

## Phytochemical Screening and Antibacterial Activity of *Viscum album* (Mistletoe) Extracts

<sup>1</sup>S.O. Oguntoye, <sup>1</sup>G.A. Olatunji, <sup>2</sup>O.M. Kolawole and <sup>2</sup>K.I. Enonbun

<sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Medical Microbiology,  
University of Ilorin, Ilorin, Nigeria

**Abstract:** *Viscum album* is a green parasitic plant belonging to the family of Loranthaceae. It grows as a partial parasite on the branches of many deciduous lemon trees especially apple and has various ethnomedical uses. Phytochemical screening has shown the presence of Alkaloids, Carbohydrates, Tannins and Flavonoids. Phytosterols are scanty. Thin-layer chromatography of the ethanolic extract (Petroleum spirit and Diethyl ether, 2:1) showed four spots, while the aqueous extract (Petroleum spirit and Diethyl ether, 2:1) showed the presence of three spots. The ethanolic and aqueous crude extracts inhibited the growth of *Klebsiella aerogenes*, *Proteus* sp., *Escherichia coli* and *Pseudomonas aeruginosa*. *Gentamycin* and *Cloxacillin* did not exhibit any activity against *Pseudomonas aeruginosa*.

**Key words:** *Viscum album*, phytochemical, bacterial, antibiotics

### INTRODUCTION

*Viscum album* L. (Synonyms: *Viscum*, *Mistletoe*) is a common bushy plant of the family Loranthaceae, which grows as an epiphyte on the branches of deciduous trees. The plant is widely distributed in tropical and subtropical Africa, in Asia and in Europe (Duke, 1985). The plant is used in the treatment of nervous complaints, bleeding and tumours (Duke, 1985; Pamplona-Roger, 1999).

The extracts from the leaves stimulate insulin secretion from pancreas cells and help in the treatment of arthritis (Blumenthal *et al.*, 1998; Gray and Flatt, 1999).

Earlier research has shown that the leaf contains choline, acetylcholine, lectins, polypeptides and polysaccharides. The lectins, peptides and polysaccharides have shown immune-stimulating activity in human studies when *Mistletoe*s extracts are given by injection (Hajto, 1986; Bocci, 1993). Other studies suggested that *Mistletoe*'s alkaloid can help to kill cancer cells both *in vitro* and *in vivo* (Khwaja *et al.*, 1986; Jurin *et al.*, 1993). Other authors also reported some flavanones and chalcones as constituents of *Viscum album* (Haas *et al.*, 2003). Cuticular waxes of *Viscum album* sp. show a high content of oleanolic acid, with aliphatic constituents such as alkanes, esters, aldehydes, primary alcohols and free fatty acids present in much lesser amounts (Wollenweber *et al.*, 2002; Haas *et al.*, 2003).

In local application, it eases rheumatic aches and is very effective for acute attacks of Lumbago. It is employed in menstrual disorders, excessive menstruation

and uterine haemorrhage due to homeostatic effect (Pamplona-Roger, 1999). The decoction of the leaves is one of the most effective extract used in treating high blood pressure in man, epilepsy and other nervous conditions (Gill, 1992).

The efficacy of this plant in the treatment of various diseases necessitated the present work in order to identify the classes of natural products present in the leaf of the plant.

### MATERIALS AND METHODS

Fresh leaves of the plant were collected at various locations within the Premises of University of Ilorin on *Parinari curatellifolia* tree [PAX326]. They were identified and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin.

**Preparation of extracts:** The fresh leaves were dried and chopped into pieces and powdered. A 200 g each was extracted separately using 500 mL cold deionised water and 95% ethanol as solvent. The extraction lasted 3 days at 28°C. The crude extracts were concentrated to give 30 g of ethanolic and 45 g of aqueous extracts. Thin-layer chromatography (0.25 mm silica gel/UV<sub>254</sub> pre-coated polygram plate, Merck, Germany) of the crude extract (Petroleum ether/Diethyl ether 2:1) gave 4 major spots for ethanolic crude extract, while three main spots were identified on the plate for aqueous crude extract.

**Test organisms:** Five bacteria species were employed as test organisms. These include; *Staphylococcus aureus*, *Klebsiella aerogenes*, *Proteus* sp., *Escherichia coli* and *Pseudomonas aeruginosa*. They were collected from clinical specimens in University of Ilorin Teaching Hospital (UITH). The organisms were maintained on Muller Hinton agar slants at 4°C prior to testing.

**Preparation of filter paper disks:** Filter paper disks were prepared by cutting paper disks of about 7 mm in diameter from Whatman filter paper No. 1 (110 mm) using a punch. The disks were sterilized in a hot air oven and to these were added a little quantity of each of the crude extracts. The disks were allowed to absorb the extracts.

**Antibacterial assay:** Each test organism was cultured and incubated for 3 h. These were used to inoculate plates of Muller Hinton agar. The disks containing the different crude extracts were transferred using sterilized forceps onto the surface of the inoculated agar plates. Sterile water and 95% ethanol were used as control. Standard antibiotic disks were also used for comparison. The plates were incubated at 37°C for 24 h before been examined for zones of inhibition of growth.

**Phytochemical analysis:** The extracts were subjected to analysis for the presence of saponins, steroids, alkaloids, flavonoids, tannins, carbohydrates, lipids and terpenoids, using standard methods (Harborne, 1998; Trease and Evans, 1978).

## RESULTS AND DISCUSSION

Table 1 shows the result of the preliminary phytochemical analysis. The results showed the presence of flavonoids, tannins, alkaloids and carbohydrates.

While saponins, lipids and terpenoids were absent. Steroids were scanty present due to the formation of a brown ring without bluish upper layer as reported in the literature (Harborne, 1998). When the crude extracts (ethanolic and aqueous) of the leaf were heated with magnesium turnings and concentrated hydrochloric acid, intense red colour was formed. This confirms the presence of flavonoids.

The results of the antibacterial screening are shown in Table 2. It could be seen that none of the extracts inhibits *Staphylococcus aureus*. *Pseudomonas aeruginosa* was resistant to both *gentamycin* and *cloxacillin*. The susceptibility test showed that the cold aqueous and ethanolic extracts have similar activities on the test organisms, although cold aqueous extract had a slightly higher inhibition zones than ethanolic extract. The antibacterial activity of the *Mistletoe* extracts correlated well with its traditional use (Pamplona-Roger, 1999).

Also, the antibacterial activity of *Mistletoe* extracts compared well with the standard antibiotic used (*Gentamycin* and *Cloxacillin*).

The resistant strain of *Pseudomonas aeruginosa* has been known to pose a major threat to hospitalized patients with underlying disease such as cancer (Hentges, 1986).

From this study, it could be seen that both the ethanolic crude extract and aqueous crude extract inhibits *Pseudomonas aeruginosa*. Based on this result, *Mistletoe* extracts administration may do well for cancer patients who are prone to *Pseudomonas aeruginosa* infection. The anticancer activity of *Mistletoe* alkaloids had earlier been reported (Khwaja *et al.*, 1986). The medicinal action of *Mistletoe* can therefore be attributed to the combined presence of flavonoids, alkaloids, carbohydrates and tannins in the leave extracts.

Table 1: Phytochemical analysis of the crude extracts of the leaves of *viscum album*

Extract	Class of natural product							
	Saponins	Steroids	Flavonoids	Tannins	Alkaloids	Terpenoids	Carbohydrates	Lipids
Aqueous	-	+	+++	+++	+++	-	+++	-
Ethanolic	-	+	+++	+++	+++	-	+++	-

Key: +++ = Present, + = weakly present, - = Absent

Table 2: Antibacterial activity and zone of inhibition (mm) of the leaf of *viscum album* extracts

Extract	Zone of inhibition (mm)				
	<i>Staphylococcus aureus</i>	<i>Klebsiella aerogenes</i>	<i>Proteus species</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
Ethanol	-	10.00	10.00	8.00	8.00
Aqueous	-	12.00	11.00	9.00	9.00
<i>Gentamycin</i>	11.00	10.50	15.00	17.50	-
<i>Cloxacillin</i>	8.00	10.00	10.50	7.50	-
95% Ethanol (control)	-	-	-	-	-
Deionised water (control)	-	-	-	-	-

### CONCLUSION

This present research has shown that the local species of *Viscum album* contains many classes of natural products. These include: Flavonoids, alkaloids, carbohydrates and tannins. Both ethanolic and aqueous crude extracts have been shown to be effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus sp.* and *Klebsiella aerogenes*. Work is in progress to isolate the individual pure compounds and characterize them.

### ACKNOWLEDGEMENT

The authors are grateful to Mr. Babatunde and Mr. Isaac Njoku for their technical assistance.

### REFERENCES

- Blumenthal, M., W.R. Busse and A. Goldberg, 1998. Therapeutic guide to herbal medicine. The complete German Commission E. Monogr., 171: 2-3.
- Bocci, B., 1993. *Mistletoe (Viscum album)* Lectins as cytokine inducers and Immuno-adjuvant in tumour therapy. A review. J. Biol. Regulatory Homoeostatic Agents, 7: 1-6.
- Duke, J.A., 1985. *Mistletoe*. In: Handbook of Medicinal Herbs CRC Press. Florida, pp: 512-513.
- Gill, L.S., 1992. *Viscum album* L. Ethnomedical uses of plants in Nigeria. University of Benin Press. Benin, pp: 244-245.
- Gray, A.M. and P.R. Flatt, 1999. Insulin-secreting activities of the traditional antidiabetic plant *Viscum album*. J. Endocrinol., 160: 409-414.
- Haas, K., M. Bauer and E. Wollenweber, 2003. Cuticular Waxes and Flavonol Aglycones of *Mistletoes*. Z. Naturforsch, 58c: 464-470.
- Hajto, J., 1986. Immunomodulatory effects of Iscador. A *Viscum album* preparation. Oncology, 43: 51-63.
- Harborne, J.P., 1998. Phytochemical Methods. 2nd Edn. Chapman and Hall, London, pp: 50-94.
- Hentges, D.J., 1986. Enteric Bacteria Medical Microbiology. Littler Brown and Company. Boston, pp: 152-153.
- Jurin, M., N. Zarkovic, M. Hrzenjak and Z. Ilic, 1993. Antitumours and Immunomodulatory Effects of the *Viscum album* L. preparation. Oncology, 50: 393-398.
- Khwaja, T.A., C.B. Dias and S. Pentecost, 1986. Resent studies on the anticancer activities of *Mistletoe (Viscum album)* and its alkaloid. Oncology, 3: 1-10.
- Pamplona-Roger, G.H., 1999. Medicinal plants: Encyclopedia of Medicinal Plants. Safeliz publication. Spain, pp: 5-247.
- Trease, G.E. and W.C. Evans, 1978. Pharmacognosy. 11th Edn. Baillirer Tindall Ltd., London, pp: 784.
- Wollenweber, E., A. Wieland and K. Haas, 2002. Epicuticular waxes and Flavonol waxes and Flavonol Aglycones of the European *Mistletoe, Viscum album*. Z. Naturforsch, 55c: 314-317.