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In Vitro Antioxidant Activities of Extracts of *Dialium Guineense* Stem Bark

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ABSTRACT:
Background: *Dialium guineense belongs to the Leguminosae family, and grows in dense forests in Africa along the southern edge of the Sahel. The bark, leaves and fruits of the plant have medicinal properties and are used to treat diseases such as stomatitis, toothache, fever, diarrhoea, palpitations, and microbial infections.*
Aim: *The present study investigated the free radical scavenging activities of aqueous and ethanol extracts of Dialium guineense stem bark.*
Methods: *Aqueous and ethanol extracts of D. guineense stem bark were prepared using standard method. The in vitro antioxidant activities of the extracts were also determined using standard methods.*
Results: *The diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity and total antioxidant capacity (TAC) of the ethanol extract were significantly higher than those of aqueous extract ($p < 0.05$). However, there were no significant differences in their nitric oxide (NO) scavenging activity, thiobarbituric acid (TBARS), and reductive potential ($p > 0.05$). The ferric reducing antioxidant power (FRAP) of the aqueous extract was significantly higher than those of ethanol extract and ascorbic acid ($p < 0.05$).*
Conclusion: *These results suggest that phenolics present in the extracts may play a role in free radical scavenging activities of the medicinal plant.*

Keywords: *Antioxidants, Dialium guineense, Diphenyl-2-picryl-hydrazyl, Free radical, Reducing power.*

I. INTRODUCTION

The search for new natural compounds with potent biological activities has been increasing with an estimated eighty percent (80 %) of the human population in developing countries relying, to some extent, on medicinal plant materials for their primary healthcare. Medicinal plant-based drugs have the added advantage of being readily available, effective, and offering a broad spectrum of activity with a greater emphasis on preventive action. These medicinal plants are usually known to exert their diverse health benefits through the numerous phytochemicals they contain (Jeyachandran *et al.*, 2010; Jothy *et al.,* 2013; Manokaran *et al.,* 2008).

Dialium guineense (Velvet Tamarind), is a tall, tropical, fruit-bearing tree. It belongs to the *Leguminosae* family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel. The bark and leaves have been reported to possess medicinal properties and are used against several diseases. Each fruit typically has one hard, flat, round, brown seed, typically 7 - 8 mm across and 3 mm thick (Dalziel and Hutchison, 1973). The seed somewhat resembles a watermelon seed (*Citrullus lanatus*). Some have two seeds. The seeds are shiny, and coated with a thin layer of starch. The pulp is edible and may be eaten raw or soaked in water and consumed as a beverage (Dalziel and Hutchison, 1973). The bitter leaves are ingredients in a Ghanaian dish called *domoda*. Its wood is hard and heavy, and used for construction. The wood is also

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used for firewood and charcoal production (Dalziel and Hutchison, 1973).

Free radicals are constantly formed in living cells and removed by antioxidant defenses. Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. When cells are exposed to oxidative stress a defense system ensures the expression and regulation of antioxidant enzymes as a defense mechanism to protect them from the damaging effect of free radicals. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components (Teixeira *et al.,* 1998). They act by reducing the energy of the free radical or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt the oxidizing chain reaction to minimize the damage caused by free radicals. It has been reported that a substantial link exist between free radicals and more than sixty different health conditions, including aging, cancer, diabetes mellitus, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, the body's potential to reducing the risk of free radical-related health problems is made more palpable (Grazioli *et al*., 1998). The present study investigated the free radical scavenging activities of aqueous and ethanol extracts of *Dialium guineense* stem bark.

II. MATERIALS AND METHODS

Plant Sample Collection and Preparation

The plant leaves were obtained from Iyekogba area in Benin and identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria with voucher number UBH_D330, after which the bark was obtained. Preparation and extraction was carried out using the method of Abu *et al.* (2015). The aqueous and ethanol extracts were concentrated using rotary evaporator and made into powder by lyophilisation.

DPPH Radical Scavenging Assay

The free radical scavenging capacity of the plant extracts against 1, 1–diphenyl–2–picrylhydrazyl (DPPH) free radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations of the extracts (0.2 - 1.0 mg/mL). The test tubes were shaken and incubated in the dark for 15 min at room temperature, and the absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid (vitamin C) was used as control, with similar concentrations as the test samples. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated as shown in Equation 1:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_o - A_1 \times 100}{A_o} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

where A_o was the absorbance of DPPH radical + methanol; A_1 was the absorbance of DPPH radical + sample extract or standard.

Reducing Power Assay

The reducing power (RP) of extracts was determined according to the method described by Lai *et al.* (2001). Briefly, 1 mL of different concentrations of extracts (0.1- 1.0 mg/mL) in water was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 ^{o}C for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10 %) was added to the mixture to stop the reaction. Distilled water (2.5 mL) and 0.5 mL of 0.1 % $FeCl_3$ were then added and the absorbance was read at 700 nm. Higher absorbance values indicated higher reducing power. Ascorbic acid served as the control.

Ferric Reducing Antioxidant Power (FRAP)

A modified method of Benzie and Strain (1996) was used for the FRAP assay. The principle behind this assay is the ability of the sample to reduce ferric tripyridyltriazine (Fe (III)- TPTZ) complex to ferrous tripyridyltriazine (Fe (II) - TPTZ), which at low pH produces an intense blue colour that can be read at 593 nm. Briefly, 1.5 mL of freshly prepared

FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution) was mixed with 1 mL of varied concentrations of the extracts (0.2 - 1.0 mg/mL). The reaction mixtures were incubated at 37 ^oC for 30 min and the absorbance was read at 593 nm. Ascorbic acid served as the control, while $FeSO_4$ was used for calibration and values expressed as mmol $FeSO_4$ equivalents per gram of sample.

Total Antioxidant Capacity (TAC)

The TAC of the extracts was evaluated using the phosphomolybdenum method based on the procedure described by Prieto *et al*. (1999). The assay is based on the reduction of Mo (+6) to Mo (+5) by the extracts and subsequent formation of green phosphate Mo (+5) complex at acidic pH. Briefly, 0.3 mL of graded concentrations of extracts was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against a blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as the blank. The TAC was expressed as milligram equivalents of ascorbic acid and calculated as shown in Equation 2:

$$TAC\ (mg\ AAE/g\ extract) = \frac{C \times V}{m} \quad \ldots\ldots\ldots\ldots (2)$$

where c = concentration of ascorbic acid in mg/mL extrapolated from the standard calibration curve; V = volume of extract in mL; and m = weight of crude plant extract in grams.

Nitric Oxide Radical Scavenging Capacity

The method described by Makhija *et al.* (2011) was used. Briefly, 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of extract prepared in phosphate buffer. The mixture was incubated at 25 °C for 150 min. To 1 mL of the incubated solution, 1 mL of Griess' reagent was added. Then, the absorbance was read at 546 nm. The % inhibition of nitric oxide radical was calculated as shown in Equation 3:

$$Nitric\ oxide\ scavenging\ activity\ (\%) = \frac{A_{control} - A_{extract}}{A_{control}} \times 100 \quad \ldots\ldots\ldots\ldots (3)$$

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric Acid Reactive Substances (TBARS) was estimated according to the method described by Ohkawa *et al.* (1979). Egg yolk homogenate (0.5 mL of 10 % v/v) and 0.1 mL of extract were mixed in a test tube, and made up to 1 mL with distilled water. Then, 50 µL of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min. This was followed by the addition of 1.5 mL of 0.8 % TBA in 1.1 % sodium dodecyl sulphate (SDS) and 50 µL 20 % TCA and vortexed. The resultant mixture was heated at 95 ^oC for 60 min. The absorbance of the sample was read at 532 nm. Inhibition of lipid peroxidation (%) was calculated as shown in Equation 4:

$$Inhibition\ of\ lipid\ peroxidation\ (\%) = \frac{A_{control} - A_{extract}}{A_{control}} \times 100 \quad \ldots\ldots\ldots\ldots (4)$$

STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Statistical analysis was performed using SPSS (21.0). Statistical significance was assumed at $p < 0.05$.

III. RESULTS

In Vitro Antioxidant Activities of Extracts of Dialium guineense Stem Bark

The DPPH radical scavenging activity and TAC of the ethanol extract were significantly higher than those of aqueous extract ($p < 0.05$). However, there were no significant differences in their NO scavenging activity, TBARS, and reductive potential ($p > 0.05$). The FRAP of aqueous extract was significantly higher than those of ethanol extract and ascorbic acid ($p < 0.05$). These results are shown in Figures 1 to 6.

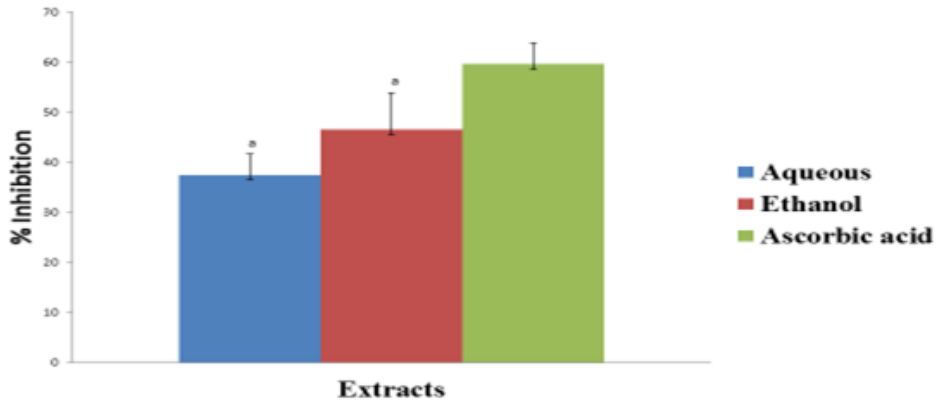

Figure 1: *DPPH* Scavenging Activity of Extracts of *Dialium guineense* Stem Bark.

$^{a}p < 0.05$, when compared with ascorbic acid.

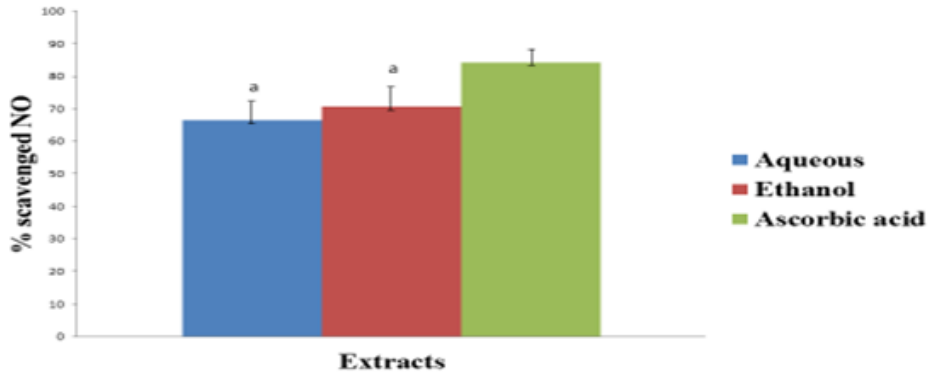

Figure 2: Nitric Oxide (NO) Scavenging Potentials of Extracts of *Dialium guineense* Stem Bark. $^{a}p < 0.05$, when compared with ascorbic acid.

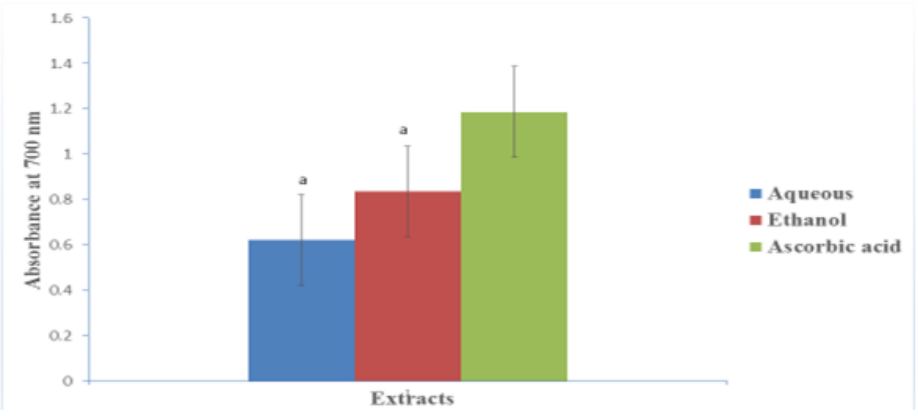

Figure 3: Reductive Potentials (RP) of Extracts of *Dialium guineense* Stem Bark. $^{a}p < 0.05$, when compared with ascorbic acid.

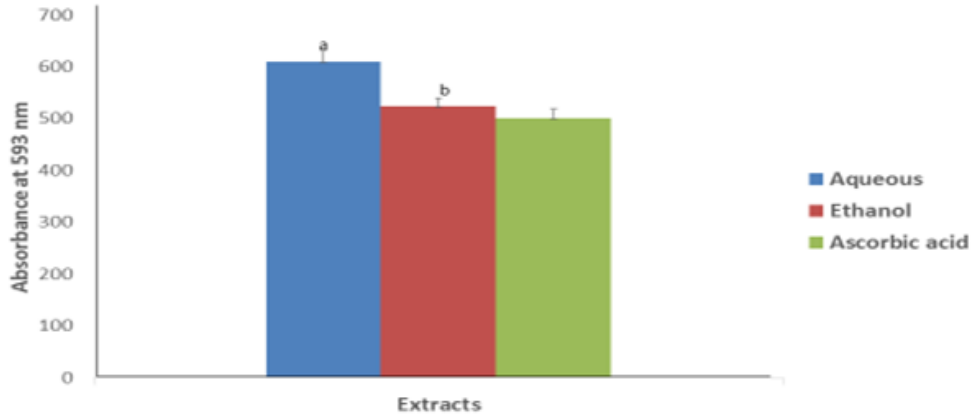

Figure 4: Ferric Reducing Antioxidant Potential (FRAP) of Extracts of *Dialium guineense* Stem Bark. $^{a}p <$ 0.05 & $^{b}p < 0.05$, when compared with ascorbic acid.

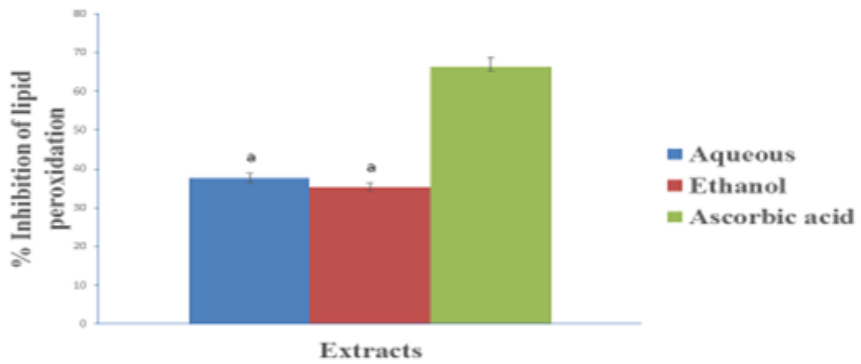

Figure 5: Thiobarbituric Acid Reactive Substances (TBARS) of Extracts of *Dialium guineense* Stem Bark. ^{a}p < 0.05, when compared with ascorbic acid.

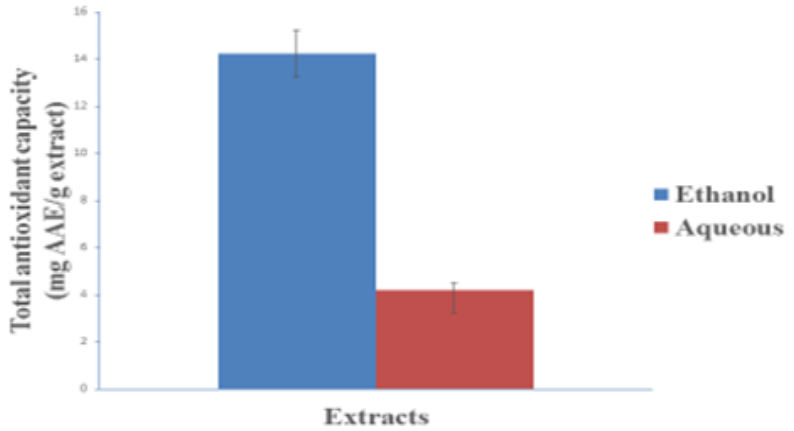

Figure 6: Total Antioxidant Capacity (TAC) of Extracts of *Dialium guineense* Stem Bark. $^{a}p < 0.05$, when compared with ethanol extract.

IV. DISCUSSION

Synthetic free radical scavengers such as butylhydroxyanisole (BHA) and butylhydroxytoulene (BHT) exist, but concerns over possible side effects necessitate the continued screening of natural plant parts for potential antioxidant properties. Antioxidants help prevent tissue damage by neutralizing the effects of free radicals. They act as scavengers. Antioxidants of nutritional origin play key roles in complementing *in vivo* antioxidant enzymes and molecules in the fight against free radicals. The DPPH radical can accept an electron or hydrogen ion to become a stable molecule (Du *et al*., 2009). Scavenging of DPPH radical is a widely used method for evaluating the free radical scavenging ability of plant or chemical materials (Lee *et al*., 2003).

The DPPH method is rapid, sensitive, and reproducible and requires simple conventional laboratory equipment for accessing antioxidant activity of samples (Du *et al*., 2009). Phenols and flavonoids represent phytochemicals whose relative abundance in plant extracts has been linked to antioxidant effect (Ayoola *et al*., 2008; Padmanabhan and Jangle, 2012). Reactive nitrogen species (RNS) are free radicals derived from the interaction of NO with oxygen or reactive oxygen species (ROS) (Tsai *et al*., 2007). Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide anion (Amaeze *et al*., 2011). It is synthesized by three isoforms of the enzyme nitric oxide synthase (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Nitric oxide (NO) is generated from amino acid L-arginine by the enzymes in the vascular endothelial cells, certain neuronal cells, and phagocytes (Nagmoti *et al.*, 2011). Low concentrations of NO are sufficient in most cases to effect the physiological functions of the radical. It is a diffusible free radical that plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities (Bhaskar and Balakrishnan, 2009). Chronic exposure to NO radical is associated with various carcinomas and inflammatory conditions such as juvenile diabetes, multiple sclerosis, arthritis, and ulcerative colitis. The toxicity of NO increases greatly when it reacts with the superoxide radical, forming the highly reactive peroxynitrite anion (ONOO−) (Amaeze *et al.,* 2011). Nitric oxide has been shown to be directly scavenged by flavonoids (Lakhanpal and Rai, 2007). Due to the reactivity of TBA with several reactive substances in a biological sample, a more widely accepted terminology called TBARS is now commonly used (Sun *et al*., 2001). Thiobarbituric acid reactive substances (TBARS) is now considered as a standard marker for lipid peroxidation-induced oxidative stress (Tsai and Huang, 2015).

Phenolic compounds are antioxidant agents which act as free radical terminators. The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron-seeking free radicals, thus down-regulating their menace in living cells (Uyoh *et al*., 2013). Studies have revealed a direct relationship between total phenol content and antioxidant effect in different plants. High phenolic content-containing plant materials have high radical scavenging abilities (Ayoola *et al*., 2008; Ghasemi *et al*., 2009; Hegazy and Ibrahim, 2012). The IC_{50} is the amount of antioxidant required to reduce the concentration of DPPH radical by 50 %. It is inversely proportional to antioxidant potential and hence a lower IC_{50} corresponds to higher antioxidant potential (Chanda *et al*., 2011). The results obtained in this study indicate that aqueous and ethanol extracts of *Dialium guineense* show great promise as important antioxidant molecules.

V. CONCLUSION

The results of this study suggest that phenolics present in the extracts may play a role in free radical scavenging activities of the medicinal plant.

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