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Chemical and Biological Assessments on *Eremomastax Speciosa* and *Lasianthera Africana* Leaf Essential Oils

***Sherifat A. Aboaba and Iniobong E. Udom**

Abstract
Eremomastax speciosa (Kocsht.) Cufod. and *Lasianthera africana P. Beauv*. are medicinal plants used in Nigeria for treating ailments like urinary tract infection, constipation, syphilis and infertility. The essential oils from the two plants were isolated by hydrodistillation in an all glass Clevenger-type apparatus and characterized by GC-FID and GC-MS. A total of 45 and 46 constituents were identified in the leaf essential oil of *E. speciosa* and *L. africana* respectively. The prominent components in *E. speciosa* were β-pinene (4.7%), nonanal (4.75%), bicyclogermarcene (5.41%), β-caryophyllene (7.58%), Germacrene D (8.86%), borneol (9.88%), and phytol (51.12%) while *L. africana* had pinene-2-ol (3.66%), myrcene (4.26%), nonanal (7.11%), thujene (10.96%), viridiflorol (12.94%) and β-pinene (27.33%). The toxicity result reveals that the oils of both plants were toxic to brine shrimp with LC_{50} of 17.59 µg/mL and 9.52 µg/mL for *E. speciosa* and *L. africana* respectively. The antimicrobial activities of the essential oils were tested using the agar well diffusion pour-plate (bacterial) and agar diffusion-surface (fungi) methods against eight microbes and both showed moderate antibacterial and antifungal activities against *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. The findings from the study support the use of these plants in traditional medical practice and increase the possibility of their use as antibacterial and antitumor agents.

Key words: *Eremomastax speciosa*, *Lasianthera africana*, Essential oils, GC-MS, Biological activity.

Introduction

Essential oils are volatile components which are extracted or distilled from odorous plant materials. Essential oils can be found in every part of plant materials like the wood, leaves, peels, stems, stem bark, seeds, fruits, roots and trunks [1]. *Eremomastax speciosa* (Hochst.) Cufod belongs to the family Acanthaceae. The plant is a robust, poly-morphous shrub which grows to a height of 2m. The stem is quadrangular, and the leaves are violet on the underside. The plant is widely distributed in tropical Africa and it is the only species of the genus *Eremomastax* [2].

The plant is utilized for the treatment of epilepsy [3], gastric ulcer and anemia [4], cough, irregular menstruation and infertility in women [5,6,7]. The stems, leaves, roots and aerial part of *E. speciosa* are used for treating anemia, irregular menstruation, dysentery, labour pain, fracture, cough, constipation, haemorrhoids, urinary track infection and for metaphysical power [8,9]. The leaves are also used for generalized pains and dermatitis by decoction [10], treatment of horns gulps, hemorrhoids, rheumatism, colic, fibromas, convulsion, syphilis, sexual weakness, dysentery, gonorrhea [11] as well as for internal, abdominal heat and post-partum hemorrhage [12]. The antidiarrhoea [13] and antiulcer [14,15] properties have also been reported.

Lasianthera africana (P. Beauv.) belongs to the family Icacinaceae. It is a perennial glabrous shrub of 61-136 cm high and widely distributed in the tropical rain forest [16]. There are four ethno-varieties distinguished by their taste, leaf colour and ecological distribution. The leaves are consumed as vegetable in southern Nigeria [17]. Ethno-botanically, *Lasianthera africana* is used as antacid, analgesic, antiplasmodic, laxative, anti-pyretic, antiulcergenic, antidiabetic and

***Sherifat A. Aboaba and Iniobong E. Udom**
**Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria*
Corresponding Author: saboaba@gmail.com

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antimalarial. *Lasianthera africana* has been reported to be bacteriostatic [18], antidiabetic and anti-plasmodial [19]. The leaves are used in Sweden to treat stomach pains [20]; female infertility [9]; as enema to children for lateness in walking [21]; treatment of gonorrhea by the infusion with *Guarea thomsoni* [22] and also to speed up and ease labour pain during child birth and to dislodge retained placenta [12].

The extensive medicinal uses of these plants encouraged us to isolate the essential volatile oils from their leaves in an attempt to characterize the chemical constituents using GC and GC-MS and also evaluate the bioefficacy to support their medicinal importance.

Materials and Methods

Plant Samples

Fresh leaves of *Eremomastax speciosa* and *Lasianthera africana* were collected from a farm land along Etinan Road, Uyo in Akwa Ibom State of Nigeria. Botanical identification and authentication was done at the Herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria.

Isolation of Essential Oils

The oils were obtained by hydro-distillation using a Clevenger type apparatus for 4 h in accordance with the British pharmacopoeia specification (1980). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4 ^{o}C until analysis. The oil yields were calculated, relative to the dry matter.

Analyses of the Essential Oils

Gas chromatography (GC): The oils were analysed by GC using a HP 6890 chromatograph. An HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness) was used with hydrogen as carrier gas at a flow rate of 1.0 mL/min. The GC oven temperature was kept at 40 ^{o}C (hold for 1 min), and programmed to reach 200 ^{o}C at a rate of 5 ^{o}C/min, then kept constant at 200 ^{o}C for 2 min being the final hold time. The split ratio was adjusted to 20:1. The injector temperature was set at 150 ^{o}C. The percentage composition were obtained from electronic integration measurements using flame ionization detector (FID), set at 300 ^{o}C.

Gas Chromatography-Mass spectrometry (GC-MS): The essential oils were analysed by GC-MS using a HP 6890 gas chromatograph system with split/splitless injection interfaced to a 5973 mass selective detector. HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness) was used with hydrogen as carrier gas (1.0 mL/min) GC oven temperature and conditions were as described above. The injector temperature was at 300 ^{o}C. Mass spectra were recorded at 70 eV, mass range from m/z 30 to 500.

Identification of Compounds

Identification of constituents of the oil was achieved on the basis of their retention time determined with a reference to a homologous series of n-alkanes and by comparison of their mass spectra fragmentation patterns (NIST database/chemstation data system) with data previously reported in literature [23].

Brine Shrimp Lethality Test

The brine shrimp eggs were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attracted to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (1000, 100 and 10 µg/mL) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. A control was in place with a mixture of brine shrimps, sea water and DMSO only. After 24 h, the vials were examined against a light background and the average number of larvae that survived in each vial was determined. The concentration that killed fifty percent of larvae (LC_{50}) was determined using the Finney computer programme.

Antimicrobial Assay

Micro-organisms

Cultures of six human pathogenic bacteria namely: *Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonae*, *Bacillus subtilis* and *Staphylococcus aureus* were used for the antibacterial assay. For the antifungal assay, *Candida albicans*, and *Aspergillus niger* were utilized. All the micro-organisms were clinical strains from the Medical Microbiology Department (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria where the anti-microbial assay was performed.

Antimicrobial Activity Determination

Agar diffusion-pour plate method (bacteria)

An overnight culture of each organism was prepared by taking two wire loop of the organism from the stock and inoculating each into the sterile nutrient broth of 5 mL, each incubated for 18-24 h at 37 ^{o}C. From the overnight culture, 0.1 mL of each organism was taken and put into 9.9 mL of sterile distilled water. From the diluted organism, 0.2 mL was taken into the prepared sterile nutrient agar cooled to about 40-45 ^{o}C, then poured into sterile petri dishes and allowed to solidify for about 45-60 min. The wells were made according to the number of the test tubes for the experiment using a sterile cork-borer of 8 mm diameter. Several concentrations of the extracts in DMSO were put into 8 wells accordingly including the controls. Gentamycin (10 µg/mL) was used as the positive control. The studies were done in triplicates to ascertain the results obtained. The plates were left on the bench for about 2 h. to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24 h at 37 ^{o}C [24,25]. The zones of incubation were measured after the period of incubation. A zone of inhibition greater than 8 mm was taken as activity.

Agar diffusion-surface plate method (fungi)

A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified properly.0.2 mL of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile petri-dish lid to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8 mm diameter. The graded concentrations of the extracts were put into the well including the controls. The positive control was 0.7 mg/mL tioconazole. All the plates were left on the bench for 2 h to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25 ^{o}C for 72 h and zones of inhibition measured as above [24,25].

Results and Discussion

The colourless essential oil (0.057% (w/w)) of *E. speciosa* yielded 45 constituents by GC and GC-MS analyses representing 100% of the oil composition (Table 1). The major components were β-pinene (4.47%), nonanal (4.75%), bicyclo-germarcene (5.41%), β-caryophyllene (7.58%), germacrene D (8.85%), borneol (9.88%), and phytol (51.12%). The oil is rich in phytol, an acyclic diterpene that is known to be used in the manufacture of synthetic vitamins E and KI. Other compounds were tetradecanoic acid (1.14%), terpine-4-ol (1.86%) and 6-hydroxyeicosane (2.21%) while the remaining composition of the oil were constituents obtained in trace levels less than 0.1%.

The leaves of *L. africana* also yielded colourless oil (0.052% (w/w)) and resulted in the identification of 46 constituents representing 100% of the total essential oil Table 2. The main constituent of *L. africana* are myrcene (4.26%), borneol (5.71%), 1, 8-cineole (6.36%), β-caryophyllene (9.91%), α-thujene (10.96%), viridiflorol (12.94%), α-pinene (27.33% while other significant compounds include α-terpinen-4-ol (1.60%), terpinene-4-ol (1.71%), citronellol (3.39%), borneol acetate (3.49%) and pinene-2-ol (3.66%) which indicate that the oil is

essentially dominated by monoterpenoids totaling 86.52% and the remaining 13.48% accounting for sesquiterpene and minor compounds.

The essential oils studied contain volatile compounds such as α-pinene, β-pinene, 1, 8-cineole, camphene, limonene, linalool, thymol. These characterized compounds have been reported in literature to demonstrate potent biologically activities [26,27,28,29,30, 31,32]. The classified biologically active com-pounds have conveniently proven the investigated plants as medicinal.

Table 1: Composition of the Volatile Oil from *E. speciosa* by GC-MS

Peak No.	Compound	RT (minutes)	% Composition
1	α- Pinene	9.83	0.03
2	β- Pinene	11.14	4.47
3	Benzyl Alcohol	11.46	0.02
4	Cis Ocimene	12.92	< 0.01
5	Myrcene	13.00	0.23
6	Allo Ocimene	13.20	0.01
7	Pinene -2- ol	13.84	0.04
8	α- Thujene	14.22	0.01
9	Thymol	14.32	0.01
10	Nonanal	14.52	4.75
11	3- Methoxyacetophenone	14.77	0.13
12	Gama Terpinene	14.95	0.02
13	Citronellal	14.96	0.01
14	Neral	15.26	0.01
15	Geranial	15.40	0.01
16	Isoartemisia	16.47	0.01
17	Borneol	16.55	9.88
18	Citronellol	17.11	0.05
19	1,8 –Cineole	17.72	0.13
20	Linalool	17.90	0.53
21	α- Terpineol	18.69	0.01
22	Terpinen-4-ol	18.79	1.86
23	Thymyl Methyl Ether	19.68	0.01
24	Ascaridole	20.09	0.01
25	Ethyl cinnamate	21.42	0.02
26	Bornoel Acetate	21.63	0.02
27	Linalyl Acetate	21.72	0.41
28	β-Bisabolene	21.88	0.02
29	β-Caryophyllene	22.44	7.58
30	β-Elemene	23.36	<0.01
31	Germacrene D	24.04	8.86
32	Bicyclogermarcene	24.68	5.41
33	α-Copan	24.72	0.01
34	Acetyleugenol	25.30	0.01
35	Elemicin	25.82	<0.01
36	Benzyl Benzoate	26.06	0.01
37	5,8-Heinicosdiene	26.39	0.01
38	7- Henicosyne	26.97	0.01
39	3-dihydroxy-2-octadiene	27.50	0.01
40	Viridiflorol	28.04	0.01
41	Torreyol	28.24	0.01
42	Tetra decanoic acid	28.39	1.14
43	Hexa decanoic acid	28.59	0.94
44	6-Hydroxyeicosane	28.90	2.21
45	Phytol	29.24	51.12
	TOTAL		**100**

Table 2: Composition of the Volatile Oil from *L. africana* by GC-MS

Peak No.	Compound	RT (minutes)	% Composition
1	Tricyclene	4.14	0.01
2	Camphene	4.78	0.01
3	Saminene	6.93	0.01
4	Limonene	7.65	0.01
5	α- Pinene	9.83	27.33
6	β- Pinene	11.21	1.03
7	Benzyl Alcohol	11.46	0.03
8	Cis ocimene	12.91	< 0.01
9	Myrcene	12.99	4.26
10	Allo ocimene	13.20	0.01
11	Pinene-2-ol	13.83	3.66
12	α- Thujene	14.16	10.96
13	Thymol	14.31	0.01
14	Nonanal	14.51	7.11
15	3-methoxyacetophenone	14.76	0.01
16	Gama terpinene	14.88	0.03
17	Citronellal	15.03	0.01
18	Neral	15.30	0.02
19	Geranial	15.39	0.02
20	Isoartemisia	16.46	0.01
21	Borneol	16.60	5.71
22	Citronellol	17.09	3.39
23	1, 8-Cineole	17.70	6.36
24	Linalool	17.89	0.06
25	α-Terpineol	18.68	1.60
26	Terpinen-4-ol	18.78	1.71
27	Thymol methyl ether	19.72	0.02
28	Ascaridole	20.12	0.01
29	Ethyl cinnamate	21.41	0.03
30	Borneol acetate	21.61	3.49
31	Linalylacetate	21.71	0.04
32	β-Bisabolene	21.92	0.03
33	β- Caryophyllene	22.38	9.91
34	β- Elemene	23.33	0.01
35	Germacrene D	24.02	0.03
36	Bicyclogermarcene	24.66	0.02
37	α- Copane	24.79	0.03
38	Acetyleugenol	25.26	0.01
39	Elemicin	25.78	0.01
40	Benzyl benzoate	26.04	0.02
41	5, 8 – Heincosyne	26.38	0.01
42	7 –Heincosyne	27.08	0.01
43	3, Dihydroxy -2- octadecene	27.49	0.01
44	Viridiflorol	28.02	12.94
45	Torreyol	28.23	0.01
46	Tetra decanoic acid	28.33	0.02
	Total		**100**

The result of the brine shrimp assay is shown in Table 3. The assay is considered a useful tool for preliminary assessment of toxicity [33]. Toxicity to brine shrimp in most cases correlates reasonably well with cytotoxic, pesticidal, antibacterial and anti-tumor properties [34]. The LC_{50} (concentration of toxicant necessary to kill 50% of the brine shrimp being tested) for the oils revealed that the oils are highly toxic with values of 9.52 and 17.59 µg/mL.

Table 3: Brine Shrimp Toxicity Assay of the Essential Oils

Sample	% Death	LC_{50}(µg/ml)	Gradient	UCL	LCL
E.speciosa	67.7	17.59	0.2706	45.1043	2.6160
L.africana	72.2	9.52	0.3231	28.6130	0.5329

UCL – Upper Confidence Limit
LCL – Lower Confidence Limit

The essential oil of *E. speciosa* inhibited the growth of six organisms to different degrees. All the bacterial strains were sensitive to *E. speciosa* at concentration ranging from 12.5 to 100 mg/mL (Table 4). *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative) and *Pseudomonas aeruguinosa* (gram negative) all showed sensitivity at concentrations ranging from 6.25 to 100 mg/mL. The oil inhibited the growth of *Bacillus subtilis* (gram positive), *Klebsiella pneumonia* (gram negative) and *Salmonella typhi* (gram negative) at concentrations ranging from 12.5 to 100 mg/mL.

The volatile oil of *L. africana* also inhibited the growth of six organisms to different degrees as indicated in Table 4. It was most effective against *Staphy-lococcus aureus* (gram positive) and *Escherichia coli* (gram negative), for their growth were inhibited at all tested concentrations (3.125 to 100 mg/mL. *Bacillus subtilis* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Klebsiella pneumonia* (gram negative) showed sensitivity to *L. africana* at the concentration ranging from 100 mg/mL to as low as 6.25 mg/mL. The oil also inhibited the growth of *Salmonella typhi* (gram negative) at concentration ranging from 100 mg/mL to 12.5 mg/mL.

Table 4: Antibacterial activities of *E. speciosa* and *L. Africana*

Bacteria isolates	Essential oil	Zone of inhibition (mm) at different oil concentration						-ve control	+ve control
		100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL		
Staphylococcus	1	22±2.83	19±1.41	17 ± 1.41	14 ± 0.00	11 ± 1.41	-	-	38
aureus	2	27 ± 1.41	22 ± 2.83	19 ± 1.41	17 ± 1.41	14 ± 0.00	11 ± 1.41	-	38
Escherichia	1	25 ± 1.41	20 ± 0.00	18 ± 0.00	15 ± 1.41	11± 0.00	-	-	38
coli	2	25 ± 1.41	19 ± 1.41	17 ± 1.41	15 ± 1.41	12 ± 0.00	10 ± 0.00	-	40
Bacillus	1	18 ± 0.00	14 ± 0.00	12 ± 0.00	10 ± 0.00	-	-	-	30
subtilis	2	23 ± 1.41	19 ± 1.41	16 ± 0.00	14 ± 0.00	10 ± 0.00	-	-	38
Pseudomonas	1	23 ± 1.41	18 ± 0.00	15 ± 1.41	12 ± 0.00	10 ± 0.00	-	-	38
Aeruginosa	2	23 ± 1.41	19 ± 1.41	17 ± 1.41	14 ± 0.00	10 ± 0.00	-	-	38
Klebsiella	1	19 ± 1.41	16 ± 0.00	14 ± 0.00	11 ± 1.41	-	-	-	36
pneumonia	2	19 ± 1.41	17 ± 1.41	14 ± 0.00	12 ± 0.00	10 ± 0.00	-	-	30
Salmonella	1	19 ± 1.41	16 ± 0.00	14 ± 0.00	11 ± 1.41	-	-	-	36
typhi	2	17 ± 1.41	15 ± 1.41	12 ± 0.00	10 ± 0.00	-	-	-	26

Key – = No inhibition 1 = *E. speciosa* 2 = *L. africana*

The antifungal activities (Table 5) with *Aspergillus niger* and *Candida albicans* against the essential oils inhibited the growth of both fungi only at high concentrations but lower than the positive control. Correlating the results obtained from the toxicity and antimicrobial assays to the ethnobotanical uses of these plants confirms their uses for the treatment of different ailments.

Table 5: Antifungal activities of *E. speciosa* and *L. Africana*

Fungi isolates	Essential oil	Zone of inhibition (mm)						-ve control	+ve control
		100mg/mL	50mg/mL	25mg/mL	12.5mg/mL	6.25mg/mL	3.125mg/mL		
Aspergillus niger	1	15 ± 1.41	13 ± 0.00	10 ± 0.00	-	-	-	-	26
	2	17 ± 1.41	14 ± 0.00	11 ± 1.41	-	-	-	-	28
Candida albicans	1	14 ± 0.00	12 ± 0.00	10 ± 0.00	-	-	-	-	28
	2	17 ± 1.41	14 ± 0.00	12 ± 0.00	10 ± 0.00	-	-	-	28

Key – = No inhibition 1 = *E. speciosa* 2 = *L. africana*

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