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Full Length Research Paper

Antioxidant properties of methanolic extracts of mistletoes (*Viscum album*) from cocoa and cashew trees in Nigeria

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Methanolic extracts of *Viscum album* leaves from two hosts (cocoa and cashew trees) were subjected to antioxidant assay. *V. album* from cocoa tree had higher total phenol content (182 mg/100 g) as against that of cashew tree (160 mg/100 g). The result of the study also revealed that the ferric reducing ability, free radical scavenging ability as well as the Fe^{2+} chelation of the extracts were all dose-dependent (0 – 1.0 mg/ml). *V. album* from cocoa tree had significantly higher ferric reducing and free radical scavenging ability than that from cashew tree, while *V. album* from cashew tree had significant higher Fe^{2+} chelating ability than *V. album* from cocoa tree. Although both methanolic extracts showed promise as a good antioxidant source, the total phenol content and the antioxidant capacity pattern of the two extracts suggest host dependency while the possible antioxidant mechanism of action is by reducing ability, free radical scavenging ability and chelation of Fe^{2+}, a major catalyst in lipid peroxidation.

Key words: *Viscum album*, antioxidant, Fe^{2+} chelation, phenolics.

INTRODUCTION

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential (Akinmoladun et al., 2007). Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process (Aruoma, 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). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Jayaprakasha et al., 2001; Gulcin et al., 2002). A profile of the chemical composition of a plant together with knowledge of its antioxidant activity will give a fair estimate of its therapeutic potential (Akinmoladun et al., 2007).

Mistletoes (*Viscum album*) are highly specialized angiosperms of the family Loranthaceae, which are well known as broad host range hemi-parasites of a variety of different gymnosperms and angiosperms (Deeni and Sadiq, 2002). They are of great economic importance due to the major damages they cause to their host which leads to economic losses (Hutchinson and Dalziel, 1972; Hussain and Musa, 1987). 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

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immunomodulatory activities have been reported for mistletoes (Onay-Ucar et al., 2006). Mistletoe teas and infusions are excellent remedy, esteemed and recommended ethno-medicinally for the prevention and management of stroke in parts of Nigeria, and it is also believed to improve the circulatory system and heart function in tradition medicine (Deeni and Sadiq, 2002). *V. album* extracts also could reduce harmful and mutagenic effect of oxygen free radicals. The physiological and biochemical effect of *V. album* has been attributed to its phytoconstituents. The medicinal effects of plants are often attributed to the antioxidant activity of the phytochemical constituents, mostly the phenolics. The antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, metal chelators and free radical quenchers (Rice-Evans et al., 1996; Oboh, 2006; Oboh and Rocha, 2007). Plants having significant medicinal values have often been found to be rich in phenolics and to have high antioxidant potentials (Akinmoladun et al., 2007). Although a lot of work has been done to elucidate the various phytoconstituents present and their biochemical activities, little effort has been channeled towards determining the antioxidant property of this plant and possible mechanism of action. Hence this present work is aimed at investigating the antioxidant properties of methanolic extracts of *V. album* isolated from cocoa and cashew trees in South Western part of Nigeria and their possible mechanism of action.

MATERIALS AND METHODS

Chemicals

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

Plant materials and extraction

Young leaves of *V. album* were harvested from cocoa and cashew trees at a local farm plantation in Akure, Nigeria and were taken to the Department of Crop, Soil and Pest Management Department of the 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. 50 g of the powdered leaves sample were weighed into a beaker and 500 ml of methanol was added. This was homogenized in a Warring blender until a homogenate was obtained. The homogenate was then filtered using Whatman no.1 filter paper and the filtrate was collected then and evaporated to dryness using a rotary evaporator. This was reconstituted in water 1:50 (w/v) and later used for the analysis.

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°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).

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 (0 – 1.0 ml) were mixed with 2.5 ml 200 mm sodium phosphate buffer (ph 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixtures were incubated at 50°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.

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 (0 – 1.0 ml) 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).

Fe^{2+} chelation assay

The ability of the sample extracts 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 150 µl of freshly prepared 500 µM $FeSO_4$ was added to a reaction mixture containing 168 µl of 0.1 M Tris-HCl (pH 7.4), 218 µl saline and the methanolic leaf extracts (0 – 500 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer.

RESULTS

The result of the total phenol content of *V. album* isolated from both cocoa and cashew trees is presented in Table 1. The result revealed that *V. album* isolated from cocoa tree had higher total phenol content (182 mg/100 g) than that from cashew tree (160 mg/100 g). Nevertheless, the two values compare favourably with that of red grape and strawberry (Sun et al., 2002) but the values were less than that of red pepper (Oboh and Rocha, 2007).

Reducing ability of both methanolic extracts is as presented in Figure 1. There was a corresponding increase in the reducing ability with increase in concentration of the extracts, indicating a dose-dependent relationship. Nevertheless, a significant difference exist between the reducing ability of the mistletoe extracts from cocoa tree and cashew tree with highest reducing ability observed at highest dose of the extracts. The reducing property of both extracts is proportional to their corresponding total phenol content (Table 1) in that, *V. album* from cocoa tree had higher total phenol content that *V.*

Table 1. Total phenol content (mg/100 g) of the methanolic extracts.

Extracts	Total phenol content (mg/100 g)
Viscum album (Cocoa tree)	182
Viscum album (Cashew tree)	160

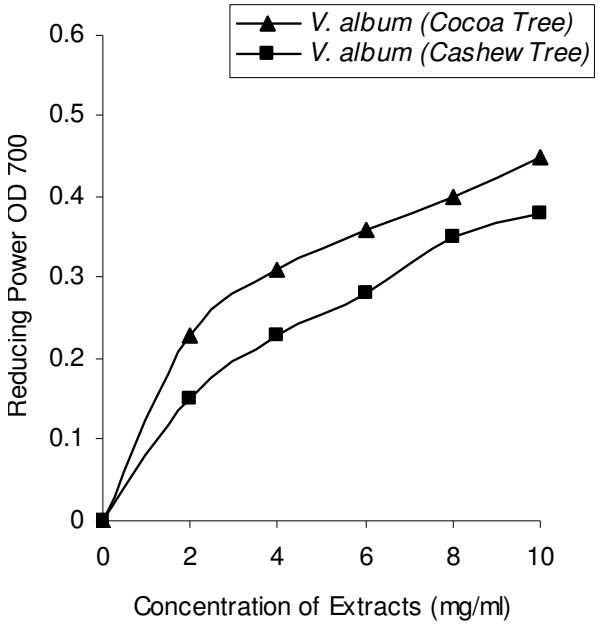

Figure 1. Reducing ability of *V. album* methanolic extracts.

album from cashew tree and this is in agreement with earlier works (Sun et al., 2002; Chu et al., 2002; Oboh, 2006; Oboh et al., 2008).

The scavenging ability of each methanolic extracts against stable DPPH in methanolic solution is presented in Figure 2, and it is expressed as percentage (%) scavenging ability. The results for both methanolic extracts followed a dose-dependent pattern. The free radical scavenging ability of the *V. album* extract from cocoa tree performs better than that from cashew tree and this also is in agreement with total phenol content and reducing property of their various 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; Oboh et al., 2008).

The ability of the *V. album* extracts to chelate Fe^{2+}, a potent free radical initiator in cell is represented in Figure 3. This also follow a dose-depended pattern as the highest % Fe^{2+} chelation was achieved at the highest dose for the two extracts of *V. album*. But extract of *V. album* from cashew tree was found to chelate Fe^{2+} better than that from cocoa tree. The reason for this cannot be readily explained but it is worth nothing.

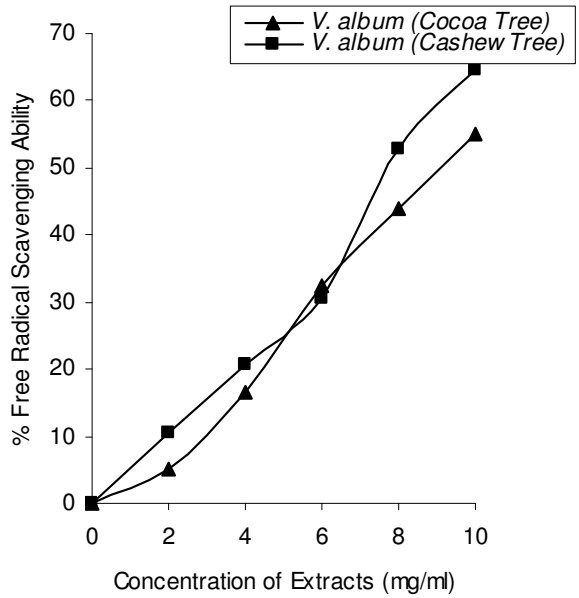

Figure 2. Free radical scavenging ability of *V. album* methanolic extracts.

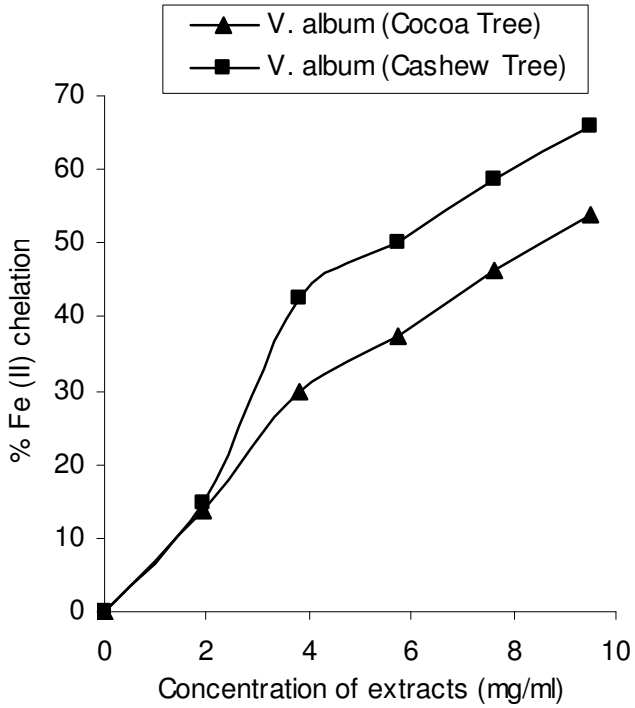

Figure 3. Fe(II) chelating ability of *V. album* methanolic extracts.

DISCUSSION

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 asso-

ciated with the normal natural metabolism of aerobic cells (Bors et al., 1996; Halliwell, 1996; Oboh and Rocha, 2007). The values obtained for both methanolic extracts indicate that *V. album* is rich in phenolic compounds. Phenolic compounds have been reported to have antioxidant properties, they act as free radical scavengers, mope-up reactive oxygen species (ROS) and they also chelate metal ions (Zhang et al., 2001; Oboh and Akindahunsi, 2004; Oboh, 2006).

Reducing ability is a measure of the ability of the methanolic extracts to reduce Fe^{3+} to Fe^{2+}; a measure of their antioxidant properties, that is, the higher the reducing property the higher the 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 oxidizing species that would otherwise damage the substrates. And the authors further treat the "total antioxidant power" as the "total reducing power". The antioxidant activity is then interpreted as the reducing capability (Oboh and Rocha, 2007).

Free radicals especially reactive oxygen species (ROS) 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 by neutralizing/scavenging free radicals produced in the body (Oboh, 2006; Oboh and Rocha, 2007). The free radical scavenging ability of both methanolic extracts is an indication that *V. album* 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 Rocha, 2007). The mechanism by which iron can cause this deleterious effect is that, Fe^{2+} reacts with hydrogen peroxide (H_2O_2) to produce the hydroxyl radicals ($OH^{\cdot}$) via Fenton reaction. Aso 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) polyphenols protect brain and liver is through their Fe^{2+} chelating ability and this gives credence to the fact that use of Fe chelators as recommended therapy for Fe overload. Extracts of *V. album* from cashew 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 extracts of *V. album* ssp. differ depending on the time of harvest and nature of the host tree. It has been suggested that pharmacologically active compounds may pass from the host trees to the parasitic plants like *V. album*. Thus, biological activities of the plant could differ, just as the apoptosis-inducing properties of *V. album* extract 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; Ademiluyi, 2006). This present study has verified that *V. album* extracts can act as primary and/or secondary antioxidants, being free radical scavengers and potent Fe chelators, and these properties of health benefit are host dependent.

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