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Comparative Antioxidant Activity of Water Extract of *Azadiractha indica* Stem Bark and *Telfairia occidentalis* Leaf

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Abstract: The antioxidant activity of *Azadirachta indica* stem bark and *Telfairia occidentalis* leaf aqueous extract was studied. The Total Phenolic Content (TPC) was determined using folin Ciocalteu method while the Total Flavonoid Content (TFC) was determined using aluminum chloride method. Antioxidant activity was determined using 2, 2-diphenyl-1-picryl hydrazine (DPPH) inhibition. *Telfairia occidentalis* extracted more phenols (11.32 g GAE/ 100 g) than *Azadirachta indica* stem bark (10.74 g GAE/100 g) but not significantly different ($p<0.05$). *Azadirachta indica* stem bark extracted more flavonoid content (5.21g QE/100 g) than *Telfairia occidentalis* leaf (0.96 g QE/100 g). *Azadirachta indica* stem bark inhibited more free radicals (83%) than *Telfairia occidentalis* leaf (65%). This study showed that *Azadirachta indica* stem bark had higher antioxidant activity compared to *Telfairia occidentalis* leaf.

Key word: Antioxidant activity, polyphenols, DPPH free radicals, *Azadirachta indica, Telfairia occidentalis*

INTRODUCTION

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds (Demiray *et al*., 2009). Reactive free radicals, such as superoxide anion (O_2^-), hydroxyl radical (OH), and peroxyl radical ($ROO^{\cdot}$), are particularly reactive and are known to be a biological product in reducing molecular oxygen. Damage mediated by free radicals results in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions, which have generally been considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis(Biglari *et al*., 2006).

Antioxidants are both natural and synthetic compounds, able to scavenge free radicals and to inhibit oxidation processes (Hayat *et al*., 2010). Many synthetic antioxidants such as Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT) are very effective and are used for industrial processing but they possess some side effects and toxic properties to human health therefore, there is an increasing interest in natural antioxidants, e.g., polyphenols, present in medicinal and dietary plants (Anagnostopoulou *et al*., 2006; Silva *et al*., 2005). Antioxidants play an important role in defending the body against free radicals damage. They work by preventing the formation of new free radical species, converting existing free radicals into less harmful molecules and preventing radical-chained reactions (Ismail *et al*., 2010).. The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body (Ismail *et al*., 2004).

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Javanmardi *et al*., 2003).

Polyphenols possess ideal structural chemistry for free radical-scavenging activity, and they have been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radical to stabilise and delocalise the unpaired electron (chainbreaking function), and from their ability to chelate transition metal ions (Rice-Evans *et al*., 1997). Hence, are capable of protecting against ROS mediated

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damage may have potential application in prevention and/or curing of diseases (Shukla *et al*., 2009).

It is an established fact that polyphenolic compounds possess remarkable antioxidant activities which are present quite commonly in the plant family Meliaceae (Nahak and Sahu, 2010). *Azadirachta indica* A. Juss, known as neem in vernacular, belongs to the family meliaceae and is widely distributed in Asia, Africa and other tropical parts of the world (Sombatsiri *et al*., 1995). Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control diseases like leprosy, intestinal helminthiasis, respiratory disorders, constipation, and skin infections (Biswas *et al*., 2002). The Neem tree contains more than 100 bioactive ingredients and the most important bioactive compound is azadirichtin (Nahak and Sahu, 2010).

The Neem tree is also considered as a natural insecticide/ pesticide plant and the quality of pesticide and pharmacological products depend upon the contents of azadirachtin and nimbin in the plant (Sidhu *et al*., 2004). The bark of the neem has been reported to have higher phenolic and antioxidant activity compared to the leaf (Ghimeray *et al*., 2009; Olabinri *et al*., 2009).

Telfairia occidentalis belongs to the family Curcubitaceae and has simple, dark green leaves that is as wide as 18 cm and long as 35 cm. *Telfairia occidentalis* contains nutrients such as proteins, carbohydrates, vitamins, minerals and fiber. It also contains oxalates, saponins, glycosides, flavonoids, alkaloids and resins (Iweala and Obidoa, 2009).

In Nigeria, the consumption of the leaf of *Telfairia occidentalis* as a leafy vegetable in the diet or as an infusion in medicinal preparation is being promoted in view of the various medicinal properties such as antianeamic, antidiaebetic and as a purgative leafy vegetable (Oboh *et al*., 2006). The choice of what medicinal plant to use as a source of natural antioxidant may dependent on well documented information of known polyphenolic compounds and antioxidant activity of various plants. Therefore, this study is aimed at comparing the antioxidant activity of *Azadirachta indica* stem bark and *Telfairia Occidentalis* leaves as part of the data that can help in the usage of these plant materials in medicine.

MATERIALS AND METHODS

Chemicals: Methanol, Gallic acid, 2, 2-diphenyl-2-picrylhydrazyl (DPPH) and Quercetin, were all analytical grades.

Materials and extraction methods: Fresh *Azadirachta indica* barks were obtained from the Agricultural garden in Babcock University while *Telfairia occidentalis* leaves were obtained in a local market in Ilisan-Remo, Ogun State. The samples were thoroughly rinsed and air dried. They were ground to fine powder and 20 g of each plant sample was soaked with distilled water for 72 h. The filtrate from each sample was concentrated using the rotary eveporator at 40ºC. The dried extracts were weighed and stored frozen.

This research was carried out in Babcock University Ilisan Remo between January and March 2010.

Determination of total phenolic content: The total phenolic content was estimated by Folin-Ciocalteu colorimetric method, based on the procedure in Singleton and Rossi (1965). Procedure: Briefly, the crude extract (50 mg) was mixed with Folin-Ciocalteu reagent (0.5 mL) and deionized water (7.5 mL). The mixture was kept at room temperature for 5 min, and then, 10 mL of 7% sodium carbonate was added to the mixture, and then incubated for 90 min at room temperature. After incubation the absorbance against the reagent blank was determined at 760 nm. The total phenolic content of the plant was expressed as Gallic acid equivalent (g/100 g dry weight. All samples were analyzed in triplicates.

Determination of total flavonoid content: The TFC were measured following a spectrophotometric method (Dewanto *et al*., 2002). Briefly, extract of each plant material (1 mL containing 100 µg/mL) were diluted with water (4 mL) in a 10 mL volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 mL) was added to each volumetric flask; at 5 min, 10% $AlCl_3$ (0.3 mL) was added; and at 6mins 1.0 M NaOH (2 mL) was added. Water (2.4 mL) was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm. TFC were determined as quercetin equivalents (g/100 g of dry weight). Three readings were taken for each sample and the result averaged.

Determination of DPPH radical scavenging activity: A solution of DPPH mixed with that of a substance that can donate a hydrogen atom, gives rise to the reduced form with change in colour, from deep violet to pale yellow colour. DPPH is a useful reagent for investigating free radical scavenging activities of phenolic compounds. The reduction of DPPH absorption is indicative of the capacity of the extract to scavenge free radicals, independently of any enzymatic activity.

Procedure: This was carried out according to the 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay System (Mensor *et al*., 2001). One ml of a 0.3 mM DPPH methanol solution was added to a 2.5 mL solution of the extract and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%), using the formula:

$$AA\% = [(Abs_{blank} - Abs_{sample}) \times 100]$$

Abs = Absorbance

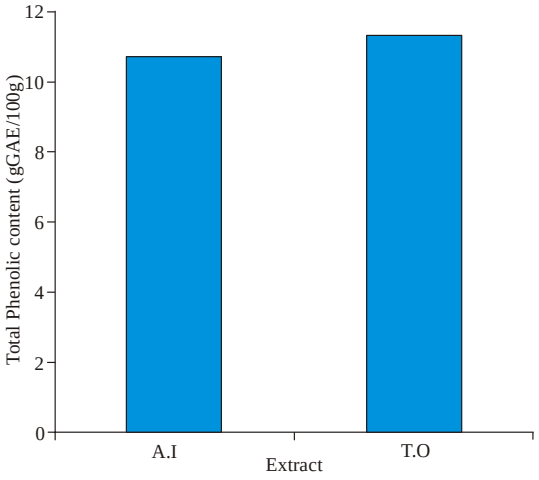

Fig. 1: Total Phenolic Content (TPC) of *azadirachta indica* bark and *telfairia occidentalis* leaves water extracts. The values represent the mean of triplicates ±SD of each extract. *Azadirachta indica* (A.I), *Telfairia occidentalis* (T.O)

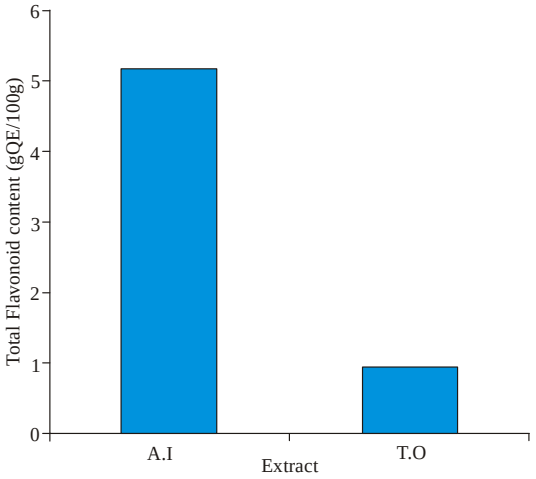

Fig. 2: Total Flavonoid Content (TFC) of azadirachta indica bark and telfairia occidentalis water extracts. The values represent the mean of triplicates ± SD of each extract

RESULTS

Total Phenolic Content (TPC): The total phenolic content of water extract of *azadirachta indica* bark and *telfairia occidentalis* leaves is shown in Fig. 1. The total phenolic contents of water extract of *azadirachta indica* bark and *telfairia occidentalis* leaves were expressed in gram Gallic Acid Equivalent per 100 g. The result of the total phenolic content of the water extract of *azadirachta indica* bark and *telfairia occidentalis* leaves showed that *azadirachta indica* bark yielded 10.74±0.36 gGAE/100 g while *telfairia occidentalis* leaves yielded 11.32±0.2 g GAE/100 g.

Total Flavonoid Content: The total flavonoid content of water extract of *A. indica* stem bark and *T. occidentalis* leaves is shown in Fig. 2. The total flavonoid content of water extract of *A. indica* stem bark and *T. occidentalis* leaf was expressed in gram Quercetin Equivalent per 100 g. The values represent the mean of triplicates ±SD of each extract.

The result of the total flavonoid content of the water extract of *azadirachta indica* bark and *telfairia occidentalis* leaves showed that *azadirachta indica* bark yielded 5.21±0.01g QE/100g while *telfairia occidentalis* leaves yielded 0.96±0.01 g QE/100 g.

Inhibition of DPPH Radicals: The DPPH radical-scavenging activity of the water extract of *azadirachta indica* bark and *telfairia occidentalis* leaves is shown in Fig. 3. *Azadirachta indica* bark extract inhibited 83.34±1.83%, while *telfairia occidentalis* leaves extract inhibited 64.51±0.09% of the DPPH free radicals.

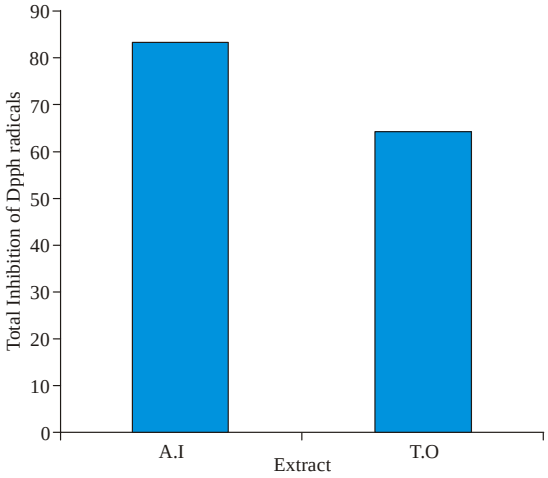

Fig. 3: Percentage inhibition of the DPPH radicals by water extracts of *azadirachta indica* bark and *telfairia occidentalis* leaves. The values represent the mean of triplicates ± SD of each extract

DISCUSSION

Polyphenolic contents: The result of this study (Fig. 1) showed that the TPC of the *telfairia occidentalis* leaves extract was not significantly higher ($p<0.05$) than *azadirachta indica* stem bark extract. This study also showed that the flavonoid content of *azadirachta indica* stem bark extract was significantly higher ($p<0.05$) than the *telfairia occidentalis* leaves extract. In the study conducted by Salawu *et al*. (2006), Protocatechiuc acid (PRA) and Caffeic acid (CA) were identified in *Telfairia occidentalis*. The phenolic content of the water extract of Azadirachta indica bark, reported by Ghimeray *et al*. (2009) was higher than the phenolic

content reported in this study. The neem tree from the different tropical parts of the world is reported to contain high level of polyphenolic compounds, but due to the wide range of geographical distribution, a large variety of morphological and biochemical characteristics have been reported. It has also been re-ported that phenolic contents of neem can be influenced by geographical locations and other abiotic factors (Ghimeray *et al*., 2009).

Antioxidaant activity: DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al*., 2002).

The result from this study showed that the antioxidant activity of *azadirachta indica* bark extract was significantly higher ($p<0.05$) than *telfairia occidentalis* leave extract. This showed that *azadirachta indica* bark extract inhibited more DPPH free radicals than *telfairia occidentalis* leave extract. This could be due to the high flavonoid contents of *azadirachta indica* bark extract compared with *telfairia occidentalis* leaf extract.

It has been recognized that flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants. They show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Pourmorad *et al*., 2006; Omale and Okafor 2008). In the study conducted by Oboh *et al*. (2006), the aqueous extract of *telfairia occidentalis* exhibited higher antioxidant activity compared to the ethanolic extract. The two plants in this study are mostly used in the aqueous form for medicinal purposes. The result of this study contributes to the medicinal claim of these plants.

CONCLUSION

In the present study, *telfairia occidentalis* leaves water extracted more phenols. However, the *azadirahta indica* bark water extract inhibited more DPPH free radicals. The higher flavonoid content of *azadirahta indica* bark water extract, suggest that flavonoids were responsible for the higher free radical inhibition of *azadirahta indica* bark water extract.

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