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A Study of the Seasonal Variation in the Antimicrobial Constituents of the Leaves of *Loranthus micranthus* Sourced from *Percia americana*

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Abstract: A comparative study of the antimicrobial constituents of the leaves of *Loranthus micranthus* (parasitic on *Percia americana*) harvested at different seasons of the year, namely, January, April, July and November, was carried out. The air-dried and pulverized leaves harvested at the stated periods were extracted with petroleum ether and the extract subjected to antimicrobial screening and phytochemical investigation. Using various solvent treatments the powdered leaves harvested in April was fractionated into four fractions, A, B, C and D; each fraction was screened for antimicrobial activity and phytochemical constituents. Phytochemical analysis of the extracts showed presence of tannins, flavonoids, alkaloids, terpenoids and saponins with some of these constituents showing variations across the seasons. Broad spectrum antibacterial activity was observed for all the extracts. However, the activity against *Bacillus subtilis* and *Salmonella kapemba* was significantly ($p < 0.001$) lower for the extracts of the leaves harvested in January when compared with the extracts of the leaves harvested in the other months. Only the extracts of the leaves harvested in April showed antifungal activity. Fractions A, B and D showed antimicrobial activity comparable ($p < 0.05$) to standard antibiotic, chloramphenicol. Fraction A is rich in alkaloids, B in terpenoids, A and D in tannins. The presence of alkaloids only in April and July may explain the higher antimicrobial activity observed in these months. In conclusion, mistletoe used for herbal remedy of nonspecific infections may be preferentially harvested in April and July.

Key words: *Loranthus micranthus*, antimicrobial, seasonal variation

INTRODUCTION

Loranthus micranthus Linn. (Loranthaceae) is the Eastern Nigerian species of the African mistletoe, which has been used widely in ethnomedicine as anti-diabetic, anticancer, anti-hypertensive, antimicrobial etc. (Oliver-Bever, 1986; Kafaru, 1993). Different research teams have worked on various species of the plant and demonstrated some pharmacological activities, which supported the claimed ethnomedicinal uses (Obatomi *et al.*, 1994; Dalziel, 1955; Osadebe *et al.*, 2004; Obatomi *et al.*, 1996; Kuttan *et al.*, 1990; Bolksman *et al.*, 1982; Osadebe and Akabogu, 2006).

Studies have shown that the composition and activities of mistletoe are host tree and harvesting period-dependent (Obatomi *et al.*, 1994; Scheer *et al.*, 1992; Wagner *et al.*, 1996; Osadebe *et al.*, 2004). In our earlier study (Osadebe and Ukwueze, 2004), we demonstrated a host tree variation in the antimicrobial activity of *Loranthus micranthus* leaves. The plant material sourced from *Persea americana* and *Kola acuminata* was found to show relatively better spectrum of antibacterial activity when compared with that sourced from the other host trees. The present study is aimed at

investigating the seasonal variation in the antimicrobial constituents of *Loranthus micranthus* leaves sourced from *Persea americana*. Considering the recent popularity of *Loranthus micranthus* as herbal remedy, such knowledge will ultimately make for more economic and optimal use of the plant in alternative medicine.

MATERIALS AND METHODS

Plant Materials

The leaves of *Loranthus micranthus* Linn. (Loranthaceae) parasitic on *Persea americana*, were harvested in April and July (the onset and peak of rainy season) and in November and January (the onset and peak of dry season) 2004 from Obukpa in Nsukka Enugu State, Nigeria. These periods represent the prominent seasons of the year in the tropics. The plant materials were authenticated by Mr. Ozioko of Bioresources Development and Conservation Project, Nsukka, Enugu State Nigeria.

Tested Microorganisms

Standard cultures of *Staphylococcus aureus* (NCTC 3761), *Bacillus subtilis* (NCTC 3610), *Escherichia coli* (NCTC 9001), *Salmonella kapemba* (laboratory strain), *Pseudomonas aeruginosa* (NCTC 6750), *Candida albicans* (laboratory strain) and *Aspergillus niger* (laboratory strain) were collected from the Pharmaceutical Microbiology Unit of the Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria.

Other Materials

Nutrient Agar, nutrient broth, Sabourand's dextrose Agar and Sabouraud's Dextrose Broth (Merck) were used. The media were prepared according to the manufacturer's instruction. Analytical grade methanol, petroleum ether (40-60°C), chloroform ethyl acetate (BDH) and freshly distilled water were used.

Preparation of Culture Media

Nutrient Agar (28 g) was dispersed in 1 L of deionized water and the mixture allowed to soak for 10 min and then mixed. The dispersion was sterilized by autoclaving for about 15 min at 121°C. It was allowed to cool to 40°C before pouring into plates. Other media were similarly prepared.

Preparation of Plant Materials

Ten gram portion each of the powdered leaves of *Loranthus micranthus* (parasitic on *Persea americana*) harvested at the stated periods were extracted with 100 mL petroleum ether (40-60°C) by cold maceration for 48 h. The extracts were concentrated *in vacuo* using rotary evaporator and stored in the refrigerator at 4°C until use.

Another portion (100 g) of the powdered leaves of *Loranthus micranthus* harvested in April was extracted with 1 L petroleum ether by cold maceration for 48 h. The resulting extract (A) was concentrated *in vacuo* (5.1%). The air-dried residue of (A) was repeatedly extracted (500 mL × 4) with 90% methanol for 4 days. The methanol extract (10.4%) was treated (washed) with chloroform and ethyl acetate to obtain the chloroform soluble, B (2.6%); ethyl acetate soluble, C (3.1%) and ethyl acetate insoluble, D (4.7%) fractions.

Antimicrobial Screening

The sensitivities of the selected bacteria and fungi to extracts and fractions of the leaves of *Loranthus micranthus* were evaluated by the cup plate agar diffusion method (Lovian, 1987). Stock solutions (10 mg mL⁻¹) of the extracts and fractions were prepared in 10% Tween 20.

Molten nutrient agar and Sabourand's dextrose Agar (20 mL each) were seeded with 0.2 mL of standardized broth cultures of bacteria and fungi, respectively. Cups, 8 mm in diameter were made in the agar using a sterile cork borer. Equal volumes (0.04 mL) of 10 mg mL⁻¹ extracts and fractions were introduced into respective cups using sterile Pasteur pipettes. The dishes were allowed to stand for one hour at room temperature for proper diffusion of the drugs before incubating at 37°C for 24 h and at room temperature (25°C) for 48 h for bacteria and fungi respectively. The experiments were done in triplicates and the average of the diameter of zone of inhibition (IZD) measured at the end of incubation period taken. Similar experiments were run using standard drugs Chloramphenicol for bacteria and nistatin for fungi. Control experiments were also done using 10% Tween 20.

Phytochemical Test

Phytochemical analysis on extracts and fractions was carried out using standard methods (Harborne, 1998). In each case, small portions of the dried extracts/fractions were extracted in a test tube and a given quantity of the reagent added. The mixtures were shaken vigorously or gently and the presence or absence of saponins, flavonoids, tannins, alkaloids etc observed.

Statistical Analysis

The mean IZD of three replicate results were used. The statistical difference between the activities of the extracts at different seasons was evaluated using one way ANOVA (SPSS 11). p<0.05 and p<0.001 were regarded as significant and highly significant respectively.

RESULTS AND DISCUSSION

The result did not show any particular trend in the distribution of phytochemical constituents across the seasons. However, judging from the intensity of the phytochemical reactions, the extract of the leaves harvested in January, April and July could be said to contain more tannins than that of the leaves harvested in November. Flavonoids are more in the extracts of the leaves harvested in July and November while alkaloids are detected only in April and July. The contents of saponins and terpenoids were similar in all the months (Table 1).

A broad spectrum antibacterial activity was observed for all the tested extracts. Activity against *Bacillus subtilis* and *Salmonella kapemba* was, however, significantly (p<0.001) lower for the extracts of the leaves harvested in January than the extract of the leaves harvested in April, July and November. Also, the extract of the leaves harvested in April showed significantly (p<0.05) higher activity against *Salmonella kapemba* when compared to that harvested in November, while that harvested in July showed significantly (p<0.05) higher activity against *Bacillus subtilis* than that harvested in November. Activity against *Escherichia coli* was significantly (p<0.05) higher for the extracts of the leaves harvested in January and July than that harvested in April and November. There was no

Table 1: Result of the phytochemical tests of the extracts and fractions of *Loranthus micranthus* leaves at different seasons

Months/Fractions	Tannins	Flavonoids	Alkaloids	Terpenoids	Saponins	Red. sugar	Glycosides
January	++	+	-	+	+	+	+
April	++	++	+	+	+	+	+
July	++	+++	+	+	+	-	-
November	+	+++	-	+	+	+	+
A	+	++	+	+	+	+	+
B	-	-	-	+	-	-	-
C	-	++	-	-	-	-	-
D	+	-	-	-	+	+	+

+++ = Present in large quantity; ++ = Moderately present; + = Present in small quantity; - = Absent

Table 2: Results of sensitivity of tests of 10 mg mL⁻¹ petroleum ether extracts of *Loranthus micranthus* leaves

Months	Inhibition zone diameter (mm) Mean±SEM						
	<i>Staph aureus</i>	<i>Bacillus subtilis</i>	<i>Salmonella kapemba</i>	<i>Pseudomonas aeruginosa</i>	<i>Eschericia coli</i>	<i>Candida albican</i>	<i>Aspergillus niger</i>
Jan.	14.7±0.67	13.3±0.33***	10.7±0.67***	16.0±0.58	18.3±0.88	-	-
April	16.7±1.67	17.0±0.58	20.7±0.67	17.3±0.67	16.0±0.58*	12.7±0.8	-
July	16.7±0.67	18.0±0.00	19.7±0.33	16.0±0.58	19.0±0.58	-	-
Nov.	14.7±0.33	16.3±0.33*	18.7±0.33*	16.0±1.0	14.7±0.33**	-	-

Each value represents the Mean±SEM, n = 3, *: p<0.05, **: p<0.01, ***: p<0.001 significantly lower when compared with values obtained at the other months

Table 3: Result of sensitivity of 10 mg mL⁻¹ of different solubility fractions of *Loranthus micranthus* leaves

Fractions	<i>Staph aureus</i>	<i>Bacillus subtilis</i>	<i>Salmonella kapemba</i>	<i>Pseudomonas aeruginosa</i>	<i>Eschericia coli</i>	<i>Candida albican</i>	<i>Aspergillus niger</i>
A	20.7±0.33	18.0±1.00	15.3±0.58	18.0±1.0	14.5±0.88	16.0±1.0	-
B	13.5±0.25 ^a	14.5±0.25 ^a	19.0±1.00	15.0±1.0	16.0±0.00	10.0±0.0 ^b	-
C	-	-	15.0±1.00	-	-	-	-
D	14.0±0.0 ^a	10.0±0.0 ^b	16.7±0.33	-	15.0±1.00	-	12.5±0.3 ^s
Chlor.	26.0±0.0	20.0±0.0	17.0±0.00	17.0±1.0	-	-	-
Nist.	-	12.0±0.0	-	-	-	17.0±1.0	22.0±2.0

Each value represents the mean±SEM, n = 3, ^a: p<0.05, ^b: p<0.01 significantly lower when compared with the control drug, chloramphenicol and nistatin; A = Petroleum ether fraction, B = Chloroform soluble fraction, C = Ethyl acetate soluble fraction, D = Ethyl acetate insoluble, Chlor. = Chloramphenicol, Nist. = Nistatin

significant (p<0.05) difference in the activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* for the extracts of the leaves harvested in all the seasons. Only the extracts of the leaves harvested in April showed mild antifungal activity (Table 2).

Plants are affected by changes in environmental factors and this is reflected in the relative differences often found in the pharmacological activities and phytochemical constituents of plant materials harvested at different seasons of the year (Longman and Jenik, 1987). Two major seasons namely rainy and dry seasons are recognized in the tropics. These periods are marked with sharp differences in environmental factors like temperature, rainfall, radiation, daylight, evaporation etc (Evans, 1993). Most likely, these environmental factors directly or indirectly affect the availability of certain precursors needed for the biosynthesis of plant secondary metabolites. The rate and/or the extent of these reactions thus vary from season to season. This could be a plausible explanation for the observed seasonal variations in the antimicrobial constituents of *Loranthus micranthus*. More so, since the different phytochemical constituents exhibit varying degrees of antimicrobial activity, any variation in kind or in proportion of these constituents across the season will likely reflect in the antimicrobial activity.

Attempt to associate the antimicrobial activity of the plant material to specific phytochemical constituent led to the fractionation of the extracts into various solubility groups as shown in Table 1. Fraction A contains tannins, flavonoids, alkaloids, terpenoids, saponins and reducing sugar. Fraction B and C contains only terpenoids and flavonoids respectively while fraction D contains tannins, saponins and reducing sugar. The antibacterial activity of A, B and D are comparable to that of chloramphenicol. Fraction C showed only mild activity against *Salmonella kapemba*, but inactive against the other microorganisms tested (Table 3). The antimicrobial activity of *Loranthus micranthus* leaves may thus be attributed to the presence of some phytoconstituents like tannins, terpenoids and alkaloids. These constituents were shown in previous studies to be responsible for the antimicrobial activity of *Loranthus micranthus* (Osadebe and Ukwueze, 2004; Osadebe and Akabogu, 2006). Moreover, tannins and alkaloids are plant secondary metabolites frequently reported to be responsible for the antimicrobial properties of most medicinal plants (Cowan, 1999; Khwaja *et al.*, 1996; Esimone *et al.*, 2003). Though some of these constituents e.g., tannins and terpenoids did not show

variation across the seasons, the presence of alkaloids only in April and July may explain the observed better antimicrobial activity of the extracts of the leaves of *Loranthus micranthus* harvested at these months.

In conclusion, significant variation in antimicrobial activity was shown by the extracts of the leaves of *Loranthus micranthus* harvested at the different seasons of the year. Generally, better antimicrobial activity was shown by the extracts of the leaves harvested in April and July. Mistletoe used for the treatment of non-specific infections may thus, be preferentially harvested both at the onset and peak of the rainy season.

REFERENCES

- Bolksman, N., P. Schmiermann and M. De Reuver *et al.*, 1982. Stimulation of humoral and cellular immunity by *Viscum* preparations. *Planta Medica*, 46: 221-227.
- Cowan, M.M., 1999. Plants products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12: 564-582.
- Dalziel, J.M., 1955. *The Useful Plant of West Tropical Africa*. Crown Agents for Overseas Government and Administration, London, pp: 297.
- Esimone, C.O., I.M. Ebebe, K.E. Chah and C.G. Onyeka, 2003. Comparative antibacterial effects of *Psidium guajava*. *J. Trop. Med. Plants*, 4: 185-189.
- Evans, L.T., 1993. *Crop Evolution, Adaptation and Yield*. Cambridge University Press, Great Britain, pp: 113-168.
- Harborne, J.B.C., 1998. *Phytochemical Methods*. Chapman and Hall, London.
- Kafaru, E., 1993. Herbal Remedies. *The Guardian*, Thursday, June 3, 1993, pp: 24.
- Khwaja, T.A., C.B. Dias and S. Pentecost, 1996. Recent studies on the anticancer activities of mistletoe (*Viscum album*) and its alkaloids. *Oncology*, 43: 42-50.
- Kuttan, G., D.M. Vasudevan and R. Kuttan, 1990. Effect of a preparation from *Viscum album* on tumor development *in vitro* and in mice. *J. Ethnopharmacol.*, 29: 35-41.
- Longman, K.A. and J. Jenik, 1987. *Tropical Trees and its Environment*. 2nd Edn. Longman Singapore Publishers, pp: 1-65.
- Lovian, I., 1987. *Antibiotics in Laboratory Medicine*. Williamms and Williams, Baltimore, London, pp: 7-22.
- Obatomi, D.K., E.O. Bikomo and V.J. Temple, 1994. Antidiabetic properties of the African Mistletoe in Streptozotoum induced diabetic rats. *J. Ethnopharmacol.*, 43: 13-17.
- Obatomi, D.K., V.O. Aina and V.J. Temple, 1996. Effect of African Mistletoe on blood pressure in spontaneously hypertensive rats. *Int. J. Pharmacognosy*, 34: 124-127.
- Oliver-Bever, B., 1986. *Medicinal Plants of Tropical West Africa*. Cambridge University, London, pp: 375.
- Osadebe, P.O. and S.E. Ukwueze, 2004. A comparative study of the phytochemical and Anti-microbial properties of the Eastern Nigeria Species of African Mistletoe (*Loranthus micranthus*) sourced from different Host Trees. *J. Biol. Res. Biotechnol.*, 2: 18-23.
- Osadebe, P.O., G.B. Okide and I.C. Akabogu, 2004. Study on anti-diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn.) sourced from five different host trees. *J. Ethnopharmacol.*, 95: 133-138.
- Osadebe, P.O. and I.C. Akabogu, 2006. Antimicrobial activity of *Loranthus micranthus* harvested from kola nut tree. *Fitoterapia*, 77: 54-56.
- Scheer, R.C., A. Schefflen and M. Ernest, 1992. Two harvesting times, summer and winter: Are they essential for preparing pharmaceuticals from mistletoe (*Viscum album*). *Planta Medica*, 58: 299-594.
- Wagner, M.L., F. Teresa, A. Elida, A.R. Rafeal, H. Silvia and A.G. Alberto, 1996. Micromolecular and macromolecular comparison of argentina mistletoe (*Ligaria cuneifolia*) acid European mistletoe (*Viscum album*). *Acta Farmaceutica Bonaerense*, 15: 99-105.