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Phenolic Contents of Extracts of *Dialium Guineense* Stem Bark

Abu*, O. D.[1], Onoagbe, I.O.[2], Obahiagbon, O. [3]
[1,2] *Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.*
[3] *Physiology Division, Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Nigeria.*

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 phenolic contents 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 phenolic contents of the plant extracts were determined using standard methods.*
Results: *The ethanol extract had significantly higher total phenol, flavonoid, flavonol and proanthocyanidin contents, relative to the aqueous extract ($p < 0.05$).*
Conclusion: *These results indicate that Dialium guineense stem bark is a good source of phenolic compounds and has the potential to be used as a natural constituent of food and medicines.*

Keywords: *Dialium guineense, Flavonoids, Phenols, Proanthocyanidin, Tannins.*

I. INTRODUCTION

Over the last few decades there has been an intense search for novel compounds with potent biological effects. Plant-derived drugs have the added advantage of being readily available, effective, and offering a broad spectrum of activity. Medicinal plants exert their health effects via 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).

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

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substantial link exist between free radicals and more than sixty different health conditions, including aging, cancer, diabetes mellitus, Alzheimer 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 phenolic content**s** 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 UBHD330, 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.

Estimation of Total Phenolic Content (TPC)

Total phenolic content was determined according to the Folin and Ciocalteau's method as described by Cicco *et al*. (2009). Varied concentrations of gallic acid (0.2 - 1 mg/mL) were prepared in methanol. Then, 0.5 mL of the sample (1 mg/mL) was mixed with 2.5 mL of a ten-fold diluted Folin- Ciocalteau reagent and 2 mL of 7.5 % sodium carbonate. The mixture was allowed to stand for 30 min at room temperature, then absorbance was read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the control.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using the method described by Ayoola *et al*. (2008). Briefly, 2 mL of 2 % $AlCl_3$ in ethanol was added to 2 mL of extracts. A concentration of 1 mg/mL of the extract prepared in methanol was used. Similar concentrations of quercetin, the standard control were used. The absorbance was measured at 420 nm after 1 h of incubation at room temperature.

Determination of Total Flavonol Content

Flavonol content was determined according to the method described by Yermakov (1987). The quercetin calibration curve was prepared by mixing 2 mL of varied concentrations of standard quercetin solution (0.2 - 1.0 mg/mL) with 2 mL of 2 % aluminium trichloride and 6 mL of 5 % sodium acetate. The absorbance was read at 440 nm after 2.5 h of incubation at 20 ^{o}C.

Determination of Proanthocyanidin Content

The determination of proanthocyanidin was carried out according to the method of Sun *et al*. (1998). To 0.5 mL of 1.0 mg/mL of each extract was added 1 mL of 4 % methanol solution and 0.75 mL of concentrated hydrochloric acid. The mixture was left undisturbed for 15 min and the absorbance was read at 500 nm. Ascorbic acid was used as standard.

III. STATISTICAL ANALYSIS

Data are expressed as mean ± SEM. Statistical analysis was performed using SPSS (21.0). Groups were compared using Student's *t*-test. Statistical significance was assumed at $p < 0.05$.

IV. RESULTS

Phenolic Contents of Stem Bark Extracts of *D. guineense*

The ethanol extract had significantly higher total phenol, flavonoid, flavonol and proanthocyanidin contents, relative to the aqueous extract ($p < 0.05$). These results are shown in Figures 1 – 4.

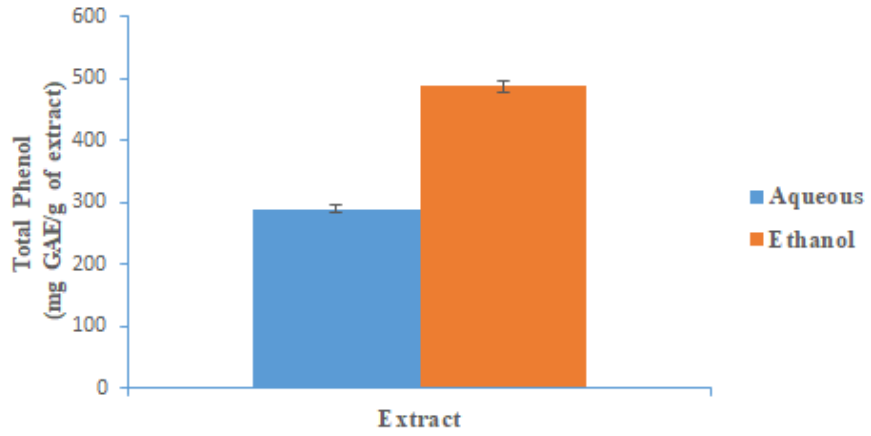

Figure 1: Total phenol content of extracts of *D. guineense* stem bark

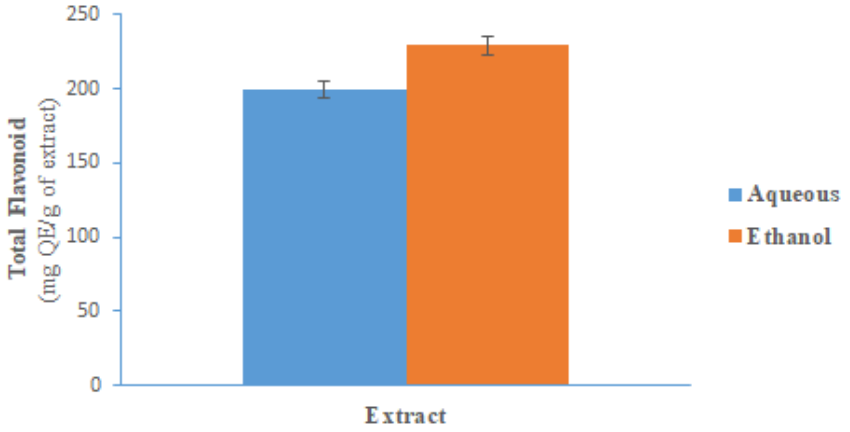

Figure 2: Total flavonoid content of extracts of *D. guineense* stem bark

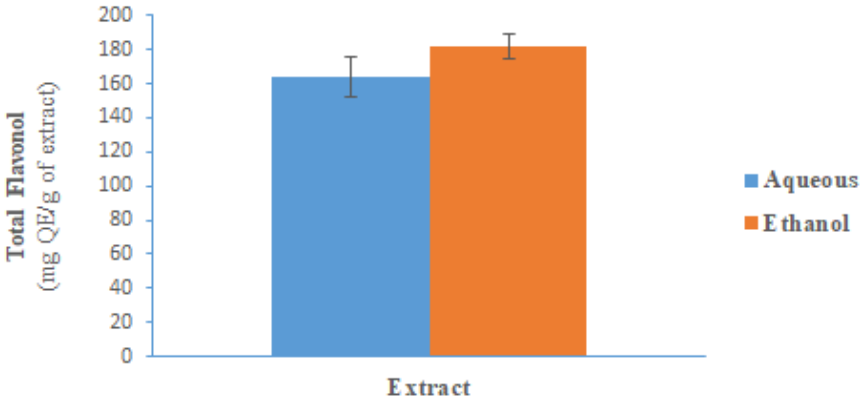

Figure 3: Total flavonol content of extracts of *D. guineense* stem bark

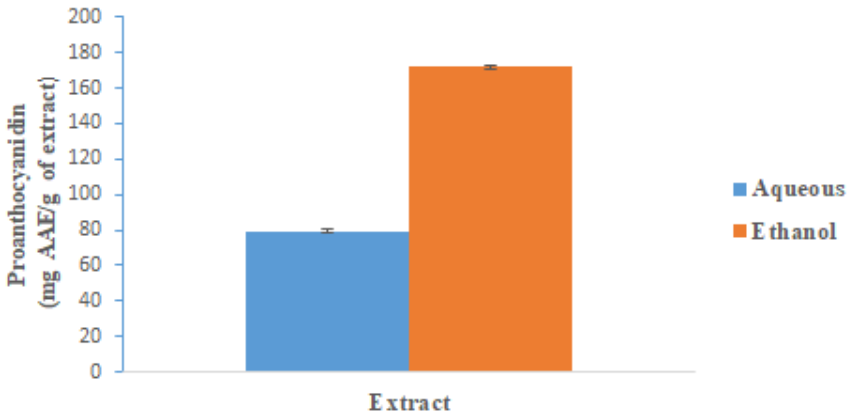

Figure 4: Proanthocyanidin content of extracts of *D. guineense* stem bark

V. DISCUSSION

Bioactive metabolites in plants contribute to the medicinal effects of plants (Akinpelu *et al.,* 2018). Phenolics possess diverse biological activities, such as antiulcer, anti-inflammatory, antioxidant, anti-tumour and antidepressant properties (Mamta *et al*., 2013). 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).

Flavonoids possess potent and appreciable antioxidant, anti-inflammatory and anticancer effects (Adetutu *et al.*, 2015; Oyedapo *et al*., 2015). In this study, the total flavonoid content of ethanol extract of *D. guineense* stem bark was significantly higher than that of the aqueous extract.

Proanthocyanidins are a class of polyphenols found in a variety of plants. Chemically, they are oligomeric flavonoids. Many are oligomers of catechin and epicatechin and their gallic acid esters. More complex polyphenols, having the same polymeric building block, form the group of tannins. Plant proanthocyanidins are involved in induced defense mechanisms against plant pathogens and predators. They possess vasodilatory, anti-carcinogenic, anti-allergic, anti-inflammatory, antibacterial, cardioprotective, immunostimulating, antiviral and estrogenic effects (Yildirim *et al*., 2015). The results of this study showed that the proanthocyanidin content of the ethanol extract was significantly higher than that of the aqueous extract, an indication that the ethanol extract may be a better antioxidant.

VI. CONCLUSION

The results obtained in this study suggest that *Dialium guineense* stem bark is a good source of phenolic compounds and has the potential to be used as a natural constituent of food and medicines.

VII. REFERENCES

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