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Comparative GC-MS analysis of two crude extracts from *Eremomastax speciosa* (Acanthaceae) leaves

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Abstract
This study was designed to identify the possible volatile compounds present in two extracts from *Eremomastax speciosa* leaves. The air-dried leaves were powdered and subjected to extraction using respective solvents; distilled water and methanol/methylene chloride. Then, each of the extracts was further subjected to gas chromatography–mass spectrometry (GC-MS). GC-MS analysis revealed the presence of 22 compounds in aqueous extract and 44 compounds in methanol/methylene chloride extract. Five compounds were found commonly in both extracts. The major constituents identified in aqueous extract were olean-12-en-3-one (28.37%), 2, 7-Dioxaisotwistane (19.77%), 9-oxabicyclo [3.3.1] nonane-2, 6-diol (15.80%) and α-Amyrin (12.09%). In methanol/methylene chloride extract the major compounds identified were n-hexadecenoic acid (17.74%), stigmasterol (12.81%), γ-sitosterol (10.50%) and α-Linolenic acid (9.01%). Some of these compounds were already reported to be pharmacologically active. From these results and given that *Eremomastax speciosa* is widely used to threat or prevent many diseases, it is obvious that this plant contains many biologically active compounds and might be exploited for the development of plant-based drugs.

Keywords: *Eremomastax speciosa*, GC-MS, aqueous extract, methanol/methylene chloride extract

Introduction
Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. History reveals that plants are sources of successful drugs and will continuously be important for screening of new lead compounds [2]. An essential part in the investigation on medicinal plant is identification of the biologically active compounds present in plant leading to further biological and pharmacological studies [3-5].

Eremomastax speciosa (Hochst.) Cufod. (Acanthaceae) is widely distributed in tropical Africa. It is a robust, polymorphous shrub that grows to 2 m high and it has a characteristic quadrangular stem and violate underside of the leaves, which has earned it the local name *Pang nyemshe*, meaning "red on one side" in the west region of Cameroon [6]. This plant is used in Cameroonian ethnomedicine for the treatment of various stomach complaints and information from tradipractitioners suggests that it possesses antiulcer effects. The antidiarrhoeic activity of *E. speciosa* leaf aqueous extract has been reported [7]. The leaf extract is used for the treatment of male infertility among the *Ifa Nkari* people of Akwa Ibom State, Nigeria (where it is known commonly as "golden seal"; "African blood tonic plant"; local name, *Edem Ididout, Ndana-edem*) [8]. Its widely-claimed anti-anemic activity has been experimentally demonstrated by authors [8] who also showed anti-microbial actions against pure clinical strains of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*. The Douala people of Cameroon employ *E. speciosa* variously for malaria, kidney pain, scabies, anemia, diabetes, and nerve pain [9]. *E. speciosa* has been cited for its local use in the treatment of female infertility in the west region of Cameroon [10], as well as for its use in the treatment of irregular menstruation by the Aguambu-Bamumbu peoples of the Lebialem highlands in the South West Region of Cameroon [11]. The plant has also been cited for the treatment of appendicitis, menstrual pains, gonorrhea, burns, as an anti-poison, and to

increase and purify blood in the mount Cameroon region [12]. Phytochemical screening of the water extract of *E. speciosa* revealed the presence of tannins, alkaloids, resins, flavonoids, anthocyanins, phenols, quinones, oils, sterols, triterpenoids, glycosides and proteins [13].

In spite of the wide ethnotherapeutic applications of *E. speciosa*, a detailed review of the literature on the plant has shown that so far there are no published reports on the chemical compounds present in *E. speciosa*. So, the aim of the present study was to investigate the volatile compounds present in aqueous and methanol/methylene chloride extracts of *E. speciosa* by using GC-MS analysis.

Material and Methods

Plant material

The leaves of *E. speciosa* were harvested in May 2017 in Yaoundé (Centre Region of Cameroon) and identified botanically by the Cameroon National Herbarium (by comparison with existing voucher specimen No. HNC/136984).

Preparation of plant extracts

The leaves were chopped and quickly dried in the shade to avoid them getting moldy and then ground in a mechanical grinder to obtain a fine powder.

For aqueous extract, eight hundred grams (800 g) of powder were extracted by infusion in 5 liters of boiled distilled water for 15 minutes. After filtration through Whatman filter paper No. 3, the filtrate was evaporated at 40 ºC using a Raven convection air oven (Jencons-PLS, UK). The brownish solid obtained (150.8 g (18.85% yield)) was stored at 4 ºC for subsequent experiments.

For methanol/methylene chloride (2v/v) extract, 500 grams of the fine ground powder was macerated in 3 L of extraction mixture for 48 hours. After filtration, the solution was evaporated in a rotative evaporator to obtain a paste which was further dried at 40 ºC, using a Raven convection air oven (Jencons-PLS, UK) (41.5 g (8.3% yield)).

Gas chromatography-mass spectroscopic analysis

The GC–MS analysis of volatile compounds from the different extracts of *E. speciosa* was done using Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length × 0.32 mm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). The carrier gas used was Helium with a flow rate of 1.0 ml/min. The initial temperature was set at 50 ºC with increasing rate of 5 ºC/min and holding time of about 10 min. Finally, the temperature was increased to 300 ºC at 10 ºC/min. Each prepared extract was solubilized in respective solvents (chloroform for aqueous extract and methanol for methanol/methylene chloride extract), filtered through a 0.45 µm filter, and then injected in a splitless mode. The compounds were identified by comparison of their mass spectra with standards available in NIST mass spectral library attached to the GC-MS instrument and the results obtained have been tabulated.

Results and Discussion

The GC-MS spectra confirmed the presence of various components with different retention times as illustrated by figures 1 and 2.

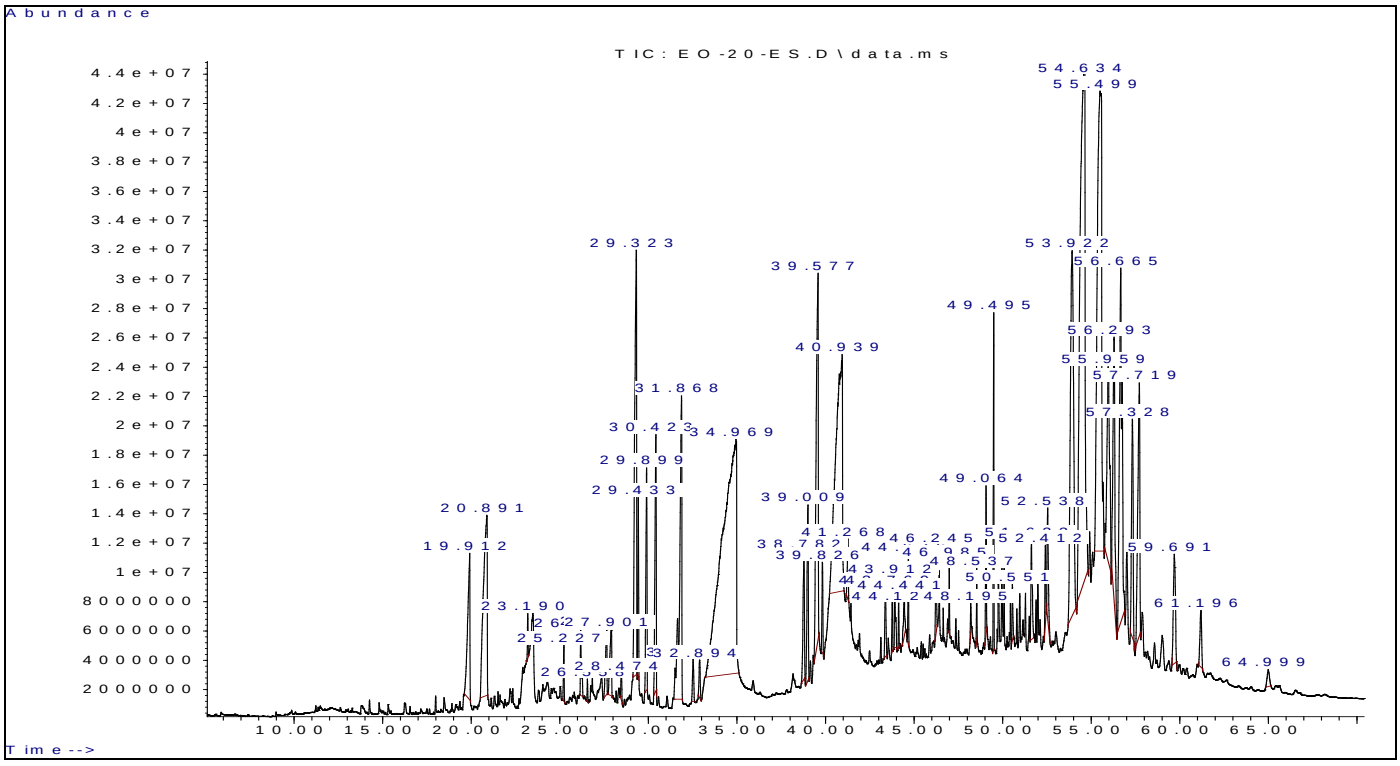

Fig 1: GC-MS chromatogram of methanol/methylene chloride extract of *Eremomastax speciosa*

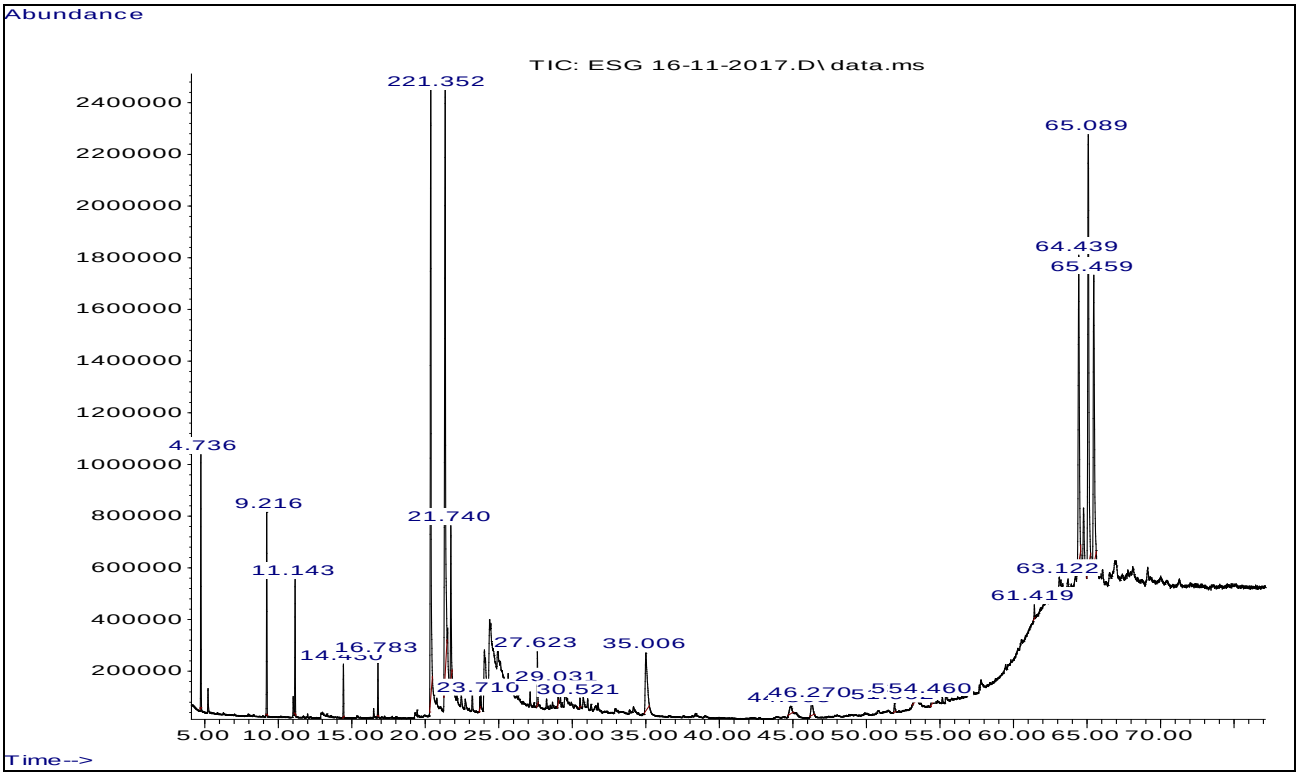

Fig 2: GC-MS chromatogram of aqueous extract of *Eremomastax speciosa*

The compounds identified in both extracts are presented in tables 1 and 2, with their retention time (RT), molecular formula, molecular weight (MW), abundance (peak area%).

Table 1: Phytoconstituents of methanol/methylene chloride extract of *E. speciosa*

No	Names of compounds	Formula	Molecular weight	Retention time (min)	Abundance (%)
1	9-Oxabicyclo[3.3.1]nonane-2,6-diol	$C_8H_{14}O_3$	158	19.91	1.75
2	2,5-Methano-2H-furo[3,2-b]pyran, hexahydro-	$C_8H_{12}O_2$	140	20.89	3.48
3	6,6-Dimethyl-10-methylene-1-oxa-spiro[4.5]decane	$C_{12}H_{20}O$	180	23.19	0.13
4	4-Hydroxy-β-ionone	$C_{13}H_{20}O_2$	208	25.22	0.20
5	2-cis-9-Octadecenyloxyethanol	$C_{20}H_{40}O_2$	312	26.26	0.63
6	N-methyl-N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl]- Acetamide	$C_{14}H_{22}N_2O_3$	266	26.56	0.07
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	27.65	0.45
8	9,10-Dimethyltricyclo[4.2.1.1(2,5)]decane-9,10-diol	$C_{12}H_{20}O_2$	196	27.89	0.57
9	3-Oxo-androsta-1,4-dien-17β-spiro-2'-3'-oxo-oxetane	$C_{21}H_{26}O_3$	326	28.48	0.09
10	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	29.43	0.69
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	30.42	6.99
12	Isophytol	$C_{20}H_{40}O$	296	32.51	0.25
13	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	$C_{16}H_{28}O_3$	268	32.90	0.23
14	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	34.96	17.74
15	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294	38.78	0.87
16	Linolenic acid, methyl ester	$C_{19}H_{32}O_2$	292	39.00	1.09
17	Phytol	$C_{20}H_{40}O$	296	39.57	3.85
18	Octadecanoic acid	$C_{19}H_{38}O_2$	298	39.82	0.38
19	α-Linolenic acid	$C_{18}H_{30}O_2$	278	40.94	9.01
20	Stearic acid	$C_{18}H_{36}O_2$	284	41.27	0.38
21	E,E,Z-1,3,12-Nonadecatriene-5,14-	$C_{19}H_{34}O_2$	294	43.38	0.42
22	Methyl 4-(2-([2-((2-[(2-pentylcyclopropyl) methyl] cyclopropyl) methyl) cyclopropyl] methyl) cyclopropyl) butanoate	$C_{25}H_{42}O_2$	374	43.76	0.23
23	Oleic Acid	$C_{18}H_{34}O_2$	282	43.91	0.38
24	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	44.13	0.14
25	Eicosanoic acid	$C_{20}H_{40}O_2$	312	44.44	0.60
26	2-Monopalmitin	$C_{19}H_{38}O_4$	330	46.24	0.42
27	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	48.19	0.29
28	Methyl lignocerate	$C_{25}H_{50}O_2$	382	48.53	0.20
29	13-Docosenamide	$C_{22}H_{43}NO$	337	49.06	0.77
30	Squalene	$C_{30}H_{50}$	410	49.49	1.1
31	Dodecanoic acid	$C_{32}H_{48}O_6$	528	50.55	0.25
32	γ-Tokoferol	$C_{28}H_{48}O_2$	416	51.62	0.60
33	cholesterol	$C_{27}H_{46}O$	386	52.40	0.31

34	Campesterol	$C_{28}H_{48}O$	400	53.92	5.72
35	Stigmasterol	$C_{29}H_{48}O$	412	54.62	12.81
36	γ-Sitosterol	$C_{29}H_{50}O$	414	55.51	10.5
37	α-Amyrin	$C_{30}H_{50}O$	426	55.98	2.26
38	Lupenone	$C_{30}H_{48}O$	424	56.29	2.66
39	4,22-Cholestadien-3-one	$C_{27}H_{42}O$	382	56.67	4.71
40	α-Tocopherol	$C_{29}H_{50}O_2$	430	57.32	2.41
41	Sitostenone	$C_{29}H_{48}O$	412	57.71	2.57
42	3β-methoxy-Stigmasta-5,22-diene,	$C_{30}H_{50}O$	426	59.69	1.19
43	5α-Stigmastane-3,6-dione	$C_{29}H_{48}O_2$	428	61.18	0.57
44	3-acetoxy-7,8-Epoxylanostan-11-ol	$C_{32}H_{54}O_4$	502	64.99	0.17

Table 2: Phytoconstituents of aqueous extract of *E. speciosa*

No	Names of compounds	formula	Molecular weight	Retention time (min)	Abundance (%)
1	2,3-Dimethylphenol, tert-butyldimethylsilyl ether	$C_{14}H_{24}OS$	236	4.73	3.96
2	Octamethyl-Cyclotetrasiloxane	$C_8H_{24}O_4Si_4$	296	9.21	3.49
3	3-methoxy-4-[(trimethylsilyl)oxy]-benzaldehyde	$C_{12}H_{19}NO_3S$	253	11.14	2.16
4	Decamethyl-cyclopentasiloxane,	$C_{10}H_{30}O_5Si_5$	370	14.40	0.85
5	1,7-Di(2,5-dimethylphenyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane	$C_{22}H_{36}O_4Si_3$	448	16.78	0.92
6	9-Oxabicyclo[3.3.1]nonane-2,6-diol	$C_8H_{14}O_3$	158	20.37	15.80
7	2,7-Dioxaisotwistane	$C_8H_{12}O_2$	140	21.35	19.77
8	5-hydroxy-9-oxabicyclo [3.3.1] nonan-2-one,	$C_8H_{12}O_3$	156	21.74	3.62
9	Boronal	$C_{14}H_{22}O$	206	23.71	0.18
10	11-Isopropylidenetricyclo [4.3.1.1(2,5)]undec-3-en-10-one	$C_{14}H_{18}O$	202	27.62	1.04
11	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	29.03	0.34
12	4-(1,5-Dihydroxy-2,6,6-trimethylcyclohex-2-enyl)but-3-en-2-one	$C_{13}H_{20}O_3$	224	30.52	0.19
13	Hexadecanoic acid	$C_{17}H_{34}O_2$	270	35.00	3.82
14	Methyl 13-octadecenoate	$C_{19}H_{36}O_2$	296	44.84	0.59
15	Octadecanoic acid	$C_{19}H_{38}O_2$	298	46.26	0.86
16	Eicosanoic acid	$C_{21}H_{42}O_2$	326	51.90	0.25
17	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	53.28	0.24
18	Methyl 11-(3-pentyl-2-oxiranyl)undecanoate	$C_{19}H_{36}O_3$	312	54.46	0.29
19	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	61.42	0.22
20	Trilinolein	$C_{57}H_{98}O_6$	878	63.11	0.93
21	Olean-12-en-3-one	$C_{30}H_{48}O$	424	65.09	28.37
22	α-Amyrin	$C_{30}H_{50}O$	426	65.45	12.09

The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compounds fragment into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprints of those compounds which can be identified from the data library [14].

The GC-MS analysis revealed the presence of 44 compounds in methanol/methylene chloride extract and 22 compounds in the aqueous extract. This significant difference can be easily explained given that recovery of natural compounds depends on the type of solvent used, its polarity index (PI) and the solubility of phenolic compounds in the extraction solvents [15]. Five compounds were found in both extracts. α-amyrin, 9-oxabicyclo [3.3.1] nonane-2,6-diol and octadecanoic acid were in higher quantities in the aqueous extract while eicosanoic acid and ethyl iso-allocholate were sensibly higher in the methanol/methylene chloride extract. Based on abundance, the major compounds present in the methanol/methylene chloride extract were n-hexadecenoic acid (17.74%), stigmasterol (12.81%), γ-sitosterol (10.50%) and α-Linolenic acid (9.01%). The aqueous extract contained olean-12-en-3-one (28.37%), 2, 7-Dioxaisotwistane (19.77%), 9-oxabicyclo [3.3.1] nonane-2,6-diol (15.80%) and α-Amyrin (12.09%) as major compounds.

Some of these major compounds and many others have been found to possess several biological activities. n-Hexadecenoic acid showed anti-inflammatory activity [16]. Stigmasterol has been shown to be anti-hypercholesterolemic, antioxidant, anti-inflammatory and hypoglycemic [17]. Antidiabetic activity of γ-sitosterol has also been reported [18]. Anti-inflammatory, anti-bacterial and anti-cancer properties of α-Amyrin were demonstrated by authors [19].

Conclusion

The biological activities of compounds present in *E. speciosa* leaves extracts support the medicinal use of the plant. The study revealed major bioactive compounds present in both extracts. Identification of these compounds in the plant serves as the basis for determination of the possible health benefits of the plant leading to further pharmacologic studies. Thus, further studies are needed to isolate active principles of the extracts as well as to elucidate their exact mechanism of action against various disorders.

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Conflicts of interest: Authors declare that there is no conflict of interests.

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