A Comparative Study Of The Phytochemical And Anti-Microbial Properties Of The Eastern Nigerian Specie Of African Mistletoe (*Loranthus micranthus*) Sourced From Different Host Trees

Osadebe, P. O. and Ukwueze, S. E.
Department of Pharmaceutical Chemistry,
Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Correspondence author: Osadebe, P. O., Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka.

Abstract

A comparative study of the phytochemical and anti-microbial properties of leaves of *Loranthus micranthus*, harvested from six host trees, namely, *Irvingia gabonensis, Pentaclethra macrophylla, Kola acuminata, Baphia nitida, Persea americana and Azadirachta indica*, was carried out using standard methods. The result showed marked variations in phytochemical constituents and anti-microbial activities of the extracts from the different host trees, both kind and in degree. The extracts from *K. acuminata*, *P. americana* and to lesser extent, *I. gabonensis* showed marked broad-spectrum activities against bacteria and fungi. When compared with standard antibiotics (amoxycillin and ketoconazole) as controls, some of the extracts were found to be significantly more active than the control. The extract from *P. americana* exhibited significant anti-pseudomonal activity ($P < 0.01$) when compared to amoxycillin while the extracts from *I. gabonensis*, *P. macrophylla* and *A. indica* all showed significant activity ($P<0.05$) against *Staph. aureus* when compared to amoxycillin. Alkaloids were found to be most abundant in *K acuminata*; *P. americana* and *I. gabonensis* The preponderance of alkaloids in the extracts from *K. acuminata, P. americana and I. gabonensis* as compared to the extract from the other host plants could be suggestive of a relationship between alkaloidal content and the antimicrobial activity. Hence, during the preparation of Pharmaceutical /herbal formulation for the treatment of non- specific infections, mistletoe may be preferentially sourced from *K. acuminata* and *P. americana*.

Keyword Words: Mistletoe, host- tree variation, antimicrobial activity, phytochemical contents, *Loranthus micranthus,* comparative study.

Introduction

African mistletoe (Eastern Nigerian version), known as *Loranthus micranthus* Linn. (Family - Loranthaceae) is a semi-parasitic evergreen plant which depends on its host for minerals and water only, but photosynthesizes its carbohydrate by means of its green leaves (Griggs, 1991). Mistletoe grows on a host of evergreen and deciduous trees all year round, around the branches of the tree. It is an obligate parasite, obtaining part of its food from the host plant. The name, African mistletoe has been used for several different plants including, *Loranthus begwensis* Linn. (a Northern Nigerian specie), *Tapinanthus vittatus* (a Southern African specie), *Loranthus micranthus* Linn. (an Eastern Nigerian specie),and *Erianthemum ulugurense* (a Kenyan specie). Several other mistletoe plants are well known worldwide. These include the European mistletoe

(*Viscum album* Linn.); Korean or Japanese mistletoe (*Viscum album coloratum*); Australian or Argentina mistletoe (*Ligaria cuneifolia* R. et T.); American mistletoe (*Phoradendron flavescens*), among several others.

Mistletoe has been used traditionally as anti-diabetic, anti-cancer, and anti-hypertensive, etc (Kafaru, 1993). In Nigeria and some other parts of Africa, it is believed that the aqueous extract of mistletoe (Loranthus species), consumed over a long time will bid farewell to the cause of hypertension, diabetes and other metabolic diseases (Obatomi et al, 1994; Dalziel, 1955). In short, Kafaru (1993) described the mistletoe plant as "an all purpose herb" because of its rich folkloric uses. Some of these uses have been verified (Obatomi et. al, 1994; Obatomi et. al, 1996; Fischer et. al, 1997; Schink et. al, 1997).

The use of the European mistletoe (*Viscum album*) as an anticancer and immune system-stimulating agent is well documented (Hulsen et al., 1987; Khwaja et al., 1986; Kuttan et al., 1990). Previous studies have shown that the composition and activities of mistletoe are host- tree, species and harvesting period -dependent. (Obatomi et. al, 1994; Scheer et.al. 1992; Wagner et al, 1996). Obatomi *et al* (1994), detected host- tree variations of the antidiabetic effects in the Northern Nigerian species of the African mistletoe (*Loranthus bengwensis*). They showed that infusions of mistletoe parasitic on lemon and guava trees significantly decreased serum glucose levels whereas that prepared from mistletoe parasitic on *Jatropha curcas* did not. Antihypertensive and anti-diabetic activities of *Loranthus bengwensis* have also been described (Obatomi et al, 1996). In our previous work (Osadebe et al, in press), we did establish a dose-dependent hypoglycaemic and antihyper-glycemic activity of Eastern Nigerian specie of mistletoe plant parasitic on *Persea americana (Avocado tree).* In the present work, we set out to investigate the variation of antimicrobial activities among extracts of L. *micranthus* growing on six different host trees, with a view to finding the host that impacts maximal anti-microbial activity on mistletoe. Such knowledge will make for more economic and optimal use of the plant in alternative medicine.

Materials and Methods

Plant material: *Loranthus micranthus* L was collected at about midday at Obukpa, a village near Nsukka, Enugu State, Nigeria in June 2001. The plant was collected from six different host-tree sources:*Irvingia gabonensis, Pentaclethra macrophylla, Kola acuminata, Baphia nitida, Persea americana and Azadirachta indica.* The plants were identified by Mr. A. Ozioko of the Department of Botany, University of Nigeria, Nsukka. Samples were deposited in the herbarium of the said Department.

Extraction procedure: The mistletoe leaves from individual host tree were dried under shade at room temperature (25 ^{0}C) for 14 days and pulverized using a mechanical grinder. Each powdered plant material (200g) was passed through a 40-mesh sieve and then extracted with petroleum ether. The petroleum ether extract was concentrated *in vacuo* using a rotary evaporator to give a yield of 12.5 %w/w (with respect to the powdered material). The extracts were stored below room temperature. A weighed quantity was suspended in 4% Tween 65 solution to be used for the experiment.

Phytochemical studies: The chemical constituents of the petroleum ether of *L. micranthus* from each host plant were investigated following the method described by Trease and Evans (1978) and Harbourne (1973). Preliminary phytochemical tests were carried out to detect the presence of steroids, alkaloids, tannins, glycosides, carbohydrates, flavonoids, saponins fats and oils.

Antimicrobial screening of the plant extracts

***Microorganism used*:** Two representatives each of gram-positive bacteria (*Staphylococcus aureus and Bacillus substilis*), gram- negative bacteria (*Pseudomonas aeruginosa and Salmonella. typhi*) and fungi (*Aspergillus niger* and *Candida albicans*) were obtained from the Pharmaceutical Microbiology Unit of the Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. The standardized cultures of the organisms were stored under the same conditions and used throughout the experiment.

Antimicrobial activity test: The extracts of mistletoe from various host trees were screened for antimicrobial activity using the agar diffusion method. Broth culture (0.1 ml) contaning1 $x10^5$ cells per ml of the required microorganism was introduced into a sterile petri dish and 20 ml of molten nutrient agar added. The content was thoroughly mixed and then allowed to set.

Four 2-fold serial dilutions of mistletoe extracts from the six plants were obtained using 4% v/v aqueous Tween 65. Each petri dish was seeded with 0.1 ml of the appropriate organism. Sterile, molten nutrient agar was then aseptically poured into it, shaken gently and allowed to solidify. Within each of the marked four quadrants, a cup (6 mm in diameter) was bored using a sterile cork borer. Two drops of each dilution were placed in each cup, allowed to diffuse and then incubated at 37^0C for 24 h (for bacteria) or for 48 h (for fungi). The diameter of the zone of inhibition was determined using a transparent meter rule. The minimum inhibitory concentration (MIC) was calculated by plotting the logarithm of the concentration against the square of inhibition zone diameter. The antilogarithm of the intercept on the log concentration axis gave the MIC values.

Statistical analysis: The mean MIC of three different results was used. The results were presented as mMean $\pm$ S.E.M and statistical difference between the activities of the extracts from host trees and control antibiotics were evaluated by the Student's t test. $P < 0.05$ was regarded as significant (Woodson, 1987)

Results and Discussions

Phytochemical tests: The result of the preliminary phytochemical analysis (Table 1) revealed the presence of alkaloids, tannins, glycosides, carbohydrates, flavonoids and little or no steroids in the all six mistletoe extracts. The chemical constituents were found to differ only in degree of content and not in kind. No regular pattern was observed. However, judging from the intensity of the phytochemical reactions, extracts of *L. micranthus* from *Kola accuminata, P. Americana* and *I. gabonensis* could be said to be richer in alkaloids than as found in *B. nitilda*, *P. macrophilla* and *A. indica*. Fats and oils were found to be generally absent. Extracts from *K. acuminata* and *A. indica* were richer in their content of glycosides and flavonoids than the rest of the sources,

Table 1: Phytochemical profiles of the test extracts of mistletoe sourced from different host trees

Host tree	Steroids	Alkaloids	Glycosides	Carbohy-drates	Flavonoids	Saponins	Tannins	Fats and oils
K. acuminata	+	++++	++	+	++	+	+	_
P. americana	_	++++	+	+	+	+	+	_
B.nitida	_	++	+	+	+	+	+	_
P.macrophylla	_	+	+	+	+	+	+	_
I. gabonensis	_	+++	+	+	+	+	+	_
A. indica	+	++	++	++	++	++	++	_

+ = present; - = absent; multiple pluses indicates degree of abundance

Table 2: Results of the anti-microbial screening of extracts of mistletoe from six different host plants (MIC determination)

Host tree	Minimum inhibitory concentrations (MIC, ug/ ml)					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*S. typhi*	*A. niger*	*C. albicans*
I. gabonensis	4.31 ± 0.17*	3.53 ±0.84	5.53 ± 2.34	5.55 ± 0.55	_	_
P.macrophylla	3.76 ± 0.25*	4.13 ± 0.59*	7.97± 1.81	_	_	_
K. acuminata	6.08± 0.23	4.19 ± 0.30**	7.16 ± 2.03	6.48 ± 0.68	5.06 ± 0.14[a]	7.98 ± 1.86
A. indica	4.45 ± 0.20*	3.99 ± 0.19***	7.41 ± 1.21	_	_	_
P. americana	5.76± 1.27	7.11 ± 1.49	6.16 ± 0.49***	4.94 ± 0.20	4.63 ± 1.0	6.05 ± 0.32
B.nitida	7.28 ± 0.50	7.70 ± 1.38*	10.32 ± 0.32	_	_	_
(Control 1) Amoxycillin	6.95 ± 0.64	1.19 ± 0.04	9.05 ± 0.74	2.43 ± 0.49	_	_
(Control 2) ketoconazole	_	3.86 ± 0.62	_	_	2.44 ± 0.07	4.26 ± 2.05

*Each value represents the mean ± s.e.m, n = 3, * P < 0.05; ** P < 0.02; ***P < 0.01 significantly different compared with control, amoxycillin; [a]P<0.01 significantly different compared with control, Ketoconazole; Blank spaces indicate no observable inhibition (i.e. lack of sensitivity)*

while saponins and carbohydrates were found to be more abundant in *A. indica* than in the other plant sources.

MIC results: The sensitivity and consequent MIC evaluation of the different extracts of *L. micranthus* leaves gave interesting profiles. All the tested organisms showed varying degrees of sensitivity to the extracts. The extracts from *K. acuminata, P. americana* and to lesser extent, *I. gabonensis* showed a marked broad-spectrum activity against bacteria and fungi (Table 2). When compared with standard antibiotics (amoxycillin and ketoconazole) as controls, some of the extracts were found to be significantly more active (p<0.01) than the standard antibiotics used as control. For instance, the extract from *P. americana* exhibited significantly better anti-pseudomonal activity (P<0.01) when compared to amoxycillin, while the extracts from *I. gabonensis, P. macrophylla* and *A. indica* were significantly more active (P<0.05) against *S. aureus* than amoxycillin. However, for *B. subtilis*, the test extracts were far less effective than the controls. The fungi (*A. niger* and *C. albicans*) appeared resistant to most of the test extracts. Only the extracts from *K. acuminata* and *P. americana* showed mild activity. The anti-typhoid activity exhibited by extracts from *I. gabonensis*, *K. acuminata* and *P. americana*, though weak, (when compared to that of the control, amoxycillin) is also worthy of note.

Most folklore usage attributed to mistletoe tended towards blood pressure control, antidiabetic activity and lately anticancer (Kafaru, 1993). However, this work reveals a great potential for its use in various systemic and non-systemic infections due to common bacteria and fungi. Its activity against staphylococcus and pseudomonas is of particular interest owing to the increasing resistance of the micro-organism to the conventional antibiotics. The observed anti-microbial activity in *L. micranthus* can be attributed to specific plant constituents of the plant. The host-tree variation in phytochemical contents may be attributable to the varying amount of the precursors for the particular plant constituents present in the various host trees (Stypinski, 1997). Although much investigative work still need to be carried out to determine the exact nature and identity of the particular constituents implicated in the antimicrobial activity observed *in L. micranthus*, suffice it to say that plant alkaloids and tannins have been reported to be responsible for most of the antimicrobial properties exhibited by plants (Khwaja et al, 1996).

Conclusion: Significant variations exist in the phytochemical content and anti-microbial activities exhibited by mistletoe from six host plants. For preparation of Pharmaceutical /herbal formulation for the treatment of non-specific infections, mistletoe may be preferentially sourced from *K. acuminata* and *P. americana.*

Acknowledgement

We are grateful to Mr. Emma Efegwo (retired) formerly of Works Department, University of Nigeria, Nsukka, for calling our attention to the rich medicinal uses of mistletoe and also for showing us a publication on the Guardian Newspaper (a Nigerian Daily) on mistletoe.

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