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Antioxidant Properties of Phenolic Extracts of African Mistletoes (*Loranthus begwensis L.*) from Kolanut and Breadfruit Trees

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Abstract
Mistletoe (*Loranthus begwensis* L.) has been used ethno-botanically for the management of several tropical diseases for centuries; and the medicinal properties have been associated with their host plant. Therefore, this study sought to investigate the antioxidant properties of mistletoe from two host plants (breadfruit and kolanut trees). The result of the study revealed that mistletoe from kolanut tree (0.69 mg/g) had higher total phenol content than that of breadfruit tree (0.49 mg/g). Furthermore, the extracts chelate Fe^{2+} and scavenge DPPH radicals in a dose-dependent (0 – 30 mg/ml) pattern. Nevertheless, the EC_{50} revealed mistletoes from kolanut as having higher DPPH scavenging (15.77mg/ml) than that of breadfruit (16.29mg/ml), while *L.begwensis* from Breadfruit tree had higher Fe^{2+} chelating ability (1.97mg/ml) than that of Kolanut tree (2.23mg/ml). Likewise, mistletoe from kolanut (27.5mg/AAE g) had higher ferric reducing ability (FRAP) than that of breadfruit (22.0mg/AAE g). Although both mistletoe extracts showed promise as good antioxidant sources, the total phenol content and the antioxidant capacity pattern of the extracts suggest host dependency.
Keywords: antioxidant, breadfruit tree, kolanut tree, *Loranthus begwensis* L.

1. INTRODUCTION

Recent research in the area of preventive medicine shows that nutrition plays a vital key role in reducing the risk factor of certain chronic disease. Discoveries in the few decades revealed that extract from plants contain not only minerals and primary metabolite but also a diverse array of secondary metabolite with antioxidant properties (Akinmoladun et al., 2007). Antioxidants are powerful free radical scavengers in the body, while free radicals are highly reactive chemical substances such as superoxide, hydroxyl radical, singlet oxygen etc. (Alia *et. al.* 2003) that travel around in the body and cause damage to the body cells. Free radical damage is one of the most prominent causes of devastating diseases that are responsible for death of many people in the world, such as cardiovascular disease, which can manifest as heart attacks, and cancer (Amic *et.al.* 2003). The therapeutic effects of several plants and vegetables, which are used in traditional medicine, are usually attributed to their antioxidant compounds. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns (Akinmoladun et al., 2007).

Phenolic compounds are diverse secondary metabolites abundant in plant tissues (Grace and Logan, 2000); they display a vast variety of structure which can be divided into three main classes, which are flavonoids, tannin and phenolic acids (Strube *et al*., 1993). They possess biological properties such as: antioxidant, antiapoptosis, anti-aging,anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Han, 2007).

African mistletoe is a semi-parasitic plant found growing on a host of evergreen and deciduous trees all the year round. It is an obligate parasite, obtaining part of its food from the host plant. It depends on its host for minerals and water only, but obtains carbohydrate by the process of photosynthesis (Osadebe and Uzochukwu, 2006). Mistletoes have been used in the treatment and management of many diseases for many years, both in traditional and complementary medicine in some part of Africa. It has also been reported to be effective in the management of chronic metabolic disorders such as diabetes (Obatomi et al., 1994). A number of biological effects such as anticancer, antimycobacterial, antiviral, apoptosis-inducing and immunomodulatory activities have been reported for mistletoes (Onay-Ucar et al., 2006). Mistletoe teas and infusion have been seen as excellent remedy with high esteem and have been recommended ethno-medicinally for the prevention and management of stroke in parts of Nigeria, and it is also believed to improve circulatory system and heart function in tradition medicine (Deeni and Sadiq, 2002). The chemical composition of mistletoe varies somewhat; depending on the host plant tree (Luczkiewics, et al., 2001, Stein and Berg, 1997). Hence, this work is aimed investigating the antioxidant properties of phenolic extract of L. begwensis L isolated from Kolanut and Breadfruit trees in South West part of Nigeria and their possible mechanism of action.

2. MATERIALS AND METHODS

2.1 Chemicals

DPPH (1, 1-diphenyl-2 picrylhydrazyl) radical and 1, 10-phenanthroline reagents were obtained from Sigma-Aldrich, USA. All other chemicals and reagent used were of analytical grade and the water used was glass distilled.

2.2 Plant materials and extraction

Young leaves of *L. begwensis* were harvested from Kolanut and Breadfruit trees at a local farm plantation in Ikeji-Arakeji, Nigeria and were taken to the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure for identification and authentication. The leaves were washed with distilled water to remove dirt; the water was later drained off and the leaves were then sun-dried to a constant weight before they were powdered and kept in an airtight container prior to analysis. The extraction of phenolic compounds was carried out according to the method reported by Chu *et al*., (2002).

2.3 Total phenol determination

The total phenol content was determined by mixing 0.5 ml of the sample extracts with 2.5 ml 10% Folin-Cioalteau reagent (v/v) and 2.0 ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45^{o}C for 40 min, and the absorbance was measured at 765 nm using a spectrophotometer. Tannic acid was used as standard phenol (Singleton et al., 1999).

2.4 Determination of reducing property

The reducing property was determined by assessing the ability of the sample extracts to reduce $FeCl_3$ solution as described by Pulido et al., (2002). Briefly, appropriate dilutions were mixed with 2.5 ml 200 mm sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixtures were incubated at 50^{o}C for 20 min. Thereafter, 2.5 ml 10% Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min. then 5 ml of the resulting supernatant was mixed with equal volume of
water and 1 ml of 0.1% ferric chloride. The absorbance was taken at 700 nm against a reagent blank.

2.5 Free radical scavenging assay

The free radical scavenging ability of the sample extracts against DPPH (1,1-diphenyl-2 picrylhydrazyl) free radical was evaluated. Briefly, appropriate dilution of the extracts was mixed with 1 ml 0.4 mM methanolic DPPH radical solution. The mixture was left in the dark for 30 min and the absorbance was taken at 516 nm (Ursini et al., 1994).

2.6 Fe^{2+} chelation assay

The ability of the sample exracts to chelate Fe^{2+} was determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al., (2005). Briefly, 150µl of freshly prepared 500µM $FeSO_4$ was added to a reaction mixture containing 168µl of 0.1M Tris-HCl (pH7.4), 218µl saline and the methanolic leaf extracts (0-500µl). The reaction mixture was incubated for 5 min, before the addition of 13µl of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer.

2.7 Determination of EC_{50}

The EC_{50} was calculated using non-linear regression analysis. EC_{50} is defined as the concentration of phenolic extracts required to cause 50 % of the antioxidant activity.

2.8 Data Analysis

The results of three replicate experiments were pooled and expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to analyze the mean and the post hoc treatment was performed using Duncan multiple range test. Significance was accepted at $P<0.05$.

3. RESULT

The result of the total phenol content of *L.begwensis* isolated from both Kolanut and Breadfruit tree is presented in Table 1. The result of the phenol content revealed that *L. begwensis* isolated from Kolanut tree had higher total phenol content (0.69 mg/g) than that from Breadfruit tree (0.49 mg/g). Nevertheless, the values compare favourably with that of red grape and strawberry (Sun et al., 2002) and were found higher than that of ethanolic extract of Struchium sparaganophora (Oboh et al., 2012).

Table 1. Total phenol content (mg/g) of extracts from *L.begwensis*

Sample	Total phenol content (mg/g)
L. begwensis (Kolanut tree)	0.69 ± 0.08^{a}
L. begwensis (Breadfruit tree)	0.49 ± 0.01^{b}

Data are mean- SD values of triplicate determinations.
Values with the same subscript letter in the same column are not significantly different (P>0.05)

Likewise, the result of the ferric reducing ability of the extracts as presented in Table 2. Also revealed that *L.begwensis* from Kolanut tree (27.50 mg/ AAE g) is significantly higher (P>0.05) than that from Breadfruit (22 mg/ AAE g). The values were higher compare to what was reported by Oboh et al (2012) for blanched and unprocessed Stuchium sparaganophora (38.6 and 43.4 mg AAE/ 100g).

Table 2. Ferric reducing properties (mg/AAE g) of *L.begwensis* extract.

Sample	Ferric reducing properties
L. begwensis (Kolanut tree)	27.50 ± 0.5^{a}
L. begwensis (Breadfruit tree)	22.00 ± 0.2^{b}

Data are mean- SD values of triplicate determinations.
Values with the same subscript letter in the same column are not significantly different (P>0.05)

The scavenging ability of each phenolic extracts against stable DPPH in methanolic solution is presented in figure 1 and it is expressed as percentage (%) scavenging ability. The results for both phenolic extracts followed a dose-dependent pattern. The free radical scavenging ability of the *L.begwensis* extract from kolanut tree performs better than that from Breadfruit tree and this also is in agreement with total phenol content of the extracts. In accordance, several reports had established a correlation between the total phenol content of plant food and their antioxidant properties (Sun et al., 2002; Chu *et al*, 2002; Oboh 2006). The ability of the *L. begwensis* extract to chelate Fe^{2+}, a potent free radical initiator in the cell is represented in Figure2. This follow a dose-dependent pattern as the highest % Fe^{2+} chelation was achieved at the highest dose for the two extract of *L.begwensis*. But, extract of *L.begwensis* from Breadfruit was found to chelate Fe^{2+} better than that from Kolanut tree. The reason for this cannot be readily explained.

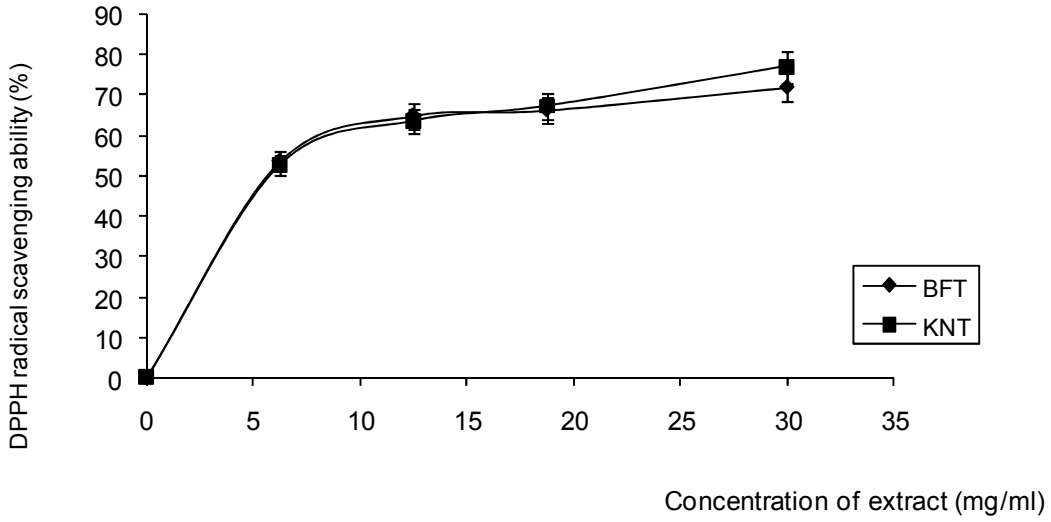

Figure 1**:** 1.1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging ability of *L. begwensis* extracts.

Table3. EC_{50} of DPPH scavenging ability of *L.begwensis* extract.

Sample	EC_{50}
L. begwensis (Kolanut tree)	15.77
L. begwensis (Breadfruit tree)	16.29

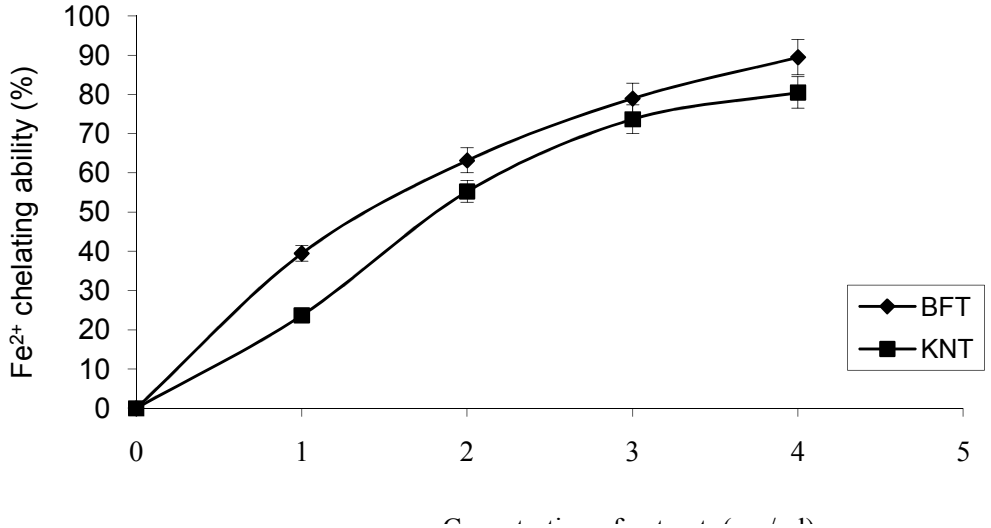

Figure 2: Iron II chelating ability of *L. begwensis* extracts

Table4: EC_{50} of Fe^{2+} chelation ability of *L.begwensis* extract

Sample	EC_{50}
L. begwensis (Kolanut tree)	2.23
L. begwensis (Breadfruit tree)	1.97

4. DISCUSSION AND CONCLUSION

In recent years, phenolic compounds have attracted the interest of researchers because they show promise of being powerful antioxidants that can protect the human body from free radicals, the formation of which is associated with the normal natural metabolism of aerobic cells (Bors et al., 1996; Halliwell, 1996; Oboh and Rocha, 2007). The values obtained for both extracts indicated that *L.begwensis* is rich in phenolic compounds. Pheenolic compounds have been reported to have antioxidant properties, they act as free radical scavengers, mope-up reactive oxygen species (ROS) and they also chelate meta ions (Zhang et al., 2001; Oboh and Akindahunsi, 2004; Oboh 2006). Reducing ability is a measure of the ability of the extracts to reduce Fe^{3+} to Fe^{2+}; a measure of their antioxidant properties, that is, the higher the reducing property the higher antioxidant activity. Antioxidants are strong reducing agents and this is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure (Rice-Evans et al., 1996; 1997; Oboh and Rocha 2007). Benzie and Strain (1999) considered the antioxidant as any species that reduces the oxidation species that would otherwise damage the substrate. The authors further treat the "total antioxidant" as the "total reducing power". The antioxidant activity is then interpreted as the reducing capability (Oboh and Rocha, 2007). Free radicals especially reactive oxygen had been implicated in a lot of degenerative diseases such as Parkinson and Alzheimer diseases. Overproduction of ROS can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation (Elmegeed et al., 2005). Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or neutralizing/scavenging free radicals produced in the body (Oboh, 2006; Oboh and Rocha, 2007). The free radical scavenging ability of both extracts is an indication that *L.begwensis* promises to be excellent dietary source of antioxidant polyphenols. Fe is necessary in relatively large amounts for heamoglobin, myoglobin and cytochrome production, but xanthine oxidase and other Fe proteins require rather small amounts of Fe for their metabolic functions. On the other hand, free Fe in the cytosol and in the mitochondria can cause considerable oxidative damage by increasing superoxide production (Oboh and Roch, 2007). The mechanism by which by which iron can cause this deleterious effect is that, Fe^{2+} react with hydrogen peroxide (H_2O_2) to produce the hydroxyl radicals (OH^-) via Fenton reaction. Also, superoxide can react with Fe^{3+} to regenerate Fe^{2+} that again goes into the Fenton reaction (Harris et al., 1992; Fraga and Oteiza, 2002; Oboh and Rocha, 2007). The hydroxyl radicals generated cause the oxidation of lipids, proteins, DNA and can directly attack the polyunsaturated fatty acids of the cell membranes and induce lipid peroxidation. Oboh and Rocha (2007) reported that the domineering mechanism through which *Capsicum annum var aviculare* (Tepin) polyphenol protect brain and liver is through their Fe^{2+} chelating ability and this gives credence to the fact that use of Fe chelator as recommended therapy for Fe overload. Extracts of *L.begwensis* from Breadfruit tree showed a stronger Fe chelating capability. The antioxidant capacity of the two extracts slightly differs depending on the host plant. Omay-Ucar et al., (2006) reported that antioxidant capacity of *Viscum album* spp. Differ depending on the time of harvest and the nature of the host tree. It has been suggested that pharmacologically active compounds may

psaa from the host tree to the parasitic plants like *L.begwensis*. Thus, biological activities of the plant could differ, just as the apoptosis-inducing properties of Viscum album has been found to be host dependent (Bussing and Schietzel, 1999). The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, free radical scavenger, singlet oxygen quenchers and metal chelators (Alia et al., 2003; Amic et al., 2003). This present study has verified that *L.begwensis* extracts can act as primary and /or secondary antioxidants, been free radical scavengers and potent Fe chelator, and these properties of health benefit are host dependent.

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