

# Phytochemistry and Toxicity Studies on Aqueous Extracts of Leaf and Stem of Mistletoe (*Viscum album*) in Albino Rats

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**Abstract:** Phytochemistry and acute toxicity studies on the aqueous extracts of leaves and stem of mistletoe (*Viscum album*) in albino rats was carried out. Phytochemical screening of leaves and stem of *Viscum album* from *Azadirachta indica*, *Acacia albida* and *Psidium guajava* trees revealed the presence of tannins, alkaloids, saponins, glycosides, flavonoids, phenols, combined anthraquinones, reducing sugars and combined sugars. Saponins had the highest scores (+++) in all the extracts followed by phenols and carbohydrates with moderate scores (++), while lower concentrations (+) was observed for reducing and combined sugars. The calculated median lethal dose (LD<sub>50</sub>) values following the intraperitoneal administration of graded doses of these extracts in albino rats were 1440mg/kg/bw and 600mg/kg/bw for *A. indica* leaf and stem; 2400mg/kg/bw each for *A. albida* leaf and stem, and 2400mg/kg/bw and 2880mg/kg/bw for *P. guajava* leaf and stem aqueous extracts respectively. The clinical signs associated with acute toxicity testing using graded doses of *Viscum album* aqueous extracts administered to albino rats were arched back, bulging eyes, weakness, depression, unsteady movement, lack of appetite, dizziness, dyspnoea, sleep, coma and death.

**Keywords:** Mistletoe, Phytochemistry, Toxicity, Intra-Peritoneal, LD<sub>50</sub>

## 1. Introduction

*Viscum album* is a parasitic plant which grows on the stem of other tree species. Mistletoe originally so named and also known as European mistletoe or common mistletoe to distinguish it from other related species. *Viscum album* grows on various deciduous trees, occasionally it grows on pines, apple, ash and hawthorn trees [1]; [2].

Mistletoe is used as a treatment for degenerating and inflamed joints and as palliative for malignant tumors [3] and the leaves are helpful in the treatment of diabetes [4]. In local application, it eases rheumatic aches and is very effective for acute attacks of lumbago. It is employed in disorders like excessive menstruation and uterine haemorrhage due to

homeostatic effect [5]. It is also used to treat high blood pressure in man, epilepsy and other nervous conditions [6]. Mistletoe has been used in Nigerian folk medicine for many generations as remedies for various ailments including inflammation, infection and fever [7]. In view of the wide medicinal usage of the plant *Viscum album*, this study was carried out to assess the phytochemical components and its toxicity to provide a safe reference for its use.

## 2. Materials and Methods

### 2.1. Collection, Preparation and Aqueous Extraction of *Viscum album*

Fresh leaves and stems of *Viscum album* from three

different host plants viz: *Azadirachta indica* (Neem), *Psidium guajava* (guava) and *Acacia albida* were collected from within the University of Maiduguri Campus and authenticated by a botanist from the Department of Biological Sciences, University of Maiduguri, Nigeria. Voucher specimen has been kept in the herbarium of Faculty of Science, University of Maiduguri, Nigeria.

The leaves and stems of *Viscum album* collected were rinsed with clean water and dried under shade for a period of one week. The dried leaves and stems were then ground, using mortar and pestle to obtain a fine powder of each sample which was extracted with 1000mls of distilled water for 8 hours at 60°C using a Soxhlet extractor (Quickfit, England<sup>R</sup>) as described by [8]. The *Viscum album* extract was concentrated on an aluminium tray, placed into an oven and maintained overnight at 60°C. The drying process removed the water, leaving only the extract of *Viscum album* from *Azadirachta indica*, *Psidium guajava* and *Acacia albida*. The extracts were stored at room temperature (27°C) until required.

## 2.2. Phytochemical Studies

Standard procedures as described by Trease and Evans [9] were used to determine the bioactive constituents of the *Viscum album* extracts in this study.

## 2.3. Acute Toxicity Testing

The modified arithmetic method of Karber as described by Aliu and Nwude [10] was used to evaluate the median lethal dose (LD<sub>50</sub>) of the aqueous extracts of stem bark and leaves of *Viscum album* (sourced from 3 host plants) in albino rats.

Twenty five (25) adult albino rats of both sexes weighing between 110 and 220 grams were used for each extract, giving a total of 150 rats. The experimental albino rats were divided into five (5) groups (A-E) of 5 rats each and kept within ambient conditions (temperature: 27±1°C.,

photoperiod: 12 hours natural light and 12 hour dark, humidity 40±5%). They were fed with standard feed (Grant Cereal Ltd, UAC Nigeria PIC, Jos, Nigeria) and portable water was provided *ad libitum*. The rats were allowed for 4 weeks to acclimatize to the laboratory conditions before the toxicity study. Groups A, B, C and D were each administered intraperitoneally with graded doses of 100, 200, 400 and 800mg/kg of extract respectively at an extract concentration of 10g/100ml, while group E rats received only distilled water intraperitoneally and served as control. All rats were observed over a period of 24hours for clinical signs of toxicity and death. All experimental albino rats were handled according to the international guiding principles for biomedical research involving animal use and care [11].

## 3. Results

Table 1 shows the phytochemistry of *Viscum album* leaf and stem extracts from *A. indica*, *A. albida* and *P. guajava*, containing tannins, alkaloid, saponins, glycosides, flavonoids, phenols, combined anthraquinones, carbohydrates, reducing sugar and combined sugar. Alkaloids was absent in leaf of *Viscum album* obtained from *P. guajava*. Saponins had the highest scores (+++) in all the extracts followed by phenols and carbohydrates with moderate scores (++) , while reducing and combined sugars had the lowest concentrations (+) (Table 1).

Table 2 shows the calculated median lethal dose (LD<sub>50</sub>) values of *Viscum album* leaf and stem from *A. indica*. The calculated value for the leaf and stem are 1440mg/kg/bw and 600mg/kg/bw respectively. The calculated median lethal dose (LD<sub>50</sub>) value of *Viscum album* leaf and stem from *P. guajava* was 2400mg/kg (table 3). Finally, table 4 shows the LD<sub>50</sub> values of *Viscum album* leaf and stem from *A. albida*, to be 2400mg/kg/bw and 2880mg/kg/bw respectively.

**Table 1.** Phytochemistry of Leaf and Stem Aqueous Extracts of *Viscum album* obtained from *Azadirachta indica*, *Acacia albida* and *Psidium guajava* Plants.

Component	Test	<i>Viscum album</i>					
		<i>A. indica</i>		<i>A. albida</i>		<i>P. guajava</i>	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
Tannins	Ferric Chlorine	++	++	+	+	+	+
Alkaloids	Mayer's	+	-	+	-	-	-
	Dragendorff's	++	++	+	+	-	+
Saponins	Frothing	+++	+++	+++	+++	+++	+++
Glycoside	Salkowski's	+	+	+	++	++	+
Flavonoids	Pew's	++	++	+	+	+	+
Terpenes	Lieberman						
	Buchard's	-	-	-	-	-	-
Steroids	Acetic anhydride	-	-	-	-	-	-
Phenol	Ferric Chloride	++	++	++	++	++	++
Ketones	Resorcinol						
	Selivanoff's	-	-	-	-	-	-
Phlobatannins	Standard	-	-	-	-	-	-
Anthraquinones	Borntrager's	-	-	-	-	-	-
Combined	Standard	+	++	++	++	+	+
Anthraquinone							
Carbohydrate	Molisch's	++	++	++	++	++	++
Monosaccharide	Barfoed's	-	-	-	-	-	-
Reducing sugar	Fehlings	+	+	+	+	+	+

Component	Test	<i>Viscum album</i>					
		<i>A. indica</i>		<i>A. albida</i>		<i>P. guajava</i>	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
Combined sugar	Standard	+	+	+	+	+	+

KEYS: +Present in low concentration ++Moderate Concentration +++Present in high concentration – Absent.

**Table 2.** LD<sub>50</sub> Values of *Viscum album* Leaf and stem extract from *A. Indica* for albino rats.

Group n = 5	Dose of extract (mg/kg bw)	Dose difference (Dd)		Death		Mean dead (md)		Md x Dd	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
A	100	-	-	0	0	-	-	-	-
B	200	100	100	0	0	0	0	0	0
C	400	200	200	0	0	0	0	0	0
D	800	400	400	0	5	0	2.5	0	1000
E	1600	800	800	4	-	2	-	1600	-
F	3200	1600	1600	5	-	2.5	-	7200	-
TOTAL								8800	1000

**Table 3.** LD<sub>50</sub> Values of *Viscum album* Leaf and stem extract from *P. guajava* for albino rats.

Group n = 5	Dose of extract (mg/kg bw)	Dose difference (Dd)		Death		Mean dead (md)		Md x Dd	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
A	100	-	-	0	0	-	-	-	-
B	200	100	100	0	0	0	0	0	0
C	400	200	200	0	0	0	0	0	0
D	800	400	400	0	0	0	0	0	0
E	1600	800	800	0	0	0	0.5	0	400
F	3200	1600	1600	5	5	2.5	3	4000	4800
TOTAL								4000	5200

**Table 4.** LD<sub>50</sub> Values of *Viscum album* Leaf and stem extract from *A. albida* for albino rats.

Group n = 5	Dose of extract (mg/kg bw)	Dose difference (Dd)		Death		Mean dead (md)		Md x Dd	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
A	100	-	-	0	0	0	0	0	0
B	200	100	100	0	0	0	0	0	0
C	400	200	200	0	0	0	0	0	0
D	800	400	400	0	0	0	0	0	0
E	1600	800	800	0	0	0	0	0	0
F	3200	1600	1600	5	4	2.5	2	-	4000
G	6400	3200	3200	5		2.5		3200	8000
TOTAL								3200	17,600

## 4. Discussion

The results of the phytochemical screening in this study has shown that the aqueous extracts from the leaf and stem of *Viscum album* species from *Azadirachta indica*, *Acacia albida* and *Psidium guajava* contained tannins, alkaloids, saponins, glycosides, flavonoids, phenols, combined anthraquinones, carbohydrates, reducing sugars and combined sugars. This agrees with the findings by Hass *et al.*, [12]; [13] and Taiga, [14] who expressed the presence of these compounds from *Viscum album* species from apple, guava, kolanut, orange, pear and cocoa trees. However, Yusuf *et al.*, [15] also reported the presence of terpenoids, phytates, oxalates and cardiac glycosides from *Viscum album* from cocoa and cola trees in Nigeria. Furthermore, Florence and Olawoye, [16] reported the presence of saponins, tannins, anthraquinones, cardinolides in the stem and leaves of *Viscum album* of orange and guava but observed that phlobatannins was only present in guava. Meanwhile Luczkiewicz *et al.*, [17]; [18] and Vicas *et al.*, [19]

emphasized that the phytochemical profile of *Viscum album* species depends on the harvesting time and the host trees of the plant.

Phytochemicals are compounds that are also known as secondary plant metabolites and have biological properties such as antioxidant activity [20]; [21]; [19]; [22], antimicrobial effects [23], modulation and detoxification enzymes, stimulation of the immune system [23]; [22], modulation and hormone metabolism and anti-cancer properties [24]; [25]; [26]; [22], and also are oxygen free radical quenchers and inhibit lipid peroxidation [22].

Following the administration in the extract, the animals exhibited clinical signs of weakness, depression, arched back, gait, anorexia, insomnia, dizziness and dyspnoea. Terminally, there were coma and death after a 2 hour period. These findings agree with Ohaeri and Agaoru, [32] who observed similar signs, and attested that death could be due to intravascular obstruction of the circulatory system resulting into asphyxia.

The results on acute toxicity testing indicated an LD<sub>50</sub> of 1440, 2400 and 2400mg/kg for the leaf aqueous extracts, and

600, 2400 and 2880mg/kg for the stem aqueous extracts of *Viscum album* from *Azadirachta indica*, *Psidium guajava* and *Acacia albida* *Viscum album* species respectively. These values agree with the findings of Eno *et al.*, [27] that *Viscum album* species in Nigeria had a very wide safety margin due to its high LD<sub>50</sub> value of 417.5mg/kg in mice. Also, according to Clarke and Clarke, [28]; [29]; [30]; [31]. Substances with LD<sub>50</sub> values between 500mg/kg and 5000mg/kg are moderately or less toxic and could be administered with some degree of safety especially through the oral route where absorption might not be complete due to inherent factors limiting absorption in the gastrointestinal tract.

## 5. Conclusion

Conclusively, intra-peritoneal administration of graded doses of *Viscum album* extracts from different plants appears to be safe and validate its folkloric application in the management of various conditions in humans and animals.

## References

- [1] Bocci, B. (1993). Mistletoe (*Viscum album*) lectins as cytokins inducers and immuno-adjuvant in tumour therapy. A review. *Journal of Biologic Regulatory Homeostatic Agent* 7:1-6.
- [2] Hajto, J. (1986). Immuno modulatory effects of Iscador. A *Viscum album* preparation. *Oncology* 43:51-63.
- [3] Kafaru, E. (1994), *Immense help from nature's workshop*. Elikaf Health Services Ltd, Nigeria. Pp. 32- 35.
- [4] Obatomi, D. K., Bikomo, E. O. and Temple, V. (1994). Antidiabetic properties of African Mistletoe in Streptozotocin induced diabetic rats. *Journal of Ethno –Pharmacology* 43:13-17.
- [5] Pamplona-Roger, G. H (1999). Medicinal Plants: Encyclopedia of Medicinal Plants. *Safelife Publications*. Pp. 343.
- [6] Gill, L. S (1992) *Viscum album* L. *Ethnomedicinal uses of plants in Nigeria*. University of Benin Press 3:89-91.
- [7] Guardian (2007). Herbal routes to beating diabetes. Lagos, Nigeria Published 13<sup>th</sup>. December, 2007.
- [8] Mittal, G. C., Aguwa, C. N., Ezeiru, V. U., Akubue, P. I., (1981). Preliminary pharmacological studies on antivenin action of *Diodi siandens* leaves. *Nigerian Journal of Pharmacology* 12, 432–436.
- [9] Trease, E. G. and Evans, W. C. (1978). *Pharmacognosy*. 11th Edition, Balliere Tindall, London, pp. 115-222.
- [10] Aliu, Y. O and Nwude, N. (1982). *Veterinary Pharmacology and Toxicology Experiments*. Baraka Press Nigeria Ltd, Zaria 1<sup>st</sup> ed. Pp. 104–109.
- [11] C. I. O. M. S (1995) Council for International Organisation of Medical Sciences. International Guiding Principles for Biomedical Research Involving Animals. Pp 98-134.
- [12] Haas, K., Bauer, M. and Wollenweber, E. (2003). Curricular waxes and flavonol aglycones of mistletoes. *Z Naturforsch* 58c: 464-470.
- [13] Oguntoye, S. O., Olatunji, G. A., Kolawale, O. M. and Enonbun, K. I. (2008). Phytochemical and antibacterial activity of *Viscum album* (mistletoe) extract. *Plants Sciences Medwell Journals* 1 (3):44-46.
- [14] Taiga, A. (2013) Quantitative phytochemical properties of Mistletoe (*Viscum album*) from five different plants. *Research Journal of Agricultural and Enviromental Management* 2 (6): 150-153.
- [15] Yusuf, L., Oladunmoye, M. K., Ogundare, A. O., Akinyosoye, F. A., Hassan, G. F., & Momoh, A. O. (2013). Comparative antibacterial studies of mistletoes growing on two diffrent host plants in Akure North, Nigeria. *International Journal of Medicine and Medical Science*, 3 (5), 009-011.
- [16] Florence I F and Olawoye, I. T. (2011). A preliminary phytochemistry of metabolites of orange (*Citrus sinensis*) and guava (*Psidium guajava*) mistletoes and their host plant. *Journal of Medicinal Plant Reseach* 5 (3):340:343.
- [17] Luczkiewicz, M., Cisonski, W., Kaiser, P., Ochocka, R. and Piotrowski A. (2001). Comparative analysis of phenolic contents in methanol extracts from medicinal plants. *Food Science Technology* 41 (3): 385-390.
- [18] Ekhaize, F. O., Ofoezie, V. G. and Enokhare, D. A. (2010). Antibacterial properties and preliminary phytochemical analysis of methanolic. extract of mistletoe (*Tapinanthus bangwensis*). *Bayero Journal of Pure and Applied Science*, 3 (2): 65-68.
- [19] Vicas, S. I., Rugina, D., Leopold, L., PlnTEA, A., & Socaciu, C. (2011). HPLC fingerprint of bioactive compounds and antioxidant activities of *Viscum album* from different host trees. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 39 (1), 48.
- [20] Orhan D. D., Aslan, M., Sendogdu, N., Ergun, E. and Yesilada, F. (2005). Evaluation of the hypoglycemic effect and antioxidant activity of three *Viscum album* species (European Mistletoes) in streptozotocin-diabetic rats, *Journal of Ethnopharmacology*, 98 (1-2): 95-102.
- [21] Shaboddin, M. Pouramir, M., Moghadamnia, A, A. Prsian, H. Lakzaei, M. (2011). *Pyrus biossieriana bulise* leaf extract: an antioxidant, antihyperglycemic and anti hyperlipidemic agent. *Food Chemistry* 126:1730–3.
- [22] Saxena, M. Saxena, J., Nema R., Sigh, D., and Gupta, A. (2013). *Phytochemsitry of Medical Plants*. *Journal of Pharmacy and Phytochemistry* 1 (6): 168-182.
- [23] Lavastre, V., Pelletier, M., Saller, R., Hostanska, K. and Girad, D. (2004). Mechanism involved in spontaneons and *Viscum album* agglutinin-1- induced human neutrophil opoptosis: *Viscum album* agglutinin-1 accelerates the loss of anti apoptotic MCL-1 expression and the degredation of exoskeletal paxillin and vimentin proteins via caspases. *Journal of Immunology* 118: 1419-1427.
- [24] Burger, A. M, Mengs, U., Schullar J. B. and Feibig, H. H. (2001). Anticancer Activity of an Aqueous Mistletoe Extract (AAME) in Syngeneic Murine Tumor Models. *Anticancer Research* 2:196–8.
- [25] Cetin E. S. and Ozcelik, N. (2000) Apoptotic Mechanism of Mistletoe (*Viscum album*) extracts. *Turkish Klin Tip Bilim* 27:533-9.

- [26] Sabova, L., Pilatova, M., Szilagyi K.; Sabo, R. and Mojzis, J. (2010). "Cytotoxic effects of mistletoe (*Viscum album L.*) extracts on jurket cells and its interaction with doxorubicin" *Phytotherapy Research*, 24 (3):365-368.
- [27] Eno, A. E., Ibovette, U. E., Offem, O. E., Unoh, F. B., Nkanu, E., Azah, N. and Ibu, J. O. (2004). The effects of a Nigerian species of *Viscum album* (mistletoe) leaf extract on the blood pressure of normotensive and doca- induced hypertensive rats. *Nigerian Journal of Physiological Science*. 19 (1-2): 33-38.
- [28] Clarke, E. G. C. and Clarke, M. I. (1979). Factors affecting the actions of poison. In: *Veterinary Toxicology*, Bailliere Tindall, London. Pp. 9-13.
- [29] Myre, L. C, Harvey, O. G. and Humphrey, D. J. (1981). *Veterinary Toxicology*, (2<sup>nd</sup> eds) Bailliere Tindall, London. Pp. 8.
- [30] Adewoye, R. O. (1999). Pesticides and the Nigerian environment: A paper presented at a training workshop on "The Petroleum Trust Fund (P. T. F) Agricultural and Veterinary Inputs Revolving Fund Scheme" (A. V. I. R. F. S). 23<sup>rd</sup> -25<sup>th</sup> February, Zodiac Hotel Enugu. 20 pages.
- [31] Biu, A. A., Yusufu, S. D. and Rabo, J. S. (2010). Acute toxicity studies on neem (*Azadirachta indica*, A Juss) leaf aqueous extract in chicken (*Gallus gallus*). *African Scientist* 11:241-244.
- [32] Ohaeri, C. C and Agaoru, C. G. (2012). Antitrypanosomal effects of neem (*Azadirachta indica*) extract in rats infected with *Trypanosoma brucei*. *International Journal Advanced Semantic and Technical Research* 2 (5):309-315.