracts (250, 125 and 62.5 µg/ml) was applied into each labeled well. 100 µl each of 20% DLMSO and standard drug griseofulvin (100 µg/ml) (Clarion Medicals Ltd, Lagos, Nigeria), served as negative and positive control, respectively. The plates were incubated at ambient temperature for 1 to 7 days and observed for growth. Anti-fungal activities of the extract as well as the controls were measured and recorded as mean diameter of zones of inhibition around the three wells.

Evaluation of antioxidant activity

The determination of the free radical scavenging activity of each of the crude extract was carried using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as described by Mensor et al. (2001) with slight modifications. Various concentrations of 250, 125, 50, 25 and 10 µg/ml of the extract in methanol were prepared. 1.0 ml of a 0.3 mM DPPH in methanol was added to 2.5 ml solution of the extract or standard, and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 518 nm on a spectrophotometer (Jenway, 6025). The decrease in absorbance was then converted to percentage Scavenging activity (%) using the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Blank = Methanol (1.0 ml) plus sample solution (2.0 ml), Negative control = DPPH solution (1.0 ml, 0.25 mM) plus methanol (2.0 ml), ascorbic acid and gallic acid were used as standards.

Reducing power assay

This method described by Akinmoladun et al. (2007) was followed in the determination of the reductive power assay of the extract. Different concentrations of the methanolic extract of *D. guineense* (250, 125, 50, 25 and 10 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Trichloro-acetic acid (10%, 2.5 ml) was added to the mixture. A portion of the resulting mixture was mixed with $FeCl_3$ (0.1%, 0.5 ml) and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicates higher reductive potential.

Determination of total phenolic content

The total phenols content was determined using Folin-Ciocalteu method (Stanly et al., 2011) with little modifications, using gallic acid as a standard phenolic compound. The extracts were diluted with distilled water to a known concentration in order to obtain the readings within the standard curve range of 0.0 to 800 µg of gallic acid/ml. 250 µl of diluted extract or gallic acid solution was mixed with 1 ml of distilled water in a test tube followed by the addition of 250 µl of Folin-Ciocalteu reagent. The samples were mixed well and then allowed to stand for 5 min at room temperature in order to allow complete reaction with the Folin-Ciocalteu reagent. Then, 2.5 ml of 7% sodium carbonate aqueous solution was added and the final volume was made up to 6 ml with distilled water. The absorbance of the resulting blue colour solution was measured at 760 nm using spectrophotometer after incubating the samples for 90 min. The result was expressed as mg of gallic acid equivalents (GAE)/g of extract by using an equation that was obtained from standard gallic acid graph. All the experiment was conducted in three replicates.

Statistical analysis

The experiments were carried out in triplicates. The results were given as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was carried out to test for significant differences between the means of samples and standards at $P < 0.05$.

RESULTS

The results of the free radical scavenging activity of the DPPH assay showed the percentage antioxidant activities of 55.22, 64.34, 73.78, 82.78 and 85.35 for 10, 25, 50, 125 and 250 µg/ml extract, respectively (Figure 1). There was no significant difference ($p < 0.05$) antioxidant activity of the extract at 50, 125 and 250 µg/ml when compared with those of the standard ascorbic and gallic acids. The reducing power of the extract (0.069 ± 0.002 nm) was significant when compared with gallic acid standard (0.078 ± 0.023 nm) (Figure 2). The total phenolic content of the extract was found to be 69.45 ± 0.002 mg/g expressed as gallic acid equivalent (GAE) (Figure 3)

The antimicrobial activity results showed that the entire test micro-organisms were inhibited in a concentration-dependent pattern at the various dilutions of the plant extracts. The antimicrobial diameter zones of inhibition ranged between 10.2 to 25.9 mm, with *S. mutans* (25.9 mm) and *P. mirabilis* (10.2 mm) being the most and least sensitive isolates (Table 1). The results also revealed that significant ($P < 0.05$) diameter zones of inhibitions were obtained with 125 and 250 µg/ml against *S. aureus, S. mutans, E. coli, B. cereus, P. mirabilis, S. typhi, C. albicans* and *T. mentagrophytes*, when compared with the reference drugs. Both *E. coli* and *P. mirabilis* were however found to be resistant to ciprofloxacin (20 µg/ml) antibiotics in this study. Generally, the Gram positive bacteria (*S. aureus, S. mutans* and *B. cereus*) were found to be the most sensitive organisms, followed by the fungi

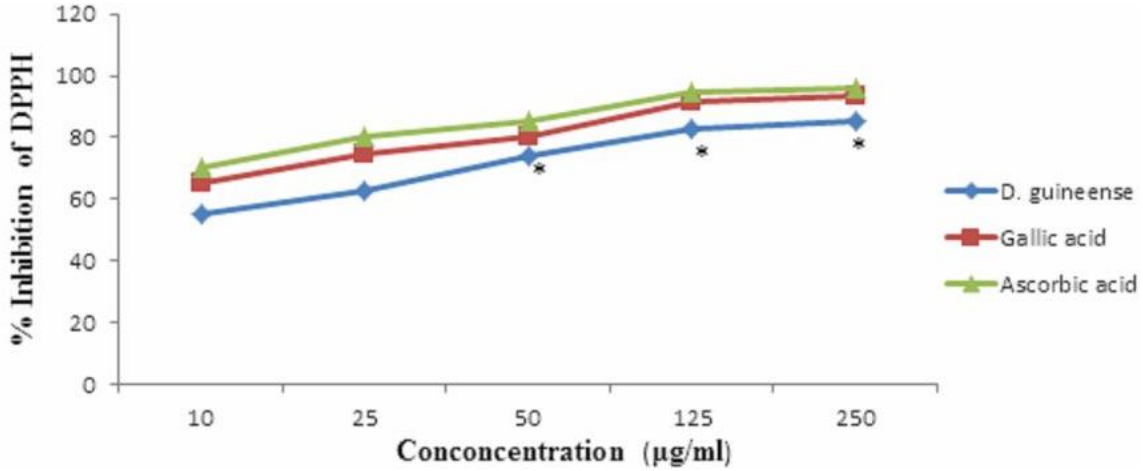

Figure 1. Inhibition of DPPH radical by methanolic leaf extract of *Dialium guineese*. *(P < 0.05) no significant difference with control.

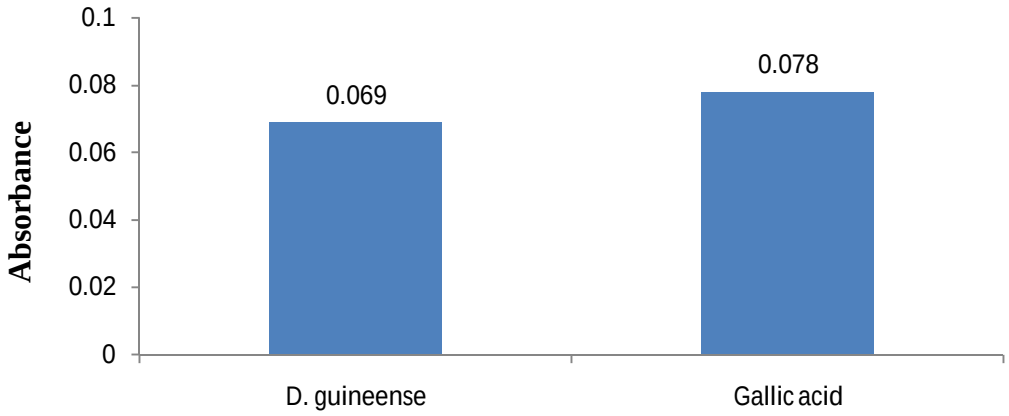

Figure 2. Reducing potential of Gallic acid and *D. guineense.*

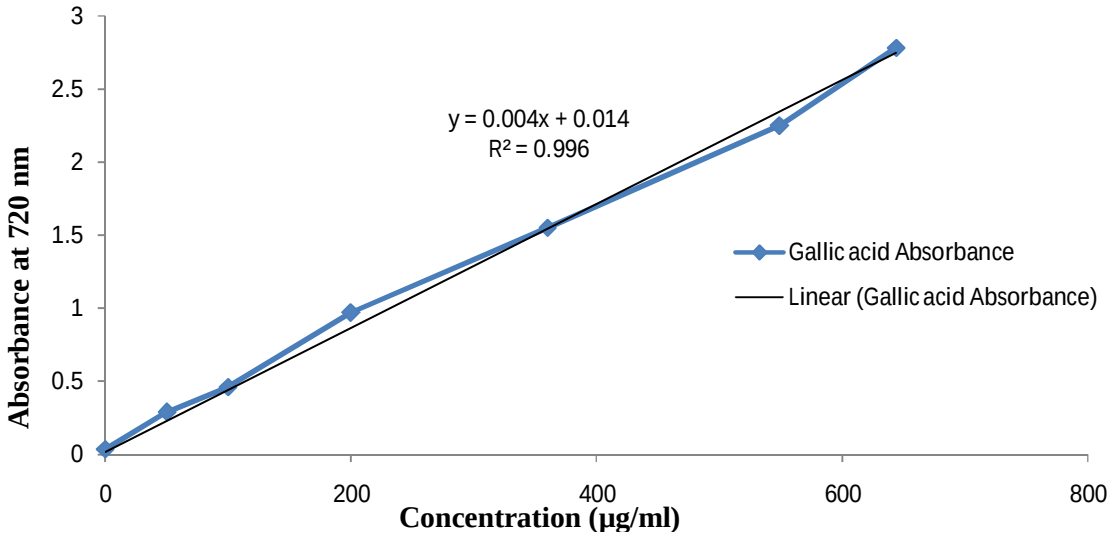

Figure 3. Gallic acid calibration curve for total phenolic compounds.

Table 1. Antimicrobial activity of methanolic leaf extract of *D. guineense,* Ciprofloxacin (Cip) and Griseofulvin (Gri).

D. guineense extract	Mean Diameter Zone of Inhibition ± S.D				
	Reference			Drug	
	62.5 µg/ml	125 µg/ml	250 µg/ml	Cip 20 µg/ml	Gri 100 µg/ml
S. aureus	16.2 ± 0.6	20.9 ± 0.1*	22.5 ± 1.6*	25.5 ± 0.8	ND
S. mutans	17.8 ± 1.2	23.0 ± 1.4*	25.9 ± 0.9*	24.0 ± 0.4	ND
E. coli	13.9 ± 0.2*	17.8 ± 0.2*	19.2 ± 1.8*	-	ND
B. cereus	16.9 ±2.2*	20.0 ± 2.7*	23.5 ± 0.7*	22.0 ± 0.6	ND
P. aeruginosa	11.0 ± 0.2	14.8 ± 0.3	18.9 ± 1.5*	24.0 ± 0.5	ND
K. pneumonia	10.2 ± 0.2	13.9 ± 0.7	17.0 ± 0.7*	19.0 ± 0.6	ND
P. mirabilis	10.0 ± 0.9*	13.2 ± 2.2*	16.8 ± 0.4*	-	ND
S. typhi	12.9 ± 1.2	15.9 ± 0.2*	18.2 ± 1.9*	20.5 ± 0.4	ND
C. albicans	15.0 ± 0.2	18.9 ± 0.6*	22.4 ± 0.7*	ND	24.0 ± 0.4
M. gypseum	13.2 ± 0.9	14.5 ± 0.8	16.9 ± 0.2*	ND	21.3 ± 0.4
T. mentagrophytes	14.9 ± 0.2	17.8 ± 1.9*	19.9 ± 2.2*	ND	23.0 ± 0.4
T. rubrum	11.0 ± 1.7	13.0 ± 0.2	15.8 ± 0.5*	ND	21.5 ± 0.4

ND = Not Determined, * Values significant ($P < 0.05$) compared with reference drugs.

(*C. albicans, M. gypseum, T. mentagrophytes,* and *T. rubrum*) and the Gram negative bacteria (*E. coli, P. aeruginosa, K. pneumonia, P. mirabilis* and *S. typhi*) in that order.

The results of the MIC values revealed that methanolic leaf extract of *D. guineense* had MIC values of 7.81 µg/ml (against *S. aureus, S. mutans* and *C. albicans*), 15.68 µg/ml (*E. coli, B. cereus, S. typhi, M. gypseum* and *T. mentagrophytes*), 31.50 µg/ml (*T. rubrum*) and 62.5 µg/ml (*P. aeruginosa, K. pneumonia* and *P. mirabilis*).

DISCUSSION

There is a growing interest in the pharmacological evaluation of various plants used in traditional system of medicine globally. In the present study, the methanolic leaf extract *D. guineense* exhibited comparable DPPH free radical scavenging ability in a dose-dependent manner. Similar findings were reported by Aliyu et al. (2009) and Ibeh et al. (2013) in their studies on the antioxidant activity of leaf extracts of *Bauhinia rufescens* and *Axonopus compressus,* respectively. DPPH radical scavenging activity assay assesses the capacity of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and on reacting with an antioxidant compound which can donate hydrogen becomes reduced to diphenylpicrylhydrazyl (DPPH). The observed antioxidant of the extracts may be due to the neutralization of free radicals (DPPH), either by transfer of hydrogen atom or by transfer of an electron (Jao and Ko, 2002; Naik et al., 2003). The scavenging ability of the extracts may be a reflection of the total activities of various components present in the plant (Omoregie and Osagie, 2012). Earlier researchers have reported that the antioxidant activity of most plants with therapeutic properties may be due to the presence of natural substances mainly phenolic compounds (Atrooz, 2009; Rached et al., 2010; Omoregie and Osagie, 2012).

The reductive potential of the extract showed that the methanolic leaf extract *D. guineense* possess a relatively high reductive potential as compared with the standard. The reductive potential value of leaf extract of *D. guinnense* (0.069 ± 0.003 nm) obtained in this study was slightly lower than 0.071 ± 0.03 nm obtained by Aliyu et al. (2009) in their study. This finding reflects the semblance in the chemical composition of the leaf extract (Aliyu et al., 2009; David et al., 2011). The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir et al., 1995). The relatively high reductive potential as compared with that of gallic acid, thus suggest that the leaf extract has high redox potentials and can acts as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans et al., 1995; Omale and Omajali, 2010).

The antioxidant capacity of phenolic compounds is mainly attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators (Oboh et al., 2009; Omoregie and Osagie, 2012). Phenolics are the largest group of phytochemicals and have been reported to account for most of the antioxidant activity of plants. According to Oki et al. (2002), they observed that the radical scavenging activity increased with the increase of phenolic compound content. Thus, the high total phenolics content discovered in this plant extract, and the detection of flavonoids, saponins and tannins in earlier study (David et al., 2011), possibly suggest the involvement of phenolics and these bioactive agents in the bioactivities of this plant and might partly be responsible for the folkloric use of the plant in traditional

medicine. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Haq et al., 2011). Therefore, the strong antioxidant potentials displayed by this extract, at least in these experimental models, could be linked to the phenolic content and or the phytochemicals detected therein. High antioxidant activity of plant extracts has previously been attributed to the phenolic compounds (Odabasoglu et al., 2004) and flavonoids, saponins and tannins (Di Carlo et al., 1999; Aliyu et al., 2009).

The significant antibacterial and antifungal activities of this extract could as well be attributed to the phenolic content and or the phytochemicals detected by earlier researchers (David et al., 2011). Plant phenolics have been implicated in antibacterial activity of plants (Adesegun and Rotibi, 1998). They react with proteins like microbial enzymes through hydrogen bonding and can cause iron deprivation, leading to the death of microorganisms (Scalbert, 1991; Haslam, 1996). Also, the presence of saponins and other phytochemical compounds have been reported to exert potent antifungal activities (Ogu et al., 2011).

It is worth noting that the extracts showed greater potential antibacterial activities against Gram-positive bacteria than Gram-negative bacteria. This result was supported by the fact that Gram-negative bacteria have an outer membrane consisting of lipoprotein and lipopolysaccharide, which is selectively permeable and thus regulates access to the underlying structures (Chopra and Greenwood, 2001). This renders the Gram-negative bacteria generally less susceptible to plant extracts than the Gram-positive bacteria (Pelczar et al., 1993; Chan et al., 2007). Furthermore, the significantly antimicrobial activities further supports the traditional application of the plant extract in the management of tooth-ache, diarrhoea, severe cough, bronchitis, wound, stomach-aches, malaria fever, jaundice, antiulcer and haemorrhoids (Idu et al., 2009; Ogu and Amebiomo, 2012).

Conclusion

This study has demonstrated the antioxidant and antibacterial activities of methanolic leaf extract of *D. guineense.* The considerable content of phenolic compounds of the extract could possibly be linked their antioxidant and broad spectrum antibacterial and antifungal activities. The significant antimicrobial activity justifies its use in traditional medicine for the treatment of diverse ailments. This plant may therefore be exploited as an alternative potential future agent to synthetic preservatives in the pharmaceutical and food industries.

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