The Antioxidant Properties and Polyphenol Content in Leaf and Bark of Neem (*Azadirachta Indica*)

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Abstract- ***Several researches have shown that Azadirachta indica (neem, family: Meliaceae) is one of those candidate plants which their different parts have protective effect and strong antioxidant potential used in traditional medicine. This study evaluated the antioxidant properties and polyphenol content in leaf and bark of methanol extracts (0- 25 µg/ ml) of neem, using 2, 2-diphenyl- 1 picrylhydrazyl (DPPH) - scavenging assay, ferric reducing antioxidant power (FRAP), total phenol and flavonoid contents. The dry powder of neem leaf and bark tree were extracted using methanol by maceration (1:6, m/v) method followed by concentration of filtrate using rotary evaporator. The extracts were tested for antioxidant activity using DPPH-scavenging assay, ferric reducing antioxidant power; flavonoid and total phenol contents by Folin Ciocalteu's reagent method. The experimental results revealed that both extracts (bark and leaf) exhibited antioxidant activities and possess polyphenols in a concentration dependent manner. Conversely, the bark extract showed the utmost activities compared to the leaf extracts (p< 0.05). Thus, the polyphenol concentrations of bark extracts revealed a positive correlation with its antioxidant capacity. Neem plant and its bark may be exploited for clinical medicine as potent factor because of its high antioxidant activity.***

Indexed Terms- ***Azadirachta indica, antioxidant, polyphenol, extract, bark, leaf.***

I. INTRODUCTION

Neem extracts are popular folk medicines in Africa, particularly in Nigeria (Prakash *et al*., 2007). The extracts, produced from various parts of the plant have been found to contain a varied selection of polyphenols (such as flavanoids, tannins, lignins) possessing strong antioxidant, antibacterial as well as anti inflammatory and immunomodulatory properties (Prakash *et al*., 2007; Brahmachari, 2008; Heyman *et al*., 2017). The beneficial use of chewing sticks made of Neem tree twigs has been recommended by the 2000 World Health Organization (WHO) Consensus Report on Oral Hygiene might be related to the mechanical act of plaque removal itself and enhanced salivation (Heyman *et al*., 2017). Nevertheless, it is likely that the antibacterial and antioxidant components in these chewing sticks also play a role (Chattopadhyay, 2003; Sithisarn *et al*., 2005). The influence of aqueous Neem preparations on oral streptococci has been explored in several in vitro studies. Neem bark aqueous extracts have shown to inhibit oral streptococci and induced a significant reduction in their surface adhesion (Schumacher *et al*., 2011). In fact, a significant decrease in plaque accumulation and bacterial counts were found following oral treatment with Neem extract (Pai *et al*., 2004). Moreover, their antimicrobial capacity, potent anti-inflammatory and antioxidant properties have shown to suppress oxidative stress that accompanies pathologies (Alzoreky *et al*., 2003). Thus, one may assume that in addition to their antibacterial activity Neem extracts may ameliorate periodontal diseases by the attenuation of inflammation through the suppression of reactive oxygen species (ROS), exerted by the plant-derived antioxidants. Also, current studies have shown that a variety of microbial species, platelets and red blood cells (RBC) have the ability to avidly bind to their surfaces a large assortment of plant-derived antioxidant flavonoids and such coated cells show marked antioxidative capacities (Biswas *et al*., 2002; Heyman *et al*., 2017).

However, there are no much studies on the possible antioxidant properties and polyphenol contents of methanol extracts of leaf and bark of this plant in an *in vitro* model.

II. MATERIALS AND METHODS

MATERIALS

- Chemicals and Reagents

1,1-diphenyl-2picrylhydrazyl (DPPH), 2,4,6-tris (2-pyridyl)-S- triazine (TPTZ), sodium trioxocarbonate (iv) (Na_2CO_3), Folin reagent, methanol, distilled water, aluminum chloride ($AlCl_3$), acetate buffer (0.2M, pH of 3.6), ferric chloride ($FeCl_3$), and hydrochloric acid (HCl) were obtained from Sigma-Aldrich (St. Louis, MO USA). All other chemicals and solvents used were of analytical grade.

- Plant Collection and sample preparation

The fresh leaves and barks of *Azadirachta indica* were collected from neem tree from the Federal Polytechnic, Auchi, Nigeria. The leaves and barks were air dried and pulverized into coarse powder. After which, extractions were done by maceration for 72 hours using methanol (1:6, w/v), at room temperature and filtered (filter paper Whatman No. 2) under vacuum. The filtrate was then evaporated and lyophilized to obtain a dry extract based on the methods of Vietmeyer (2000).

METHODS

- Determination of Total Phenol

The total phenol content was determined according to the method of Singleton *et al.,* (1999). Briefly, appropriate dilutions of the extracts were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45 ^oC and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

- Determination of Total Flavonoid

The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda *et al.,* (2005). About 0.5 ml of appropriately diluted sample extract were mixed with 0.5 ml methanol, 50 µl 10% $AlCl_3$, 50 µl 1 M potassium acetate and 1.4 ml water and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid content calculated as quercetin equivalent.

- Free Radical Scavenging Ability (DPPH)

The free radical scavenging ability of the cerebral tissue against DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radicals were evaluated. Tissue homogenates were mixed with 600µL, 0.3mMm methanolic solution containing DPPH radicals, the mixtures was left in the dark for 30 min and the absorbance of the resulting golden yellow was measured at 517nm (Brand-Williams *et al*., 1995).

- Reducing Power Ability

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^oC for 20 min and then 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 801 × g for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing power was subsequently calculated using ascorbic acid equivalent.

III. STATISTICAL ANALYSIS

All values obtained were expressed as mean ± SEM of three independent experiments carried out in different days using Duncan's New Multiple Range Tests where appropriate.* Represent significant difference from control at $p < 0.05$.

IV. RESULTS

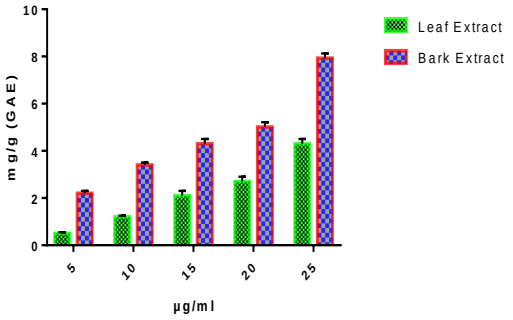

Figure 1: The Total phenol content of the leaf and bark extracts of *Azadirachta indica***.** Data are presented as mean ± SEM values from three independent experiments done in duplicate in different days with significant difference from control at $p < 0.05$.

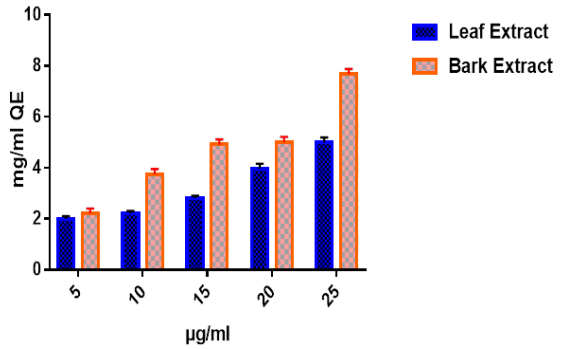

Figure 2: The flavonoid content of the leaf and bark extracts of *Azadirachta indica***.** Data are presented as mean ± SEM values from three independent experiments done in duplicate in different days with significant difference from control at $p < 0.05$.

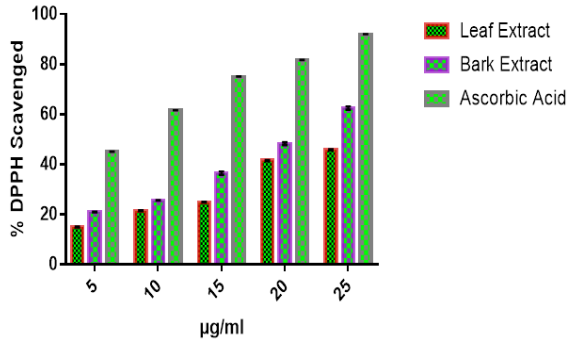

Figure 3: The % DPPH radical scavenged of the leaf and bark extracts of *Azadirachta indica***.** Data are presented as mean ± SEM values from three independent experiments done in duplicate in different days with significant difference from control at $p < 0.05$.

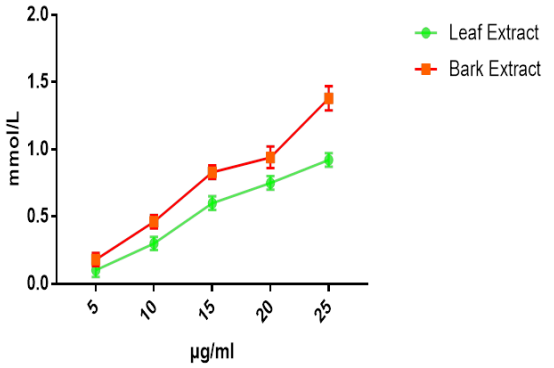

Figure 4: The ferric reducing antioxidant power of the leaf and bark extracts of *Azadirachta indica***.** Data are presented as mean ± SEM values from three independent experiments done in duplicate in different days with significant difference from control at $p < 0.05$.

V. DISCUSSION

It is an established fact that polyphenolic compounds possess remarkable antioxidant activities which are commonly present in plants like Neem. (Siddiqui, *et al.*, 1992; Sultana, *et al*., 2007). Neem is one of those candidate plants which has polyphenols such as phenol and flavonoid with strong antioxidant potential (Chattopadhyay, 2003; Sithisarn *et al*., 2005). Flavonoids are broadly spread and form major colouring components of plants. They are a large group of phenolic compounds and are responsible for a variety of pharmacological activities (Cook and Samman, 1996; Pandey, 2007; Kumar and Pandey, 2013). They exist as aglycones, glycosides and methylated derivatives and can be further divided into different groups like the flavones, flavonols and flavanones (Kumar and Pandey, 2013). Some plant flavonoids have been shown to exhibit protective effects against infectious, cardiovascular, carcinogenic and age-related diseases (Pandey, 2007; Kumar and Pandey, 2013). Moreso, reports have revealed that there are active compounds in neem plant, like nimbin, azadirachtin, nimbidiol, quercetin, and nimbidin (Chattopadhyay *et al*., 1992; Bhanwra *et al*., 2000). In addition, the components of the neem tree and its parts such as bark, seed, leaf, fruit, gum and oil have been revealed to contain compounds offering some notable therapeutic applications

(Biswas *et al*., 2002). Consequently, Figures 1- 2 explain the phenolic and flavonoid contents of methanol extracts of leaf and bark of *Azadirachta indica*. The leaf and bark extracts reveal the presence of phenol and flavonoids in concentration dependent manner. However, the bark extract tend to have higher phenolics and flavonoids contents than the leaf extract. Several reports have shown that *Azadirachta indica* leaf and bark extracts exhibit antioxidant property (Ghimeray, *et al.,* 2009; Sultana, *et al*., 2007). The anti-oxidant activity of neem leaf and bark extracts by cold maceration method is very important and has not been evaluated. DPPH free radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and β-carotene bleaching model are some of the assays used to evaluate antioxidant capacity of plant materials (Chen *et al.,* 2012). Specific mechanism to measure the antioxidant capacity of plant materials are possessed by each of these assays, where the concept of the antioxidant capacity evaluation is based on the ability of the antioxidant to scavenge free radical compound since free radicals are main cause of the propagation phase in the oxidation process. In this study, DPPH free radical-scavenging and FRAP assays were used to evaluate the antioxidant extracts in the *Azadirachta indica.* DPPH is stable organic nitrogen radical (1, 1-diphenyl-2-picrylhydrazyl). It free radicals are reduced by an antioxidants extract to their hydroxyl group within the assay time, thus the remaining DPPH free radicals are measured spectrophotometrically at 515mm. The ability of the extract to scavenge for free radicals may be through hydrogen or electron – donating mechanism (Brand-Williams *et al*., 1995; Ho *et al.,* 2013). More so, FRAP assay is another conventional method used in evaluating the antioxidant capacity of plant extracts. The principles of the FRAP assay is based on the antioxidant strength in reducing ferric tripyridyltriazine complex to its ferrous form. The intensity of the blue colour formation is proportional to the concentration of the ferrous form and the antioxidant capacity of the extract. Antioxidant compounds that exhibit antioxidant capacity in FRAP assay are usually election donors as they reduce the oxidized intermediate to the stable form in order to eliminate the oxidation chain reaction (Brand-Williams *et al*., 1995). Figures 3 and 4 represent DPPH radical scavenging properties and ferric reducing antioxidant power leaf and bark of *Azadirachta indica*.

We observed that both leaf and bark extracts were able to exert a significant effect on DPPH radicals and in formation of ferrous ions from ferric ions in the concentration range tested (5 - 25µg/ml). The bark extract scavenged free radicals; reduce ferric ions to its ferrous state more than the leaf extract in concentration dependent way. This research findings agree with the works of Usha, (2001), that used different parts of leaf stem bark and seed of neem extracted with methanol and their efficacy were tested against pathogens and stress mediated disaeses, as the neem stem bark and neem leaf recorded about 56s-87% and 51-80% parasitemia inhibition respectively. This correlates with Chi -Chun *et al*., (2006) position, that the higher the reducing power, the greater the antioxidant activity of the plant being tested.

CONCLUSION

This research work has justified some traditional uses of *A. indica* in the management of stress induced human diseases. The exploitation of this plant may offer solutions to some prevailing clinical and nutritional conditions, since it is becoming obvious that the natural vegetation around us is enriched with solutions of most of our health challenges.

DECLARATIONS

- Author Contribution statement

Olaitan D. Johnson: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Lawal Taofeeq, Zubair Sadiya and Akpaka Charles Participated in the experiments; Contributed reagents, materials, analysis tools or data.

- Funding statement

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- Data availability statement

Data will be made available on request.

- Declaration of interests statement

The authors declare no conflict of interest.

- Additional information

No additional information is available for this paper.

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