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Full Length Research Paper

Antibacterial activity and phytochemical analysis of leaf extracts of *Lasienthera africanum*

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The phytochemical analysis of both the aqueous and methanolic extracts of edible indigenous medicinal plant *Lasienthera africanum* ("Editan") and their antibacterial activities against clinical isolates, *Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Staphylococcus aureus* were investigated. The phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones and cyanogenetic glycosides in varying concentration. Both the methanolic and aqueous extracts inhibited the growth of the test organisms with *Sal. typhi* showing the highest susceptibility. This research supports the local use of the leaf of the plant, *L. africanum* ("Editan") for prophylactic and therapeutic purposes against bacterial infection.

Key words: *Lasienthera africanum,* phytochemical analysis, antimicrobial activity, activity index, therapeutic purpose.

INTRODUCTION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value (Nostro et al., 2000). Some of them are also used for prophylactic purposes. An increasing interest in herbal remedies has been observed in several parts of Nigeria and many of the herbal remedies have been incorporated into orthodox medicinal plant practice. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections (Sofowora, 1996).

The plant *Lasienthera africanum* ("Editan") belongs to the order Celestrales which comprises thirteen families of trees and shrubs with simple leaves. It belongs to the family Icacinacea. These kinds of plants and shrubs are of both nutritional and medicinal importance (Sofowora, 1993). *L. africanum* is consumed as vegetables in the South-Easern State of Nigeria. It is believed to have cooling effects on the body, "purifying effects" and prevent internal bleeding.

Although synthetic and semi synthetic antimicrobial drugs abound in various markets today, there is need for continuous search for new ones to cope with the increased evolution of multiple antimicrobial resistant strains of organisms (Hart and Kariuki, 1998). The research objectives were to investigate the phytochemical composition of the plant, *L. africanum* and its antimicrobial effects on the four test microorganisms.

MATERIALS AND METHODS

Collection and authentication of plant materials

The leaves of the plant species were obtained locally from the farmland of Faculty of Agriculture, University of Uyo. The plant species were identified locally as „Editan" and scientifically by experts at Department of Pharmacognosy, University of Uyo as *L. africanum*.

Preparation of extracts

To prepare the methanolic extracts, 150 g of each of the ten plant material was collected, dried in the oven at 70^{0}C for 4 h and reduced to powder. It was separately macerated with methanol, allowed to stand for 72 h and then filtered. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extracts were stored in labeled sterile screw capped bottles at 5°C in the refrigerator, until when required for use.

For the aqueous extraction, 50 g of the plant powder was weighed into 50 ml Eylen-Mayer flask and to this was added 400 ml

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of distilled water. This was heated to boil using hot plate. The mixture was stirred at regular intervals (3-5 min) for one hour after which it was filtered with No. 1 Whatman filter paper (W and R Balson Ltd, England). The filterate was then filtered sterilized using a membrane filter of pore size 0.45 cm diameter (millipores corp, England). The extracts were concentrated in a hot water bath at 80^{O}C for 5 h during which 0.5 g charcoal was added to decolourise it. Sterile decolourized filtered extract was then refrigerated at 5^{O}C until required for use.

Microorganisms

The species of bacterial organisms were *Staphylococcus aureus, E. coli, K. pneumoniae* and *Salmonella typhi*. They were clinical isolates obtained from Microbiology Laboratories of St. Luke General Hospital and University of Uyo Medical Center, Uyo Metropolis. The cultures of bacteria were maintained on nutrient agar slants at 4°C, re-identified by biochemical tests (Cheesbrough, 1982; Cowan and Steel, 2004) and sub-cultured on to nutrient broth for 24 h prior to testing.

Phytochemical screening

To test for alkaloids, about 0.5 g of the extract was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. A few drops of Dragendorff"s reagent were used to treat 1 ml of the filterate. Turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. Exact 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins. Also, to test for presence of tannins, about 0.5 g of the extract was dissolved in distilled water and about 10 ml of bromine water added. Decolourization of bromine water indicated the presence of tannins.

Borntrager"s test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone.

The presence of cardiac glycosides was confirmed by Liberman"s test, Salkowski test and Keller-Killani test (Culer, 1982; Sofowora, 1993; Trease and Evans, 2002).

Antimicrobial susceptibity test

The spreading method of Cruickshank et al. (1980) was used. Twenty four hours old cultures of the organisms to be tested were used. A loopful of the cultures was uniformly spread over the surface of a sterile Muller-Hilton agar with a sterile bent rod. The extract was diluted to obtain different concentration of 500, 250, 125 and 62.5 mg/ml using sterile peptone water. Various concentrations of the prepared extracts were used to fill hole bored by 5 mm cork borer in the inoculated agar. The plates were made in triplicate with one for the test organism- tetracycline, standard drug. All plates were incubated at 37^{O}C for 24 h. The diameter of the zones of inhibition in the triplicate plates was measured by calculating the difference between core borer (5 mm) and the diameters of inhibition (Hewitt and Vincent, 1989) and their mean designated as ZI. The activity indices, designated as AI, were calculated as the division of zone of inhibition of the extract by that of the standard drug (Singh et al., 2002).

Tube dilution method

The extracts were diluted into different concentrations of 500, 250, 125 and 62.5 mg/ml with sterile peptone water in test tubes. Methanol and water were used as the control. To each of the dilution was added 0.2 ml broth culture of the test organism. The tubes were incubated at 37^{O}C for 24 h after which turbidity reading was taken using turbidiometer. Extracts added with peptone water served as control

Table 1. Phytochemicals in *Lasienthera africanum* extracts.

Phytochemical	Occurrence
Alkaloids	++
Tannins	++
Flavonoids	++
Saponnins	++
Anthraquinone	++
Cardiac glycosides	+
Cyanogenetic glycosides	+

++ = Present in appreciable quantity; + = present in low quantity.

RESULTS

Phytochemistry of the plant extracts

The result of the phytochemical analysis shows that the six plants are rich in at least one of alkaloids, flavonoids, saponins, anthraquinones, plobatannins, tannins, cyanogenetic glycosides and cardiac glycosides. Table 1 shows the phytochemical screening results of ethanolic extracts of the six plants used in this study.

Antimicrobial activity

Both crude methanolic and aqueous forms of the extracts of *L. africanum* exhibited varying degree of antimicrobial activities against the test organisms. On a general note, methanolic extracts exhibited higher degree of antibacterial activities than the aqueous extracts. At 62.5 mg/ml, crude ethanolic extract had higher antibacterial activity with mean zones of inhibition 3.8 ± 0.1 mm (A.I = 0.5) and 4.0±0.5 mm (A.I = 0.4) than crude aqueous extract with mean zones of inhibition 3.0 ± 0.3 mm (A.I = 0.4) and 3.5 ± 0.6 mm (A.I = 0.4) against *E. coli* and *Staph. aureus,* respectively. Besides that, aqueous extract had higher antibacterial activities [mean zone of inhibition 4.4±1.0 mm (A.I = 0.6) and 4.0 ± 0.6 mm (A.I = 0.6) and 4.0±0.6 mm (A.I = 0.7)] than ethanolic extract [4.0±0.3 mm (A.I = 0.5) and 3.0 ±0.6 mm (A.I = 0.5)] against *Sal. typhi* and *K. pneumonia*, respectively.

Equal or sometimes higher activities were observed at concentration of 500, 250 mg/ml by the crude methanolic extracts than the standard drug, gentamycin. Hence, the activity index, A.I ≥ 1 against *E. coli, Sal. typhi* and *K. pneumoniae*. Consistently high activity indices were ob-

Table 2. Antibacterial properties of *Lasienthera africanum* extracts using the agar diffusion technique (mm).

Isolate	Gentamycin	500 mg/ml				250 mg/ml				125 mg/ml				62.5 mg/ml			
		A		B		A		B		A		B		A		B	
		Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I	Z.I	A.I
E. coli	7.5±1.0	9.5±1.0	1.3	6.0±0.5	0.8	6.0±0.5	0.8	4.8±0.3	0.6	5.0±1.0	0.7	4.0±0.2	0.5	3.8±0.1	0.5	3.0±0.3	0.4
Staph. aureus	9.5±1.0	8.0±1.1	0.8	6.5±1.0	0.7	6.5±1.0	1.0	5.5± 0.6	0.6	6.4±1.0	0.7	4.0±1.0	0.4	4.0±0.5	0.4	3.5±0.6	0.4
Sal. typhi	7.8±1.1	9.0±1.0	1.2	7.5±1.2	˜1.0	8.5±1.0	1.1	6.6±1.0	0.9	6.8±0.5	0.9	5.8±0.5	0.74	4.0±0.3	0.5	4.4±1.0	0.6
K. pneumonia	6.1±1.1	7.0±1.0	1.2	6.6±0.5	1.1	6.6±0.7	1.1	5.2±0.3	0.9	6.0±0.2	1.0	5.0±0.6	0.8	3.0±0.6	0.5	4.0±0.6	0.7

A = Methanolic extract, B = aqueous extract, Z.I = mean zone of inhibition in mm ± SD, A.I = activity Index with respect to Gentamycin.

Table 3. Antibacterial properties of *Lasienthera africanum* extracts using the tube dilution method.

Isolate	500 mg/ml		250 mg/ml		125 mg/ml		62.5 mg/ml	
	A	B	A	B	A	B	A	B
Staph. aureus	1.50*	2.21	2.80	3.10	3.70	4.22	4.60	5.60
E. coli	2.30	2.90	3.85	3.60	4.60	4.40	5.90	5.90
Sal. typhi	1.20	1.90	2.40	2.80	3.70	3.60	4.82	4.76
K. pneumonia	3.10	3.85	4.20	4.35	5.62	5.82	6.60	6.20
Control	4.00	4.12	4.76	4.92	4.94	5.14	5.10	5.20

A = Methanolic extract, and B = aqueous extract.
*Values are in Nephelometric Turbidity Unit (NTU).

served against the etiology of pneumonia at crude concentration of 250 and 125 mg/ml (Table 2). The high activity indices were enduring with decrease in concentration from 500 to 62.5 mg/ml. Just low reduction in activities were observed as the crude extract concentration were reduced gradually from 500 to 62.5 mg/ml in both the agar diffusion set up (Table 2) and tube dilution method (Table 3). The same trend of activity in agar dilution was equally observed in tube dilution method. Methanolic extract inhibited the growth of the four bacteria with lower turbidity than the aqueous extract. For instance at 500 mg/ml, the turbidity readings were 1.50, 2.30, 1.20, 3.10 and 4.00 NTU for crude methanolic extract, while the reading for crude aqueous extracts were 2.21, 2.90, 1.90, 3.85 and 4.12 NTU against *Staph. aureus, E. coli, Sal. typhi* and *K. pneumoniae,* respectively. The slight higher potency observed in methanol (control) than water was expected due to antimicrobial activity of alcohol in general.

Like the agar diffusion set up, the trend of antimicrobial activity continues until the crude extract concentration of 62.5 mg/ml where both methanolic and aqueous extracts had equal turbidity of 5.90 against *E. coli*. Meanwhile at this same concentration of 62.5 mg/ml, higher turbidity was observed in ethanolic extract tube i.e. 4.82 and 6.60 than in aqueous extract tube i.e. 4.76 and 6.20 against *Sal. typhi* and *K. pneumonia* in that order.

DISCUSSION

The results obtained from this work revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants (Benjamin et al*.,* 1981). These agents are alkaloids, saponins, flavonoids, tannins, Research work revealed that tannins from the barks, roots etc of many plants especially euphorbiaceae are used to treat cells that have gone neoplastic (Duke and Wain 1981). It is obviously interesting to observe the result of high antibacterial effects

of both methanolic and aqueous extracts the four potential pathogens of public health importance. *Staph. aureus,* no doubt, is frequently connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis, furuncle, etc. It is also frequently involved in both nosocomial and community acquired infections. The successful inhibition of this bacteria and its contemporary aetiology of gastroenteritis (*E. coli)* is a good development, especially when we consider the records of resistance to various conventional antibiotics by them over the years (Wiedemann, 1996; Voss; 1996; Ayliffe, 1997). This extract could therefore be of use in management of opportunistic infection in HIV/AIDS involving these two isolates.

Similarly, the extract showed appreciable level of potency against the commonest aetiology of enteric fever. Records have it that the enteric fever had mortality rate of 10 - 15% in developing countries (Brooks et al., 2004). Both methanolic and aqueous extract could be put into fixed dosage combination therapy for treating the salmonella infection. This extracts is already in use by the traditional medicine practitioners in Nigeria, though in either methanolic or aqueous form. By virtue of high activity indices above unitary value even in crude forms, the extracts have more promising therapeutic advantages than the likes of gentamycin and its aminoglycoside relations when refined to produce antibiotics.

In conclusion, this finding justifies the traditional use of this plant, *L. africanum*, for prophylactic and therapeutic purposes. The findings could also be of commercial interest to both pharmaceutical companies and research institutes in the production of new drugs.

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