

# Phytochemical Investigation and the Effects of Aqueous Plant Extracts of *Viscum Album* on Antioxidant Property and Biochemical Profile as a Measure of its Therapeutic Value

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**Abstract:** The aim of the research was to investigate the phytochemical compounds inherent in the aqueous plant extracts of *Viscum album*, its antioxidant property and the effects on biochemical parameters in wistar rats. Phytochemical investigation revealed the presence of alkaloid, flavonoid, saponin, tannin, carbohydrate, reducing sugar, protein, cardiac glycoside and steroidal aglycon in both the leaf and stem extracts, although the stem extract showed higher concentration of the phytochemical compounds compared to that of the leaf extract. The plant extracts possessed antioxidant properties using the DPPH free radical scavenging method. Biochemical results showed significant reduction in the concentrations of liver enzyme markers such as Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline Phosphatase (ALP) suggesting that the aqueous plant extracts possessed hepatoprotective property. There were also significant reduction in the levels of glucose, cholesterol, Triglyceride, Total protein, Albumin, Creatinine and Urea.

**Keywords:** *Viscum album*; DPPH; Reactive Oxygen Species; Alkaline Phosphatase; Aspartate transaminase; phytochemical compounds.

## 1. Introduction

*Viscum album* is a bi-perennial shrub distributed widely in tropical and subtropical regions of Africa, Asia and Europe. *Viscum album* is in the family of Loranthaceae. It is an evergreen semi-parasitic plant obtaining its nutrients and water from its host. Pharmacologically, it has been used as anticancer, antimycobacterial, antiviral, immunomodulatory agents (Hajto et al., 2005). It has also been reported to be effective in the management of chronic metabolic disorders such as diabetes mellitus (Obatomi et al., 1994).

Medicinal and therapeutic effects of several plants which are used in traditional medicine are usually attributed to their antioxidant compounds. Antioxidants have been used as food preservatives, mainly because they arrest oxidative deterioration of lipids. These factors have engineered the 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). Therefore, a good phytochemical profile of a plant together with knowledge of its antioxidant activity will give a fair estimate of its therapeutic potentials. Therefore, the aim of this work was to determine the constituent phytochemical compounds present in aqueous extract of *Viscum album*, its antioxidant properties and its effects on biochemical parameters in order to ascertain the therapeutic value(s) of the plant.

## 2. Materials and Methods

### 2.1 Preparation of plant extracts

Fresh leaves of *viscum album* was obtained in Mushin market and was botanically identified by Mr. Adeleke in the department of pharmacognosy, college of medicine, University of Lagos. The leaves were removed from the stem and were weighed. Both the leaves and stem weighed 178g and 284g respectively. The different portions were sun-dried and extracted by aqueous extraction. The different filtrates were then freeze-dried to obtain a powdered form. The powder yield of the leaf and stem were 8g and 5g respectively.

#### 1) Animals

21 albino rats were bought from the Animal Laboratory center, college of medicine, University of Lagos. The rats weighed between 150-200g and were acclimatized for two weeks (14days). The rats were fed with well formulated rat feed and water.

#### 2) Phytochemical Screening of *Viscum album* (Qualitative investigation)

The phytochemical investigation of aqueous extracts of leaf and stem of *Viscum album* was carried out using the standard procedures (Trease and Evans, 1983)

#### 3) Alkaloid Test:

0.5g of each extract was boiled with 5ml of 2% HCL on a water bath. 1ml portion of each filtrate was treated with 2 drops of Mayer's reagent (mixture of 36g of HgCL<sub>2</sub>, 5g of KI and 100ml distilled water) and a cream coloured precipitate was observed indicating the presence of alkaloid.

**4) Flavonoid Test**

0.5g of each extract was heated with 10ml ethyl acetate in a boiling water for 3mins. 4ml of each filtrate was shaken with 1ml of 1%  $\text{AlCl}_3$  solution, a light yellow coloration in ethyl acetate layer was observed showing the presence of flavonoid.

**5) Saponin Test (frothing test)**

Each extract of the plant was boiled with 5ml of distilled water. 1ml of the filtrate was diluted with 4ml of distilled water. On shaking vigorously, a stable froth was observed after a while indicating the presence of saponins.

**6) Tannin Test (Hydrolyzable test)**

0.5g of each extract was boiled in 10ml of distilled water for 5mins and then filtered. The filtrates were added a drop of bromine water and an orange precipitate was observed.

**7) Protein Test**

5ml of distilled water was added to some quantities of the extracts and were allowed to stand for 3hrs. 2ml of the solution was added to 0.1ml of millon reagent (mixture of  $\text{HgNO}_3$  and  $\text{HNO}_3$ ). On shaking vigorously, a yellow precipitate was observed showing the presence of protein.

**8) Carbohydrate Test (Molisch Test)**

0.5g of each plant extracts was added water. Two drops of molisch reagent (mixture of 15g of  $\alpha$ -naphatol and 100ml of chloroform) was added to the solutions and shaken vigorously. 2ml of 10% concentrate  $\text{H}_2\text{SO}_4$  was carefully added and a layer was formed below the solution forming a deep violet coloration at the interface, indicating the presence of carbohydrate.

**9) Reducing Test (Fehling Test)**

0.5g of each extract was added 5ml of distilled water. 5ml of both Fehling A (17.3g of  $\text{CuSO}_4$  dissolved in distilled water and made up to 250ml) and Fehling B (86.5g of sodium potassium tartrate was dissolved in warm water, 30g of NaOH was dissolved in distilled water and both solutions were then added and volume made up to 250ml) was added to the solutions. On vigorously shaken, a brick-red precipitate was observed indicating the presence of reducing sugars.

**10) Steroidal Aglycon (Salkowski Test)**

0.5g of each extract were dissolved in 2ml of chloroform. 10% concentrated  $\text{H}_2\text{SO}_4$  was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring that is aglycon portion of the cardiac glycosides.

**11) Cardiac glycosides (Keller- Killian Test)**

0.5g of each extract was dissolved in 2ml of glacial acetic acids containing few drops of 10%  $\text{FeCl}_3$  solution. This was then underplayed with 1ml of 10% of concentrated  $\text{H}_2\text{SO}_4$ . A brown ring obtained at the interface indicates the presence of deoxy sugar characteristic of cardenoides.

**12) Cyanogenic glycosides Test**

0.5g of the plant extracts were put into a conical flasks and 20ml of distilled water was added to cover the extracts. A piece of sodium picrate paper was suspended in the flask on a water bath for 1hour. A colour change from yellow to orange was observed showing the presence of cyanogenic glycosides.

**2.2 Anti-Oxidant Properties Of Viscum Album Using Dpph Method**

The method of Vicas et al., 2008 was used. Different concentrations of the plant extracts were prepared that is 25, 50, 75, 100, 200, 250 and 500 $\mu\text{g/ml}$ . Each concentration was mixed with 0.1mM DPPH solution in methanol. The mixture was allowed to react at room temperature in the dark for 30mins. Blank solution was prepared with each test sample solution only when negative control was DPPH solution. Gallic was the positive control and/ or has been used as standard reference. The absorbance was measured at 517nm using spectrophotometer. Values obtained were converted to percentage antioxidant activities as given by the formular

$$\% \text{AOXA} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}}} \times 100$$

where A = Absorbance

$$\% \text{I or \% DPPH scavenged} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

where I = Inhibition

**2.3 Biochemical Assay**

Glucose level was determined by the glucose oxidase method of Sharma et al., 1997. The protein content in the sample was assayed by the method of Lowry et al., 1951. Urea and Creatinine levels were determined by the method of Talke and Schubert, 1951 and Jaffe, 1886 respectively. The activities of ALT and AST were assayed for by the combined methods of Mohun and Cook, 1951 and Reitman and Frankel, 1951. Serum triglyceride and cholesterol levels were determined using commercial diagnostic kits (Randox). Finally, the activity of ALP was determined based on the method of Williamson, 1972.

**2.4 Stastical analysis**

The data obtained were analysed using Mean  $\pm$  SD while ANOVA test was used to determine the significant difference at confidence limit of 95% ( $p=0.05$ ).

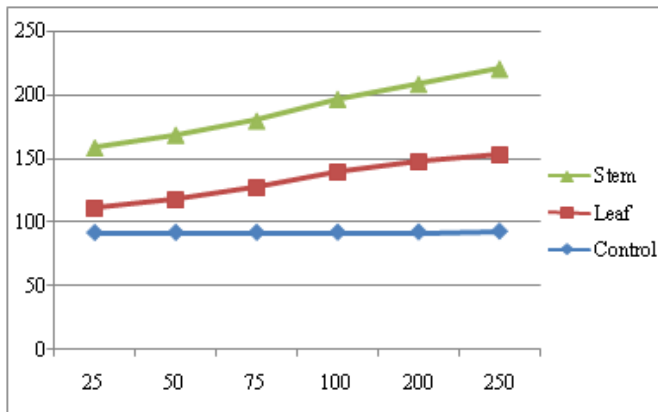
**3. Results**

**Table 1:** Phytochemical Constituents Of Aqueous Plant Extracts Of *Viscum Album*.

Phytochemical constituents	Leaf extract	Stem extract
Alkaloid	+	+++
Flavonoid	++	++
Saponin	+	++
Tannin	+	++
Protein	+	+++
Carbohydrate	++	++
Reducing sugar	+	++
Cardiac glycoside	++	+++
Steroidal aglycon	++	++

**Table 2:** Percentage Inhibition of DPPH Free Radical Scavenging Activities of the Aqueous Plant Extracts of *Viscum Album*

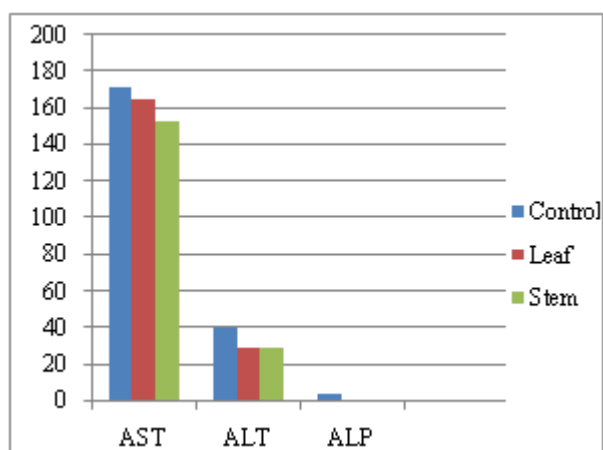
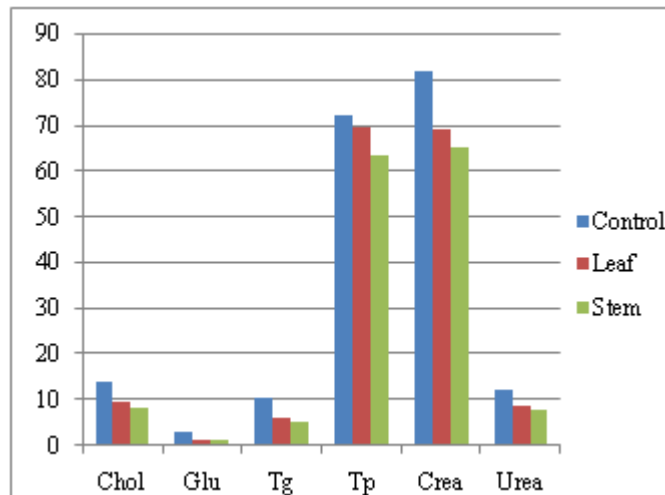
Concentrations	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	200 µg/ml	250 µg/ml	500 µg/ml	Mean ±SD
Control	92.05	92.23	92.23	92.40	92.58	93.11	93.46	92.58±0.27
Leaf	19.96	26.15	35.69	47.34	56.00	60.25	64.31	44.24±0.24
Stem	46.82	50.53	52.12	57.60	60.60	68.02	83.22	59.84±0.93

**Figure 1:** Effect of aqueous plant extracts of *Viscum album* on Antioxidant activity using DPPH free radical scavenging method.**Table 3:** Effects of 250mg/Kg Aqueous Plant Extracts of *Viscum Album* On Liver Enzyme Markers In Wistar Rats.

Liver enzyme markers	Control	Leaf extract	Stem extract
AST (unitsL <sup>-1</sup> )	171.97± 0.62	165.10± 0.37	152.50± 0.93
ALT (unitsL <sup>-1</sup> )	40.63± 0.64	29.56± 0.80	29.36± 0.80
ALP (unitsL <sup>-1</sup> )	3.93± 0.19	1.21± 0.66	1.20± 0.66

**Table 4:** Effects of 250mg/kg aqueous plant extracts of *viscum album* on biochemical parameters in wistar rats

Biochemical parameters	Control	Leaf extract	Stem extract
ALB (g/L <sup>-1</sup> )	51.10± 0.50	37.81± 0.85	36.87± 0.77
Cholesterol (mg/dL)	14.00± 0.37	9.49± 0.28	8.40± 0.63
Glucose (mmol <sup>-1</sup> )	3.20± 0.82	1.37± 0.93	1.31± 0.96
Triglyceride (mmol <sup>-1</sup> )	10.67± 0.68	6.06± 0.40	5.14± 0.46
Total protein (g/L <sup>-1</sup> )	71.90± 0.17	69.54± 0.65	63.54± 0.21
Creatine (mg/dL)	81.91± 0.63	68.94± 0.89	65.16± 0.73
Urea (mmol <sup>-1</sup> )	12.27± 0.75	8.71± 0.66	7.94± 0.33

**Figure 2:** Effect of Aqueous plant extracts of *Viscum album* on liver enzyme markers**Figure 3:** Effect of Aqueous plant extracts of *Viscum album* on serum Biochemical parameters in wistar rats

#### 4. Discussion

From Table 1, it was observed that the aqueous plant extracts of *Viscum album* contained the following phytochemical constituents such as alkaloid, flavonoid, saponin, tannin, protein, carbohydrate, reducing sugar, cardiac glycoside and steroidal aglycon. Though the stem extract showed higher of these phytochemical constituents compared with that of the leaf extract.

The results in Table 2, showed that DPPH free radical scavenging effect of aqueous plant extracts of *Viscum album* increased as the concentrations increased. DPPH scavenging activity of aqueous stem extract (59.84%) was higher than that of the aqueous leaf extract (44.24%). This implied that the stem extract has more antioxidant property compared to that of the leaf extract.

The liver and heart releases Aspartate transaminase (AST), Alkaline Phosphatase (ALP) and Alanine transaminase (ALT) and an elevation in their serum concentrations are an indication of liver and heart damage (Mythilypriya et al., 2007, Wasan et al., 2001). The significant decrease in the levels of AST, ALP and ALT suggested that aqueous plant extracts of *Viscum album* has hepatoprotective effects and equally could not have caused some toxic effects on the heart tissue (Crook, 2006).

The physiological and biochemical effects of aqueous extracts of *Viscum album* has been attributed to its phytochemical constituents, mostly the phenolics (Akinmoladin et al., 2007). Aqueous plant extracts of *Viscum album* showed significant reduction in the biochemical parameters. The percentage reduction in the level of glucose was found to be 69.2% compared with the control. The hypoglycemic effect of the aqueous extract of *Viscum album* could either be by increasing the peripheral utilization of glucose or by stimulating the secretion of

insulin by the  $\beta$ -pancreatic cells (Iheanacho et al., 2008). Serum cholesterol and triglyceride levels decreased which might be attributed to the presence of hypolipidemic agents in the aqueous plant extracts of *Viscum album* (Ogbonnia et al., 2011). The protein levels in the aqueous plant extracts compared to the control was observed to be reduced. This suggested that there was no sign of impaired renal function (Tilkian et al., 1979). Creatinine level significantly reduced which directly suggested no kidney damage, specifically by renal filtration mechanism (Waswan et al., 2001). This is a clear indication that the drug did not cause renal impairment. High urea level is an indication of hyper-hepatic activity and if not checked could result in liver damage. From the result showed in Table 3, the levels of urea in both the aqueous leaf and stem extracts decreased compared to the control. This suggested that the aqueous plant extracts of *Viscum album* are liver friendly.

## 5. Conclusion

In conclusion, aqueous plant extracts of *Viscum album* contained phytochemical compounds. Using the DPPH free radical scavenging method, the aqueous plant extracts of *Viscum album* showed an elevated antioxidant property and finally showed effects on the biochemical parameters in wistar rats.

## 6. Acknowledgement

My appreciation goes to Mr Elensi of the department of Pharmaceutical chemistry, University of Lagos for his technical assistance.

## References

- [1] Akinmoladin A.C, Ibukun E.O, Afor E, Akinrinlola B.L, Onibon T.R, Akinboye A.O, Obuofor E.M, Farombi E.O : Chemical constituents and antioxidant activity of *Alstonia boonei*. *Afr. J. Biotechnol.* 2007, 6(10), 1197-1201.
- [2] Crook, M.A., 2006. Clinical Chemistry and Metabolic Medicine. 7th Edn., Hodder Arnold, London, pp: 426.
- [3] Gulcin I, Buyukokuroglu M.E, Oktay M, Kufrevioglu O.I : On the *in vitro* antioxidant properties of melatonin. *J. Pineal Res.* 2002, 33: 167-171.
- [4] Hadjo T.K, Hostanska, T, Berki, L, Palinska, F, Boldizsar, P, Nemeth: Oncopharmacological perspectives of a plant lectin (*Viscum album*). Overview of recent results from *in vitro* experiments and *in vivo* animal models and their possible relevance for clinical applications. *Evid based complement Alternat Med.* 2005, 2, 59-67.
- [5] Iheanacho K.M.E, Udebuani A.C, Ihegboro G.O: Phytochemistry and effect of aqueous leaf extract of Mistletoe (*Tapinanthus bangwensis*) on serum glucose and cholesterol concentrations in normoglycaemic rats. *Nig. J. Biochem. Mol. Biol.* 2008, 23(2): 35-39.
- [6] Jayaprakasha G.K, Singh R.P, Sakariah K.K.: Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. *Food Chem.* 2001, 73: 285-290.
- [7] Lowry, OH, Rosebrough, NJ, Farr, AL, Randall, RJ: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951, 193(1), 265-275.
- [8] Mohun, AF, Cook, LJ: Simple method for measuring serum level of glutamate-oxaloacetate and glutamate-pyruvate transaminase in Laboratories. *J. Clin. Pathol.* 1951, 10(2), 394-399.
- [9] Mythilypriya, R., P. Shanthi and P. Sachdanandam: Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation, on rats. *J. Health Sci.* 2007, 53: 351-358.
- [10] Obatomi D.K, Bikomo E.O, Temple V.J: Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* 1994, 43: 13-17.
- [11] Ogbonnia Steve, O., I. Odimegwu Joy and N. Enwuru Veronic: Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. and their mixture on streptozotocin (STZ)-induced diabetic rats. *Afr. J. Biotechnol.* 2008, 7: 2535-2539.
- [12] Reitman, S, Frankel, S: A Colrimetric method for the determination of serum level of glutamate-oxaloacetate and pyruvate transaminase. *Am. J. Clin. Pathol.* 1951, 28(1), 56-63.
- [13] Sharma, SR, Dwived, SK, Swarup, D: Hypoglycemic, antihyperglycemic and hypolipidemic activities of *Caesalpinia bonducella* seeds in rats. *J. Ethnopharmacol.* 1991, 58(1), 39-44.
- [14] Talke H, Schubert, GE: Enzymatische Harnstoffbestimmung in blut and serum in Optischen test nach Warburg. *Klin. Wochschr.* 1965, 43(1), 174.
- [15] Tilkian Sarko, M., Conover Boudreau Mary and G. Tilkian Ara: Clinical implication of laboratory tests. 2nd Edn. 1979. The C.V Mosby Company, St Louis, Missouri, USA.
- [16] Wasan, K.M., S. Najafi, J. Wong and M. Kwong: Assessing plasma lipid levels, body weight and hepatic and renal toxicity following chronic oral administration of a water soluble phytosterol compound FM-VP4, to gerbils. *J. Pharma. Sci.* 2001, 4: 228-234.
- [17] Williamson, T: A comparison between the phosphatase and phenyl-phosphate methods of Alkaline Phosphatase assay. *Med. Lab. Technol.* 1972, 29(2), 182-187.