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Research Paper

Chemical composition of *Lasianthera africana* and their Immunomodulatory and antileishmanial activities

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ABSTRACT

The leaf extract and fractions of *Lasianthera africana* were investigated for immunomodulatory activity of the in whole blood, neutrophils and macrophages using luminol/lucigenin-based chemiluminescence assay. The extract and fractions were similarly screened for antileishmanial activity against promastigotes of *Leishmania major* in vitro. GCMS analysis of the most active fraction was carried out. The leaf extract exhibited prominent pro-oxidative activity in whole blood and macrophages with moderate antioxidative burst activity in neutrophils. The extract also exhibited significant antileishmanial activity against promastigotes of *L. major* in vitro. GCMS analysis of active fraction revealed pharmacologically active compounds. These results suggest that leaf extract/fractions of *L. africana* possess immunomodulatory and antileishmanial activities boths.

Keywords: *Lasianthera africana*; Immunomodulatory; Antileishmanial.

INTRODUCTION

Lasianthera africana (P.Beav.) (Family-*Icacinaceae*) is widely distributed in the tropical rain forest (Hutchinson and Dalziel, 1973).There are four ethno varieties distinguished by their taste, leaf colour and ecological distribution. The leaves are consumed as vegetable in southern Nigeria. *L. africana* is commonly used as antacid, analgesic, antispasmodic, laxative, antipyretic, antiulcerogenic, antidiabetic and antimalarial (Okokon, et al., 2007). Biological activities reported on *Lasianthera africana* include bacteriostatic (Itah, 1997), fungicidal (Itah, 1996) antidiabetic (Ekanem, 2006), antiplasmodial (Okokon, et al., 2007), antimicrobial (Andy, et al., 2008) and antiulcer (Okokon, et al., 2009). The leaf extract has been reported to contain alkaloids, terpenes, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides with LD_{50} value of 5000mg/kg (Okokon, et al., 2007). Although some researchers have worked on this plant to confirm its activity, the present study was aimed at studying the antioxidant/pro-oxidant and antileishmanial activities as well as GCMS analysis of the active fraction of the ethanolic leaf extract of the dark green variety. This type of work on this plant has not been reported previously in literature.

MATERIALS AND METHODS

Plant collection: The plant material *Lasianthera africana* (leaf) were procured from a market in Uyo metropolis, Akwa Ibom State, Nigeria in April, 2011. The plant was identified and authenticated by Dr. Magaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

***Extraction*:** The leaves were washed and shade-dried for two weeks. The dried plant materials were reduced to powder. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness in vacuum at 40°C using rotary evaporator. The crude ethanolic extract (100g) was further partitioned successively into 1L each of n-hexane, dichloromethane, ethyl acetate and butanol to give the corresponding fractions of these solvents.

***Immunomodulatory activity*:** The ethanolic crude extract was screened for cellular antioxidant activities in whole blood, neutrophils and macrophages using chemi-luminescence assay. Briefly, luminol or lucigenin-enhanced chemi-luminescence assay were performed as described by Helfand et al., (1982) and Haklar et al., (2001). Briefly, 25µl diluted whole blood (1:50 dilution in sterile $HBSS^{++}$) or 25µl of PMNCs (1×10^6) or MNCs (5×10^6) cells were incubated with 25µl of serially diluted plant extract with concentration ranges between 6.25 and 100µg/ml. Control wells received $HBSS^{++}$ and cells but no extract. Tests were performed in white 96 wells plates, which were incubated at 37ºC for 30min in the thermostated chamber of the luminometer. Opsonized zymosan-A or PMA 25µl, followed by 25µl luminol (7×10^5M) or lucigenin (0.5mM) along with $HBSS^{++}$ was added to each well to obtain a 200µl volume/well. The luminometer results were monitored as chemi-luminescence RLU with peak and total integral values set with repeated scans at 30s intervals and 1s points measuring time.

***Antileishmanial activity*:** of the extracts and fractions were evaluated against promastigotes of *L. major* (DESTO) in culture using micro plates. *L. major* promastigotes were grown in bulk, early in modified NNN biphasic medium, using normal physiological saline. Then the promastigotes were cultured with RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS). Parasites (*L. major)* were harvested at log phase and centrifuged at 3000rpm for 10 min. They were washed three times with saline at same speed and time. Finally the parasites were counted with the help of Neubauer chamber under the microscope and diluted with fresh culture medium to give a final density of 10^6cells/ml. In a 96-well micro titer plate, 180ml of the culture medium was added in different wells. The extracts and fractions were dissolved in PBS (Phospate buffered saline, pH 7.4 containing 0.5% MeOH, 0.5% DMSO) to make a stock concentration of 1000mg/ml. 20µl of each extract/fraction concentration was added to the wells and serially diluted to get working concentrations ranging between 1-100µg/ml. 100ml of parasite culture (final density of 10^6cells/ml) was added in all wells. Two rows were left, one for negative and other for positive control. Negative controls received the medium while the positive controls received Pentamidine and amphotericin B as standard antileishmanial compounds. Plate was incubated between 21-22°C for 72h. The culture was examined microscopically for cell viability by counting the number of motile cells on an improved Neubauer counting chamber and IC_{50} values of compounds were calculated (Atta-ur-Rahman, et al., 2001).

Gas chromatography-Mass spectrometry analysis: Quantitative and qualitative data were determined by GC and GC-MS, respectively. The fraction was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/ split less injector. The column used was an DB-5 (Optima-5), 30m, 0.25mm i.d., 0.25µm

df, coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following oven temperature programme: 50°C, held for 1 min, rising at 3°C/min to 250°C, held for 5min, rising at 2°C/min to 280°C, held for 3min; injection temperature 250°C and volume 1.0µl respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8KPa; detector temperature, 280°C; hydrogen, flow rate, 50ml/min; air flow rate, 400 ml/min; make-up (H2/air), flow rate, 50ml/min; sampling rate, 40ms. Data were acquired by means of GC solution software (Shimadzu). Agilent 6890N GC was interfaced with a VG Analytical 70-250s double-focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250°C. The GC was fitted with a 30mm x 0.32mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

Identification of the compounds: present in the various active fractions of the plants' extracts was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley and Nist Libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literatures (Adams, 2001; Setzer, et al., 2007).

RESULTS

***Immunomodulatory activity*:** Ethanolic leaf extract of *Lasianthera africana* was observed to exhibit weak antioxidant activity in whole blood, neutrophils (intracellularly) and macrophages. However, significant antioxidant effect was observed extracellularly in the neutrophils (Table 1).

Antileishmanial activity: Crude extract and fractions of ethanolic leaf extract of *Lasianthera africana* exerted significant antileishmanial activity when tested against promastigotes of *Leishmania major*. Ethyl acetate fraction exerted a higher activity than other fractions and crude extract though incomparable to the standard drugs, pentamidine and amphotericin B (Table 2).

***GC-MS analysis*:** The GCMS analysis of the ethyl acetate fraction of *Lasianthera africana* revealed the presence of 7 bioactive compounds as represented in Table 3.

DISCUSSION

Lansianthera africana is used primarily as vegetable. In folk medicine this is used in the treatment of various diseases especially infections. Its activities against pathogenic microorganisms like fungi, bacteria and protozoa have been reported (Itah, 1997; Okokon, et al., 2007; Andy, et al., 2008). In this study, the leaf extract has been found to demonstrate predominant pro-oxidant activity and also exert significant antileishmanial activity against *Leishmania major*, a protozoan. GCMS revealed the presence of some pharmacologically active compounds in the ethyl acetate fraction.

The leaf extract demonstrated significant antileishmanial activity which is known to be promoted by pro-oxidant state. In this study, different concentrations of the extract were observed to exhibit pro-oxidant activity predominantly which could have resulted in the generation of reactive oxygen species (ROS) such as H_2O_2 and superoxide radicals. The generated ROS have tendencies of destroying the cell wall and membrane structure of the microorganism through lipid peroxidation of the cell membrane leading to the loss of membrane integrity and affecting membrane permeability. This will result in loss of ions and vital cellular components of the microorganism, thus killing it (Cohenand, 1987). Pro-oxidant activity has been reported to enhance antimicrobial activity (Anderson, et al., 1981) and promote host defense system against microorganisms. Compounds such as hexadecanoic acid,

hexadecanoic acid ethyl ester, and linoleic acid which have been found to be present in the leaf extract have been reported to possess antimicrobial activity (Kumar, et al., 2010). More so, many fatty acids are known to have antimicrobial properties (Russel, 1991). Dodecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid and oleic acids are among the fatty acids known to have potential antimicrobial activity (McGraw, et al., 2002; Seidel and Taylor, 2004).

These fatty acids are potential antibacterial and antifungal principle for clinical application (Altieri, et al., 2008). These compounds present in the leaf extract may have contributed to the antileishmanial activity observed in this study. Although no compound was isolated or identified in this study as the active principle, the leaf extract has been reported to contain saponins and terpenes (Okokon, et al., 2007). Triterpene and saponins from plants have been found to exert antileishmanial activity (Maes, et al., 2004; Germonprez, et al., 2005; Vermeersch, et al., 2009) and the mechanism of their action has been proposed to involve alteration of parasite membrane integrity, inducing programmed cell death (Dutta, et al., 2007). This could possibly be the mode of action of this plant as it contains saponins and terpenes. Also saponins in *Apodytes dimidiate*, a member of *Icacinaceae* family, have been reported to be the active antileshmanial principles (Foubert, et al., 2011).

Similarly, the antileishmanial activity observed in this plant may have been due to the presence of saponins and terpenes in the extract.

CONCLUSION

Thus leaf extract of *Lasianthera africana* has antileishmanial activity which is due to its pro-oxidant activity and phytochemical constituents.

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Table- 1: Immunomodulatory activity of ethanolic seed extract of *Lasianthera africana*.

Cell Type	Dose (µg/ml)	%Inhibition (RLU)
WHOLE BLOOD	1	-28.6 ±15.20
	10	-9.20±2.36
	100	0.20±6.70
NEUTROPHILS (intracellular)	0.5	0.50±0.46
	5	21.30±4.00
	50	33.20±1.14
NEUTROPHILS (extracellular)	0.5	-2.90±4.79
	5	16.50±6.76
	50	70.20±3.64
MACROPHAGES	0.5	-26.30± 4.79
	5	-11.60 ±2.60
	50	16.30±1.50

- Data are represented as Mean ± SEM of three independent experiments.

Table- 2: Antileishmanial activity of *Lasianthera africana*.

Extract / Fraction	ED_{50}(µg/ml)
Crude extract	16.73±0.13
Hexane fraction	>100
DCM fraction	>100
Ethyl acetate fraction	35.14±0.48
Butanol fraction	>100
Aqueous fraction	>100
Pentamidine	5.09±0.04
Amphotericin B	0.29±0.05

- Data are represented as Mean ± SEM of three independent experiments.
- ED_{50} means effective dose that will kill 50% of the parasites

Table-3: GCMS analysis of ethyl acetate fraction of *Lasianthera africana.*

S.N.	Name of compound	Molecular Weight	Chemical Formula	RT
1.	2-pentyl 2-Nonenal	210	$C_{14}H_{26}O$	612
2.	Hexadecanoic acid, 2-methyl methyl ester	284	$C_{18}H_{36}O$	656
3.	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	651
4.	Linoleic acid, ethyl ester	308	$C_{20}H_{36}O_2$	737
5.	9,12,15-Octadecatrienoic acid methyl ester,(Z,Z,Z)	292	$C_{19}H_{32}O_2$	740
6.	8,11,14-Eicosatrienoic acid (Z,Z,Z)	306	$C_{20}H_{34}O_2$	748
7.	Octadecanoic acid, ethyl ester	312	$C_{20}H_{40}O_2$	752

