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Evaluation of Effect of Time of Collection on the Antioxidants Values of Mistletoes (*Viscum album*) Grown on Orange Tree

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aim: To investigate the effect of time of collection on the antioxidants values of mistletoes (*Viscum album*) grown on orange tree.
Methods: Aqueous leaves extracts of different time of harvest of *Viscum album* were prepared and standard methods were used to determine quantitatively the antioxidants values in the plant extracts.
Results: In the experiments, the total phenol content in aqueous leaves extracts of *Viscum album* was 19.5840 mg/g, at 6: 00 am, 55.4061 mg/g at 1:00 pm and the value of 86.3682 mg/g was recorded at 7:00 pm. The value of 0.344 mg/g was recorded for flavonoids at 6:00 am while the value of 0.676 mg/g was observed at 1:00 pm and at 7:00 pm it was 1.458 mg/g. The % of inhibition of DPPH content in aqueous leaves extracts of *Viscum album* was 36.819 mg/g, at 6: 00 am, 49.213 mg/g at 1:00 pm and the value of 61.203 mg/g was recorded at 7:00 pm. The antioxidants values were significant (P =0.05) when compared time of plants collection.
Conclusion: The results showed that the antioxidants values depend on the time of collection which was maximum when the leaves were obtained at dawn. It is therefore advisable that the use of this extract in herbal medicine should be encouraged and the plant should be plucked at dawn to maximise the antioxidants potential of the mistletoes.

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Keywords: Viscum album; leaves extract; antioxidants; collection time.

1. INTRODUCTION

Today, most of the developing countries notable Nigeria has adopted traditional medical practice as an integral part of their culture and plant remedies are now being used as alternative in almost all branches of medicines and they are proving to be very effective, more especially in the treatment of incurable disease, such condition as male infertility hypertension, diabetes e.t.c. These ailments have been treated successfully with the use of medicinal plants and herbal preparations [1]. It is on this basis that researchers keep on working on the medicinal plants in order to produce the best medicines for physiological uses. Plants show enormous versatility in synthesising complex materials which have no immediate obvious growth or metabolic functions. These complex materials are called secondary metabolites [2]. Plants secondary metabolites were recently been referred to as phytochemicals. Phytochemicals are naturally occurring and biological active plant compounds that have potential disease inhibiting capabilities. It is believed that phytochemicals may be effective in combating or preventing disease due to their antioxidants effect [3]. Antioxidants protect other molecule (in vivo) from oxidation when they are exposed to free radicals and oxygen species which have been implicated in the aetiology of many diseases and food deterioration and spoilage [4,5].

Viscum album (mistletoe) is a hemi-parasitic shrub, frequently globular in shape. It grows on the branches of other trees, to which it is attached by a swelling called a haustorium. As a hemi-parasite it depends on its host for water and mineral nutrients but is able to photosynthesise (create its carbohydrates using sunlight) because it has green leaves and stem. *Viscum album* species contain lectins (viscumin/agglutinin), protein toxins, alkaloids and polysaccharides. A number of biological effects, such as anticancer, antimicrobials, antiviral, apoptosis inducing and immunomodulatory activities have been reported for mistletoes [6]. It was reported to be effective in the management of chronic metabolic disorder such as diabetes [7]. Obatomi et al. [7] also reported the antioxidants properties in mistletoes growing on cocoa and cashew trees in Nigeria. They found out that mistletoes growing on cocoa had more antioxidants properties than mistletoes grown on cashew tree. According to Bussing and Schietzel [8] the biological activities of *Viscum album* are dependent on the host tree, manufacturing process, and time of collection. Therefore, in this study, the effect of time of collection on the antioxidants values of mistletoes (*viscum album*) grown on orange tree was investigated.

2. MATERIALS AND METHODS

2.1 Chemicals

DPPH [2,2-diphenyl-1- picrylhydraxyl]radical, Gallic acid, ascorbic acid and folin-Ciocalteau reagent were obtained from sigma-Aldrich, USA. All other chemicals and reagent used were of analytical grade.

2.2 Experimental Plant

The fresh leaves of mistletoe plant (*Viscum album*) from host plants Orange tree were obtained from a research farm of Adeyemi College of Education, Ondo, Ondo State, Nigeria in the morning (6:00 AM), afternoon (1:00 AM) and at dawn (6:00 PM). Authentication of the plants was done in the department of Biological Sciences of the institute.

2.3 Preparation of Aqueous Extract

The fresh leaves of mistletoe plant (*Viscum album*) were sorted out to remove extraneous material and rinsed with water to remove debris and dust particles. They were air dried for two weeks, packed in a paper bag stored and pulverised. A portion (50 g) of the powdered leaves was weighed into a beaker and 500ml of warm distilled water was added and stirred for 20 minutes. This was left to stand for 24 hours. It was then filtered using Whatman filter paper to obtain *Viscum album* aqueous extract and use for antioxidants analysis.

2.4 Total Phenolic Content

The total phenolic content was determined using Folin-Ciocalteau reagent. Test solution of aqueous extract in different concentration (25 to 100 mg/ml) was mixed with 10% folin-Ciocalteau reagent (v/v) and 2.0 ml of 7.5% freshly prepared sodium carbonate. The reaction mixture was incubated at 45°C for 30 mins and the absorbance measured at 765 nm in the spectrophotometer, Gallic acid was used as standard phenol (positive control) [9]. The total phenol activity was obtained using the formular

C=As x Cs/As Where C is the total content of phenolic in the methanol extract sample in mg/g gallic acid equivalent. As is the absorbance of the sample, Cs is the concentration of standard and As is the absorbance of the standard. 0.1 mg/ml was used as the concentration of gallic acid. The total phenolic content was calculated for gallic acid and different concentrations of aqueous extract. Each test was carried out thrice.

2.5 Flavonoid Determination

The aluminium Chloride colorimetric method with some modification was used to determine flavonoid content as described by Zhanget et al. [10]. 1 ml of aqueous extract was mixed with 1ml of methanol, 0.5ml of aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 mins at room temperature; the absorbance of the reaction was measured at 415 nm. The calibration curve was prepared by preparing quercetin (5-60 µ/ml) solution in methanol. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of the extracted compound).

2.6 DPPH Radical Scavenging Activity

The free radical scavenging activities of aqueous extract was measured with stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) spectrophotometrically [11]. 0.004%. DPPH solution was prepared in methanol. A test solution of methanol extract was prepared in different concentrations (25 to 100 mg 1ml). The absorbance was read at 507nm. About 500µl of an extract of different concentrations was added to 2.950 ml of DPPH solution taken in a cuvette. The sample was kept in the dark for 30 mins and reading were measured at 517 nm. The scavenging activities was observed by bleaching of DPPH solution from violet colour to light yellow. Ascorbic acid was used as a control and 500 µl methanol as black. The DPPH radical scavenging was calculated in terms of percentage inhibition using the formula % inhibition= [Ac – As/Ac] x 100%. Where Ac is the absorbance of the blank sample and As is the absorbance of the sample. The % inhibition was calculated for ascorbic acid and different concentrations of aqueous extracts kept in the dark for 30mins. Each test was carried out thrice.

2.7 Statistical Analysis

The data obtained were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 23. Antioxidants values were expressed in mg/g as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons by Dunnett's method. The results were considered statistically significant if $P < 0.05$.

3. RESULTS AND DISCUSSION

The Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts on total phenol was measured (Fig. 1). There is a statistically significant difference (P=0.05) in the mean value of total phenol between the time of plants collection. The total phenol content in leaf of aqueous extracts of *Viscum album* was 19.5840 mg/g, at 6: 00 am, 55.4061 mg/g at 1:00 pm and the value of 86.3682 mg/g was recorded at 7:00 pm.

The Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts on flavonoid contents was measured (Fig. 2). There is a statistically significant difference (P=0.05) in the mean value of flavonoids between the time of plants collection. The value of 0.344 mg/g was recorded for early morning (6:00 am) while the value of 0.676 mg/g was observed at afternoon (1:00 pm) and at dawn (7:00 pm) it was 1.458 mg/g.

The Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts on % inhibition of DPPH contents measured (Fig. 3). There is a statistically significant difference (P=0.05) in the mean value of % of inhibition of DPPH between the time of plants collection. The % inhibition of DPPH content in the leaf of aqueous extracts of *Viscum album* was 36.819 mg/g, at 6: 00 am, 49.213 mg/g at 1:00 pm and the value of 61.203 mg/g was recorded at 7:00 pm. It was observed that the antioxidant values depend on the time of collection which was maximum when the leaf was obtained at dawn.

Antioxidants are compounds that protect cells against the damaging effect of reactive oxygen species such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species result in oxidative stress that has been linked to cellular damage leading to cancer, ageing, antherosclerosis, inflammatory and neuro-degeneratives diseases [12]. Antioxidant is absolutely essential to maintain optimal cellular well-being. Human beings have a complex antioxidant protection

system that protects cells and organs. According to Dave [12], there are different types of antioxidants. These include nutrient based antioxidants (ascorbic acid, tocopherols, carotenoids), antioxidant enzymes: which catalyse free radical quenching reactions (suproxide dismutase, gluthathione perioxidase, gluthathione reductase); metal binding proteins (ferritin, lactoferritin, albumin), which sequester free ion and copper ions that are capable of catalysing oxidative reactions.

From this study, the total phenol content was seen to increase gradually with time of collection of *Viscum album* (mistletoes) in the aqueous extracts. However, the total phenol activity was low in early morning and at afternoon respectively when compared with value at dawn. This was in agreement with previous work done by Bussing and Schietzel [8]. Phenolics are the largest group of phytochemicals and have been touted as accounting for the most antioxidant activity of plants. Phenolic compounds are known

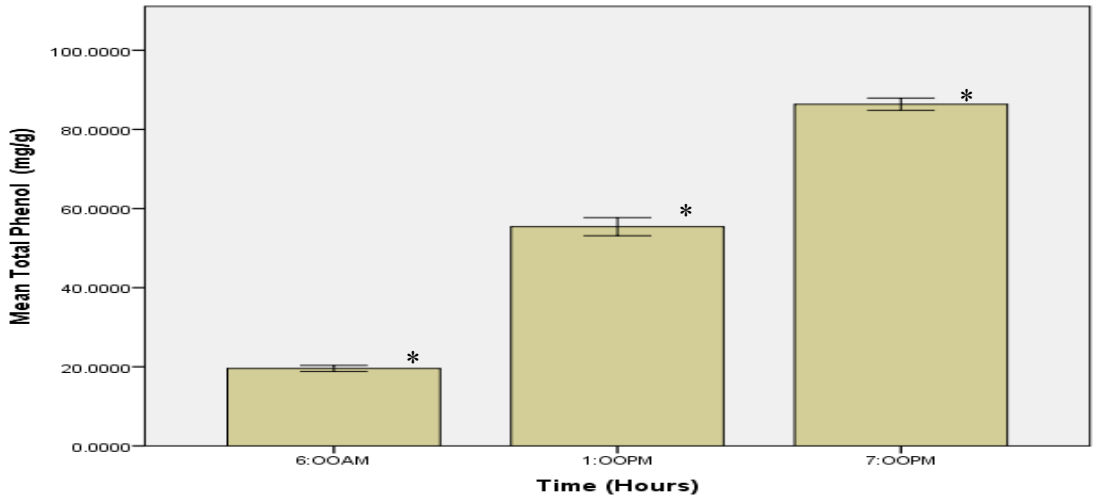

Fig. 1. Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against total phenol contents

**Significant change at P=0.05 with respect to the means*

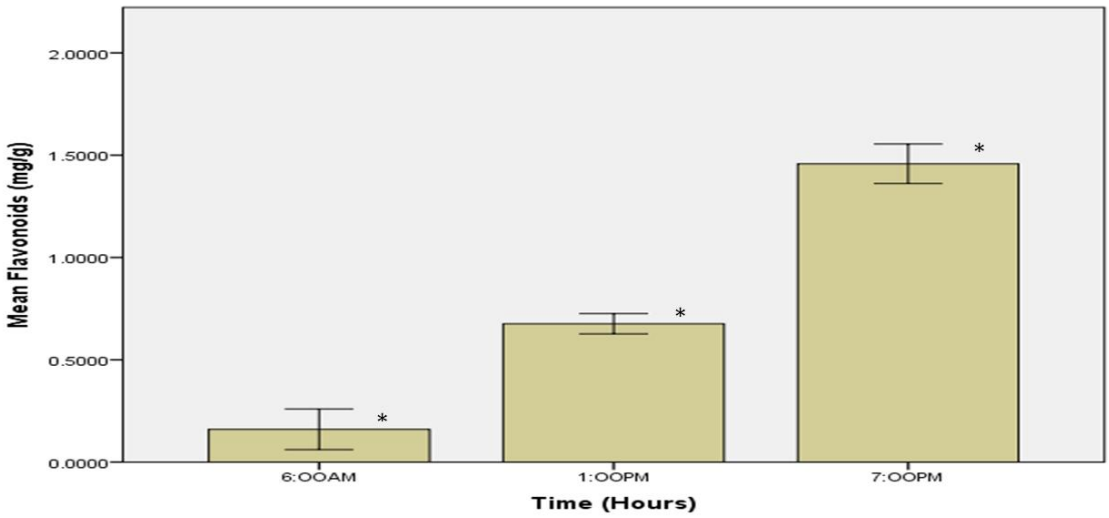

Fig. 2. Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against flavonoid contents

**Significant change at P=0.05 with respect to the means*

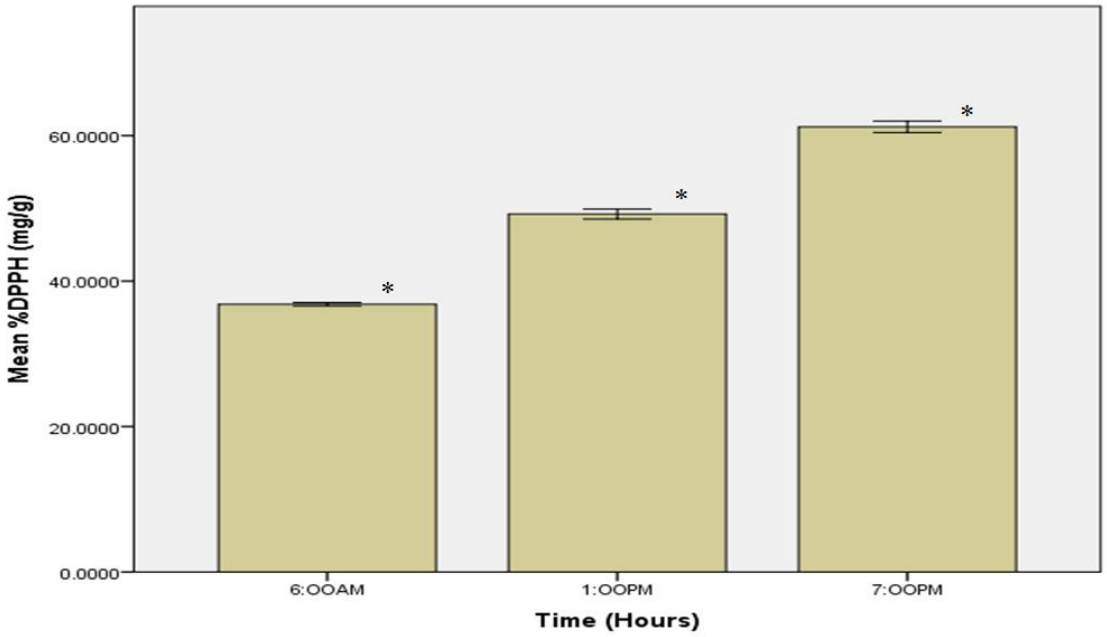

Fig. 3. Effect of time of collection of *Viscum album* (mistletoes) aqueous extracts against % inhibition of DPPH content

**Significant change at P=0.05 with respect to the means*

to possess biological effects such as antioxidants, anti-aging, cardio protection and anti-cancer activities [13,14]. Flavonoids are the most common and widely distributed group of plant phenolic compounds which are very effective antioxidants [15]. Flavonoids are also responsible for the free radical scavenging activity in plants [16].

4. CONCLUSION

It is therefore advisable that the use of this extract in herbal medicine should be encouraged and the plant should be plucked at dawn to maximise the antioxidants potential of the *Viscum album* (mistletoes).

CONSENT

It is not applicable.

ETHICAL APPROVAL

The author declares that this work was not against public interest.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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