Future Journal of
Pharmaceutical Sciences

RESEARCH **Open Access**

Eremomastax speciosa (Hochst.): GC/MS profiling, antioxidant and antimicrobial activities of stem essential oil

Michael G. Ibok[1,2*], Oluwakayode O. Odeja[3], Ejike O. Okpala[4], Juliet E. Eghwubare[3] and Eniola O. Anifalaje[1]

Abstract

Background *Eremomastax speciosa* (Hochst.) Cufod. (*Acanthaceae*) is a renowned medicinal plant used to ease menstrual cramps and treat female infertility, anaemia, dysentery, urinary tract infection and haemorrhoids. Essential oils and their constituents from herbs have also been utilised in the management of a good number of ailments in ethnomedicine. The chemical composition, antioxidant and antimicrobial activities of the stem essential oil are investigated in this study. The essential oil was obtained by hydro-distillation using an all-glass Clevenger apparatus. Identification and characterisation were done using Gas Chromatography–Mass Spectrometry, while antioxidant activity was evaluated with 2, 2-diphenyl-1 picrylhydrazyl radical (DPPH*) method. The antimicrobial property was assessed by the broth dilution method.

Results The essential oil contained forty-three compounds constituting 62.87% of the total oil composition. It was dominated by non-terpene derivatives, of which (14β)-Pregnane (17.58%) is the most abundant compound. Other significant compounds identified in the essential oil include *n*-decane (2.3%), norbornane (2.2%), (−)-α-Copaene (1.5%), 10-epizonarene (1.5%), thymol (1.25) and (−)-α-phellandrene (1.12%). The essential oil exhibited significant antioxidant activity (IC_{50} 0.7296 μg/mL), which is more active than the standards; vitamin C (IC_{50} 0.8728 μg/mL) and butylated hydroxy anisole (IC_{50} 0.8729 μg/mL) used for the assay. Also, the oil inhibited significant bacterial and fungal strains at concentrations ranging from 100 to 3.125 μg/mL with a minimum inhibitory concentration between 3.5 and 6.5 μg/mL.

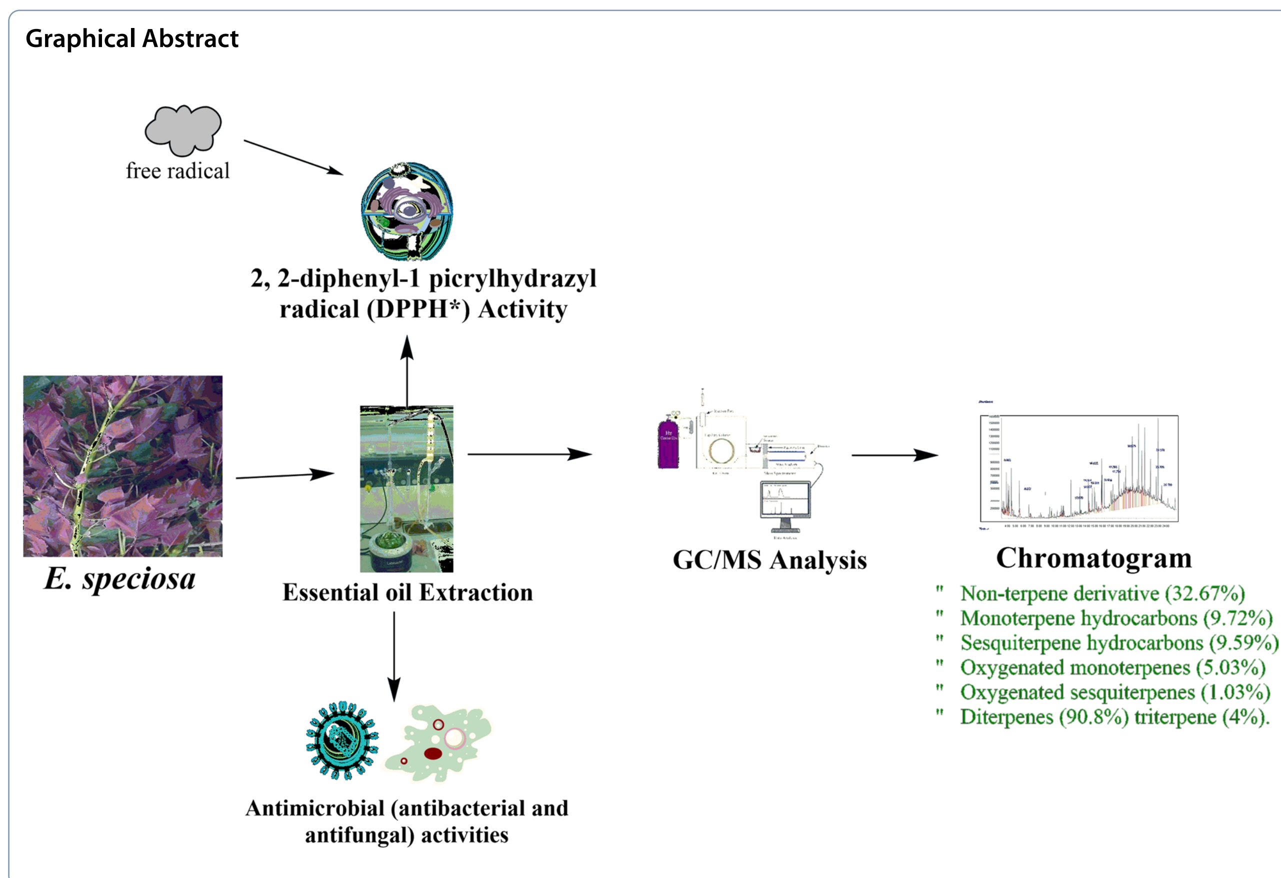
Conclusion The chemical composition of the stem essential oil of *E. speciosa* could be responsible for the pharmacological applications of the plant in ethno-medicine and the chemical constituent of the stem essential oil of *E. speciosa* is reported for the first time.

Keywords *Eremomastax speciosa* (Hochst.) Cufod, Antioxidant, Antimicrobial, Gas chromatography/mass spectrometry, Essential oil, (14β)-Pregnane

*Correspondence:
Michael G. Ibok
michaelibok08@gmail.com
Full list of author information is available at the end of the article

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

Graphical Abstract



Background

Plants are essential in treating diseases with less or no side effects [1]. They contain chemical compounds with significant biological and pharmacological properties. Plants are a source of effective medications. There will continue to be crucial for screening novel lead compounds, as has been discovered over time. However, the demand for and interest in medicinal and aromatic plants for food, medicine and other applications is constantly rising. Plant secondary metabolites include many essential oils (EOs) [1]. However, despite their rich and complex composition, essential oils are used in cosmetics and perfumes. In order to produce beneficial uses in environmental health, agriculture, biology and pharmaceutics, it is vital to understand the chemistry and biological properties of essential oils and their unique constituents [1].

Essential oils constitute a small proportion of plants' composition. They are composed of volatile compounds that typically have low molecular weight [2]. The volatile compounds are mainly terpenes with little traces of alcohols, heterocycles, aldehydes, ethers, ketones, amines, esters, phenols and amides [3]. Numerous other aromatic compounds range from aldehydes, alcohols and ketones, including fruity flavours like ((E)-nerolidol), floral (Linalool), citrus (Limonene), herbal ((+)-selinene), etc. can also be present. Additionally, non-terpenic compounds like cinnamaldehyde and safrole are produced during the biogenesis process of essential oils through the phenylpropanoids route [3, 4]. The antibacterial property of EOs and their components have been reported [5, 6] with notable characteristics—hydrophobicity. Hydrophobicity allows EOs to partition into the lipids of the cell membrane of micro-organisms, altering the structure and making it more porous [7], leading to the death of the organisms' cells. Also, the constitutions of an EO play significant role in antioxidant activity. It is widely known that phenolic compounds and secondary metabolites with conjugated double bonds typically exhibit strong antioxidative effects [7].

Eremomastax speciosa (Hochst.) Cufod. (*Acanthaceae*) (Fig. 1) is widely distributed in the tropics of Africa [8]. It is a robust, polymorphous shrub that grows up to 2 m long and has a characteristic quadrangular stem and violets on the underside of leaves. It is known in Cameroon as pang nyemshe (red on one side) and in southern Nigeria (Ibibio) as Edem ididot ("golden seal" or "African blood tonic") [8]. In addition

Fig. 1 Picture of *E. speciosa* plant (Source: Picture captured at the point of collection on 15 April, 2022)

to its application in treating female infertility and menstrual cramps, the plant has also been used in treating anaemia, dysentery, urinary tract infection, haemorrhoids and gastric ulcers [9–11]. The preliminary phytochemical analysis of aqueous leaf extract revealed the presence of flavonoids, tannins, alkaloids and saponins [12, 13]. The pharmacological relevance of the leaf includes promising fertility effect [14], antianemic, antimicrobial activities [15, 23] and antiulcer [16–22].

However, no reports on the composition of the essential oil of *E. speciosa* exist in the literature. Given the various medicinal applications in ethno-medicine. Also, our continuing search for bioactive chemical compounds from Nigerian medicinal plants [29, 30], this paper now reports the results of the analysis of the essential oil, antioxidant and antimicrobial activities of the *E. speciosa*.

Methods

Plant collection and preparation

A fresh *E. speciosa* was harvested from Utit Uruan, Akwa Ibom, Nigeria. The fresh stem was identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Oyo State, Nigeria, where a voucher specimen with the FRIN herbarium number FHI 113451 was deposited [30].

Isolation of essential oil from *E. speciosa* stem

According to the British Pharmacopoeia guidelines described by Odeja et al. [30], the fresh, chopped *E. speciosa* stem (400 g) was placed in an all-glass Clevenger-type equipment hydrodistilled for 3 h. Essential oil collected was dry over anhydrous sodium tetraoxosulphate (vi), filtered, weighed (0.5 g) and stored in a refrigerator at 4 °C before analysis. The % yield of the essential oil was calculated using the formula:

$$\%\ \text{yield} = \frac{\text{Weight of essential oil}\ (\text{g})}{\text{Weight of the sample}\ (\text{g})} \times 100 \quad (1)$$

Gas chromatography–mass spectrometry (GC–MS) analysis and identification of the essential oil constituents

About 1.5 mL of *n*-hexane was added to the GC vial and approximately 1.0 µL of the essential was added, centrifuged for about 5 min.

The constituents of the stem essential oil of *E. speciosa* were identified on an Agilent 7809A gas chromatograph hyphenated with an Agilent mass detector featuring a split/splitless injector interfaced to a mass selective detector operating at 70 eV. With a 1428 amu/sec scan rate, the ion source temperature was 200 °C with a mass spectral range of m/z 50–700. A 30 m long HP-5MS column with an internal diameter of 0.25 mm and a film thickness of 0.25 m was installed in the GC column. The oven temperature was adjusted as follows: 80 °C at first for 2 min, then 10 °C/min up to 240 °C/6 min. Helium was used as the carrier gas, with a 1 mL/min flow rate. Injection volume, linear velocity and pressure were adjusted at 1.0 µL, 362 cm/s and 56.2 kPa, respectively. The oven temperature was set at 60 °C, hold for 1 min to 180 °C for 3 min at 10 °C/min, then the final temperature was 280 °C for 2 min at 10 °C/min. Both injector and detector temperatures were fixed at 250 °C.

Based on their retention indices, which were calculated using homologous series of normal alkane and comparing the mass spectral fragmentation patterns (NIST data/base/Chemstation data system) with information previously published in the literature, the constituents of essential oil were identified [36].

Antioxidant activity

According to Brand-William et al. [33] and Odeja et al. [30], the free radical scavenging capacity of the essential oil and standards were determined by their ability to react with the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH*). 3.94 mg of DPPH* was dissolved in 100 mL of methanol, producing a methanol-DPPH* solution (0.1 mM). The oil was dissolved in methanol and serially diluted to prepare five (5) concentrations of the oil (1000, 500, 250, 125 and 62.5 mg/mL). The oil was then combined with 2.0 mL of a methanol-DPPH* solution (0.1 mM). The mixture was agitated and incubated for 30 min at room temperature in the dark cupboard. A GS UV-12 UV–Vis

spectrophotometer measured the absorbance at 517 nm. The identical approach was used in a control experiment, but no essential oil was used (DPPH* + methanol) and the absorbance was recorded as A_C. The antioxidant capacities of ascorbic acid and butylated hydroxyanisole (BHA) were used as a baseline for comparison. Each test was conducted in triplicate and the essential oil's capacity to free radical scavenging was determined using the formula to determine percentage inhibition (% I):

$$\%I = \frac{(\mathrm{Ac} - \mathrm{As})}{(\mathrm{Ac})} \times 100. \quad (2)$$

where Ac—Absorbance of Control, As—Absorbance of Sample.

50% inhibition concentration (IC_{50}) of the sample was evaluated using GraphPadPrism 5.0.

Antimicrobial activity (broth dilution method)

The minimum inhibitory concentration (MIC) and the MBC of the essential and reference standards were assessed against ten (10) micro-organisms; six bacteria—*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*, four fungi—*Candida albican*, *Aspergillus niger*, *Penicillium notatum*.

Standardisation of inoculum

The micro-organisms were revived in tryptose sulphite-cycloserine agar (Oxoid) with d-cycloserine (Sigma) under anaerobic conditions at 36 °C for 24 h following British Standards Institute [37]. The inoculum's bacterial concentration was calibrated to be 0.5 on the McFarland turbidity scale or 10^8 CFU mL^{-1}. To prepare a concentration of 107 CFU mL^{-1}, an aliquot (1 mL) of this suspension was transferred to a sterile tube and the volume was increased to 10 mL using sodium chloride solution (0.8%, w/v). Using reinforced clostridial medium (RCM; Oxoid), 200 μL aliquots of this solution were divided into three test tubes. The contents were adjusted to 10 mL to produce working inoculums with final concentrations of 2.0×10^5 CFU mL^{-1} [38, 39].

Minimal inhibitory concentrations

Anaerobic conditions were used for all microbiological experiments. MIC analyses were conducted in 96-well microplates following the Clinical and Laboratory Standards Institute [39] suggested protocols. To prepare a stock solution containing 40 mg of oil per mL, essential oil (200 mg) was dissolved in 40 μL of dimethyl sulphoxide before the volume was increased to 5 mL with sterile RCM that contained 1% Tween 80. Essential oil stock was serially diluted twice with RCM to produce final concentrations ranging from 20 to 0.625 μg/mL. The diluted samples (100 μL) were added to the microplate wells and thoroughly mixed using a micropipette. The negative controls consisted of sterile RCM alone in combination with dimethyl sulphoxide (DMSO). At the same time, gentamycin (for bacteria at 10 μg/mL) and tioconazole (for fungi at 0.07 μg/mL) were used as a positive control. The control wells had sterile RCM, but no inoculum, so aseptic conditions could be determined. The inoculated microplates were incubated in anaerobic conditions at 36 °C for 48 h. The bacterial growth was verified by adding 10 μL of a sterile 0.5% aqueous solution triphenyltetrazolium chloride (TTC, Sigma-Aldrich) and incubating at 36 °C for 30 min [40, 41]. Pink/red 1,3,5-triphenyl formazan was produced from yellow TTC by the live bacteria (TPF). Each assay was performed in triplicate.

Minimum bactericidal concentrations and Minimum fungicidal concentrations

According to the Ministério da Agricultura, Pecuária eAbastecimento's recommendations as described by Radaelli et al. [40], MBCs and MFCs were evaluated by inoculating the test mixtures from the wells showing no microbial growth onto the surface of sterile Shahidi-Ferguson Perfringens agar medium. The plates were subjected to ocular inspection after being incubated anaerobically for 24 h in an oven at 36 °C. The essential oil sample had bactericidal and fungicidal activities if there was no microbial growth on the medium, which suggested that the oil sample had bacteriostatic and fungistatic activities.

Results

Phytoconstituents of stem essential oil of *E. speciosa*

The hydro-distillation of stem essential oil of *E. speciosa* gave colourless fluid (0.13% yield) with a characteristic herbal-like scent. GC/MS qualitative and quantitative data on the stem essential oil of *E. speciosa* are shown in Fig. 2 and summarised in Table 1. Forty-three (43) compounds were identified, representing 62.87% of the oil constituents. Non-terpene derivatives were the main constituents identified in the stem essential oil (32.67%; Table 2) followed by monoterpene hydrocarbons (9.72%) and sesquiterpene hydrocarbons (9.59%). Moderate quantifications were observed in oxygenated monoterpenes (5.03%). Minor quantification of the essential oil was oxygenated sesquiterpenes (1.03%), diterpenes (0.8%) and triterpene (4%). The most abundant compound was (14β)-p (17.58%). Other significant compounds identified in the essential oil include *n*-decane (2.3%), norbornane (2.2%), (−)-α-Copaene (1.5%), 10-epizonarene (1.5%), thymol (1.25) and (−)-α-phellandrene (1.12%).

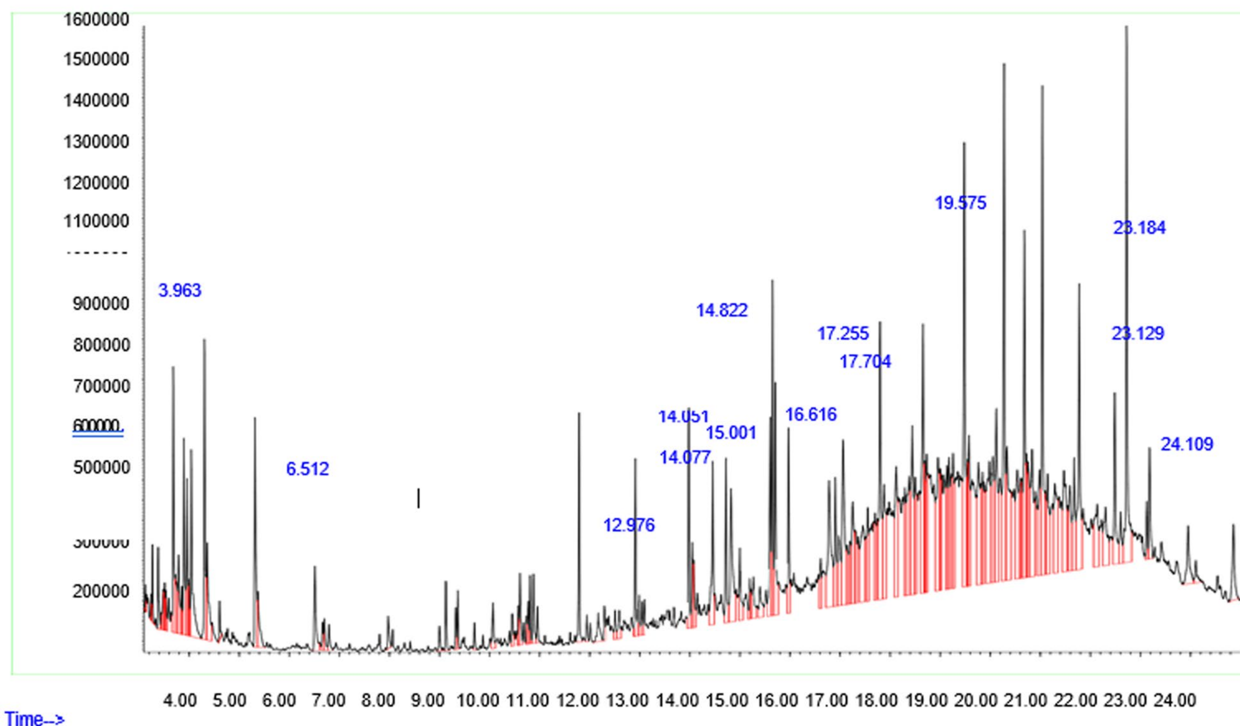


Fig. 2 GC-TIC Chromatogram of essential oil of *E. speciosa* stem

Antioxidant activity

The results of the scavenging ability to stem essential oil of *E. speciosa* on DPPH* assay and IC_{50} values are summarized in Table 3. The percentage inhibition of the essential oil was concentration-dependent, as shown in Fig. 3.

Antimicrobial activity of stem essential oil of *E. speciosa*

Experimental data obtained from the in vitro antimicrobial activity of *E. speciosa* stem essential oil are summarised in Table 4. The essential oil of *E. speciosa* stems exhibited potent antimicrobial activities with 10–28 mm inhibitory zones against bacterial strains and 10–20 mm against fungal strains compared to the controls (Gentamicin: 40–38 mm and Tioconazole: 20–28 mm). The MBCs between 4.0 and 6.5 µg/mL against bacterial strains and MFCs between 3.5 and 5 µg/mL against fungal strains when compared to the controls (Gentamicin: 3.0 µg/mL and Tioconazole: 3.5 µg/mL). It was also found that the strains of fungi (*C. albican*, *A. niger*, *P. notatum* and *R. spp.*) were more susceptible to essential oil than the strains from bacteria (*S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. typhi* and *K. pneumoniae*). Initial screening tests prove that, in the 100 µg/mL concentration, the oil had the highest fungistatic and bacteriostatic effects (Table 4). A 100% growth inhibition of the tested fungi (*R.* spp.) and almost 71% for the rest fungi, while about 68% growth inhibition was obtained in the bacteria strains. The EO shows strong growth inhibition against all the tested micro-organisms at 100 µg/mL. Additionally, the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) values were determined for the oil of *E. speciosa* stem against the pathogenic fungi tested (Table 5). The tests were conducted in the 100–3.125 µg/mL concentration range. The minimum inhibitory concentration (MIC) values of the fungi species in the tested oils were in the range of 3.5–5.0 mg/ml and the minimum fungicidal concentrations (MFC) were in the range of 100–> 3.0 µg/mL (Table 5). The highest activity of these oils was observed against *Rhizopus* spp. The MIC and MFC values of *E. speciosa* oil for individual strains were *C. albican*, *A. niger* and *P. notatum* (MIC = MFC = 5.0 µg/mL). EO was bacteriostatic against *E. coli*, *B. subtilis* and *S. aureus,* with bactericidal concentrations re-evaluated at MIC 4, 4.5 and 5 µg/mL, respectively.

Data analysis

All results were conveyed as mean ± standard deviation. Analysis of variance was used to determine any

Table 1 Essential Oil Composition of *Eremomastax speciosa* stem

S/N	Compound identified	Retention Index	% Composition
1	*n*-Decane[a]	166.22	2.3
2	Norbornane[a]	820	2.2
3	4-Ethyl-2-methylhexane[a]	833	0.25
4	3-Ethylheptane[g]	870	0.89
5	3-Methyl-2-hexanol[g]	906	1.2
6	m-Ethylmethylbenzene[g]	945	2
7	Octan-3-one[g]	966	1
8	1-Ethylbutyl hydroperoxide[g]	968	0.5
9	1-Octen-3-ol[g]	982	1.12
10	Isobutylcyclohexane[a]	983	0.7
11	β-Pinene[a]	985	0.25
12	1,2,4-trimethylbenzene pseudocumene [g]	1002.8	0.96
13	(−)-α-phellandrene[b]	1003	2.54
14	D-(+)-Limonene[a]	1018	0.78
15	β-trans-Ocimene[a]	1050	0.9
16	β-Linalool[b]	1086	1.24
17	O-Hydroxybenzoic acid[g]	1169.35	1.3
18	Thymol[b]	1265.51	1.25
19	Azulene[a]	1326	0.57
20	Panaginsene[a]	1337.9	0.27
21	(−)-α-Copaene[a]	1376	1.5
22	α-Longipinene[c]	1385	1
23	β-Elemene[c]	1390	0.75
24	Caryophyllene[c]	1423	0.58
25	δ-Selinene[c]	1496	1.1
26	p-Cresol[d]	1497	0.89
27	β-*cis*-Guaiene[c]	1504	0.27
28	α-Selinene[c]	1505	0.12
29	γ-Cadinene[c]	1511	2
30	δ-Cadinene[c]	1518	0.9
31	10-epizonarene[c]	1537	1.5
32	Cetene[c]	1588	1.2
33	Longiborneol[c]	1593	0.17
34	Valerena[d]	1715.7	0.17
35	Phytane[e]	1814	0.8
36	Cyclohexadecane[g]	1883	1.33
37	Methyl hexadecanoate[g]	1904.1	0.98
38	*n*-Hexadecoic acid[g]	1964	1.13
39	Docos-1-ene[g]	2190	2.4
40	(14β[)]-Pregnane[g]	2521	17.58
41	Hexacos-1-ene[g]	2596	0.28
42	Squalane[f]	2665	3.32
43	1-Nonacosene[f]	2888	0.68
	Total		62.87

Monoterpene hydrocarbon = a, Oxygenated Monoterpenes = b, Sesquiterpene hydrocarbon = c, Oxygenated sesquiterpene = d, Diterpene hydrocarbon = e, Ttriterpene hydrocarbon = f, Non-terpene derivatives = g

Table 2 Class of compounds identified in stem essential oil of *Eremomastax speciosa*

Class of compounds	% Composition
Monoterpenes hydrocarbon	9.72
Oxygenated monoterpenes	5.03
Sesquiterpenes hydrocarbon	9.59
Oxygenated sesquiterpenes	1.06
Diterpenes hydrocarbon	0.8
Triterpenes hydrocarbon	4.0
Non-terpenes derivatives	32.67
Total	62.87

Table 3 Absorbance reading of stem essential oil of *Eremomastax speciosa* and the reference standards

Concentration (mg/mL)	Vitamin C	BHA	ESEO
1.0	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.00
0.5	0.04 ± 0.01	0.06 ± 0.01	0.03 ± 0.01
0.25	0.05 ± 0.01	0.09 ± 0.00	0.04 ± 0.01
0.125	0.07 ± 0.01	0.09 ± 0.01	0.05 ± 0.02
IC_{50}	0.8728[a]	0.8729[a]	0.7296[a]

Blank reading: 1.20, BHA: Butyl hydroxyanisole, ± = Standard deviation, ESEO: *E. speciosa* stem essential oil; Values are mean ± standard deviation of each essential oil and standard analysed individually in triplicate. Means followed by the same alphabet in the same row represent no significant difference ($p < 0.05$) for a one-way ANOVA

significant difference between groups using the statistical analysis software package GraphPad 5.0. Values with $p < 0.05$ were regarded as significant. All graphs/charts were drawn with GraphPad 5.0.

Discussion

The phytoconstituents of essential oil of *E. speciosa* stem identified (14β)-Pregnane (17.58%) as the major component of the stem EO. (14β)-pregnane is a parent hydrocarbon for two series of steroids stemming from 5α-pregnane to 5β-pregnane. The C21 steroid has been reported in the urine of pregnant women. It exhibited anaesthetic, hypnotic and sedative effects [24]. (14β)-pregnane has been reported as the major constituent identified in the EO of *Allium rotundum* flower and the significant bacteria strains inhibition was attributed to (14β)-pregnane per cent composition [25]. Decane has been reported as the major component of *Hypericum perfortum* EO and possesses strong insecticidal activity [26]. Norbornane, a bicyclo-[2.2.1]heptane used as a pharmaceutical intermediate [27], (−)-α-Copaene-exhibited cytotoxic, antioxidant and antigenotoxic activities. Also, 10-epizonarene was identified in the essential oil of *Mentha piperita* [28]. Thymol was identified as a major component of *Asparagus flagellaris* essential oils of leaf and root [29, 30]. 1-octen-3-ol (11.9%)—a natural product derived from linoleic acid during oxidative breakdown [31] and also known as mushroom alcohol, has been confirmed as an antimicrobial [32].

Notwithstanding, the EO from *E. speciosa* stem contains several constituents with distinct antioxidant properties. Since the radical molecule is stable, the DPPH* test is a reliable, simple and affordable way to assess the antioxidants' capacity to scavenge free radicals [33, 34]. The assay's process is based on how the antioxidant agent reduces the radical, either by donating an electron or by reducing DPPH to its reduced form, DPPH-H, a stable diamagnetic molecule, changing its colour from purple to

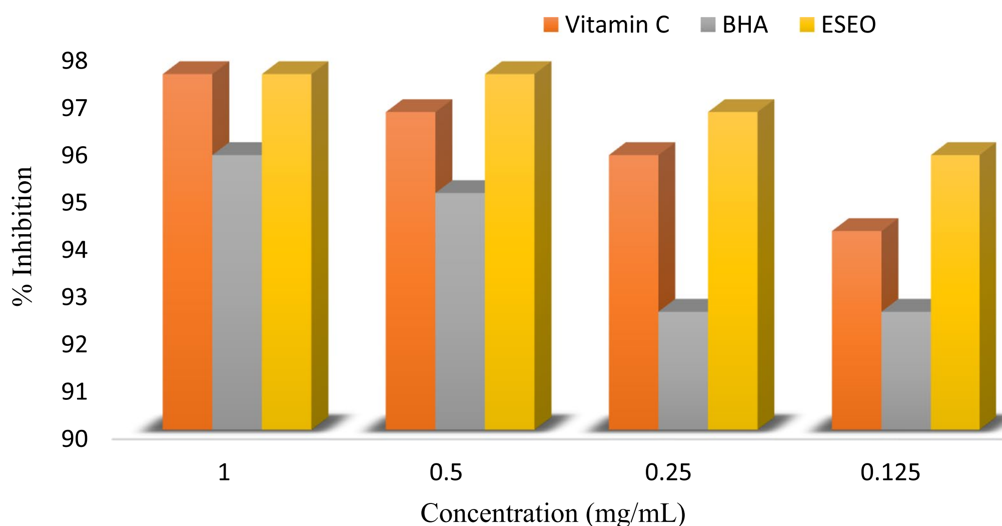


Fig. 3 Percentage inhibition of stem essential oil of *E. speciosa* and reference standards

Table 4 Inhibitory zone of the stem essential oil of *E. speciosa* and reference standards

Conc (μg/mL)	Sa	Ec	B.sab	Ps.a	Sal	Klebs	Ca	An	Rhis	Pen
100	28 ± 0.01	26 ± 0.00	28 ± 0.00	26 ± 0.01	24 ± 0.01	24 ± 0.01	20 ± 0.01	20 ± 0.01	20 ± 0.00	18 ± 0.00
50	26 ± 0.00	24 ± 0.01	26 ± 0.01	22 ± 0.01	20 ± 0.01	20 ± 0.01	18 ± 0.01	18 ± 0.01	18 ± 0.01	16 ± 0.01
25	21 ± 0.02	21 ± 0.02	24 ± 0.00	19 ± 0.01	18 ± 0.01	18 ± 0.01	16 ± 0.00	16 ± 0.02	16 ± 0.01	14 ± 0.02
12.5	18 ± 0.01	17 ± 0.01	21 ± 0.02	17 ± 0.01	16 ± 0.01	16 ± 0.01	16 ± 0.01	16 ± 0.01	14 ± 0.00	12 ± 0.01
6.25	12 ± 0.00	14 ± 0.01	18 ± 0.01	14 ± 0.01	14 ± 0.01	14 ± 0.01	12 ± 0.03	12 ± 0.01	12 ± 0.01	10 ± 0.00
3.125	10 ± 0.01	12 ± 0.00	14 ± 0.00	10 ± 0.01	10 ± 0.01	10 ± 0.01	10 ± 0.01	10 ± 0.00	10 ± 0.00	–
− ve cont	–	–	–	–		–	–	–	–	–
+ ve cont	40 ± 0.01	38 ± 0.00	36 ± 0.01	38 ± 0.00	38 ± 0.01	40 ± 0.01	28 ± 0.02	28 ± 0.01	20 ± 0.01	26 ± 0.01

Sa *Staphylococcus aureus*, Ec *Escherichia coli*, B. sab *Bacillus subtilis*, Ps.a *Pseudomonas aeruginosa*, Sal *Salmonella typhi*, Klebs *Klebsiella pneumoniae*, Ca *Candida albican*, An *Aspergillus niger*, Pen *Penicillium notatum* and Rhis *Rhizopus* spp., cont: control

Table 5 Minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration of stem essential oil of *Eremomastax speciosa* and reference standard

Micro-organisms	MIC (μg/mL)	MBC (μg/mL)	MFC (μg/mL)
Staphylococcus aureus	5.0	5.0	–
Escherichia coli	4.0	4.0	
Bacillus subtilis	4.1	4.1	–
Pseudomonas aeruginosa	5.0	5.0	–
Salmonella typhi	6.0	6.0	–
Klebsiella pneumoniae	6.5	6.5	–
Gentamycin	3.0	3.0	–
Candida albicana	5.0	–	5.0
Aspergillus niger	5.0	–	5.0
Penicillium notatum	5.0	–	5.0
Rhizopus spp.	3.5	–	3.5
Tioconazole	3.5	–	3.5

yellow [35]. It was observed that the stem EO of *E. speciosa* exhibited significant antioxidant activity at the tested concentrations (Fig. 2 and Table 2) comparable to the reference standard vitamin C and BHA. The order of activity based on the IC_{50} is as follows: Vitamin C (0.8728 mg/mL) ≤ BHA (0.8729 mg/mL) < ESEO (0.7296 mg/mL). Statistical analysis of the data subjected to a one-way analysis of variance (ANOVA) showed no significant difference between the stem essential oil and the reference standard at $p < 0.05$. The considerable antioxidant activity observed in this study can be attributed to the synergetic action of oxygenated monoterpenes and oxygenated sesquiterpenes coupled with conjugated double bond [7] constituents identified in *E. speciosa* EO either by hydrogen atom transfer mechanism or by the sequential proton loss-electron-transfer [36].

The emerging multidrug resistance of bacterial strains is critically lessening the potentially effective antimicrobials used in health care. One reason might have been the long-term consequence of the uncontrolled use and misuse of antimicrobial agents and products. Natural products like EOs can be potential alternatives to fight multidrug-resistant species. The tested essential oil showed vital to moderate effectiveness against the tested bacteria and fungi strains. The antimicrobial of *E. speciosa* stem essential oil was compared with the earlier report of the ethanolic, *n*-hexane and aqueous leaf extracts of E. *speciosa*. Okokon et al. [15] reported that all the extracts showed moderate to higher activity against the tested organism, the same trend was observed in the present study. Following evaluation of the MBC, EO has shown practically equal bactericidal and bacteriostatic effects against both gram-positive and -negative bacteria. It has been purported that EO can be used as an effective antiseptic against several micro-organism species, including *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. typhi* and *K. pneumoniae*. The significant antimicrobial activity exhibited in this study might be due to the chemical composition of the EO, such as (14β)-pregnane [25] and the synergetic effect of the identified compounds in the EO.

Conclusion

The *E. speciosa* stem essential oil comprises important constituents such as (14β)-pregnane, thymol, daucene, panaginsene, (−)-α-Copaene, α-longipinene, β-*cis*-guaiene, among others. Although the constituents identified were quantified in small amounts possessed different pharmaceutical properties including generic antimicrobial and free radical scavenging activities. The free radical activity of the *E. speciosa* oil against DPPH* radical was significantly higher than those of the reference standard (vitamin C and BHA), demonstrating activity even at a lower concentration. The inhibitory power of *E. speciosa* oil against tested micro-organisms was also exhibited at lower concentrations when

compared to the reference standard for different strains of gram-positive and -negative bacteria and fungi. The EO was bacteriostatic against *E. coli*, *B. subtilis* and *S. aureus* and fungistatic against all the tested fungi strains, but *R. spp.* Inhibitory power was comparable to the Tioconazole. Thus, this plant is a rich source of secondary metabolites of medicinal importance and the results of this study provide some scientific basis for the utilisation of the plant in ethno-medicine for menstrual cramps and treat female infertility, anaemia, dysentery and urinary tract infection. Also, this is the first study on the *E. speciosa* stem essential oil. The present study shows that *E. speciosa* EO possesses the significant antioxidant and antimicrobial capacity and can readily be used as a natural preservative to minimise or prevent product losses from oxidative processes and for infectious diseases.

Abbreviations

GC/MS	Gas chromatography/mass spectrometry
FRIN	Forestry Research Institute of Nigeria
DMSO	Dimethyl sulphoxide
IC_{50}	The half-maximal inhibitory concentration
DPPH	2,2-Diphenyl-1-picrylhydrazyl radical
RI	Retention index
TIC	Total ion concentration in percentage
GC	Gas chromatography
ANOVA	Analysis of variance
NIST	National Institute of Standards and Technology
ESEO	*Eremomastax speciosa* Essential oil
BHA	Butyl hydroxyanisole
TTC	Triphenyl tetrazolium chloride
RCM	Reinforced clostridial medium
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MFCs	Minimum fungicidal concentrations
EOs	Essential oils

Acknowledgements
The authors are grateful to the Departments of Chemistry and Pharmaceutical Microbiology, University of Ibadan, Nigeria, for providing the laboratory space and facilities for the extraction and biological analysis of the essential oils.

Author contributions
MGI carried out the sample collection, conceptualisation, extraction of the essential oil, antioxidant, and antimicrobial assays, results interpretation and write-up; OOO was involved in the GC–MS analysis, interpretation of the essential oil chromatogram and supervision; EOO was involved in the antioxidant assay; JEE carried out the extraction of the essential oil; EOA was involved in the antimicrobial assay. All authors read and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors declare no conflict of interest

Competing interests
The authors declare that they have no competing interests.

Plant material
Michael G. Ibok collected the plant from latitude: 5° 6′ 36″ N and longitude: 7° 57′ 57″ E, identified and authenticated at the Forest Research Institute of Nigeria (FRIN), Oyo State, Nigeria.

Author details
[1]Natural Products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Ibadan, Oyo State, Nigeria. [2]Organic Chemistry Section, Chemical Science and Technology Division (CSTD), CSIR-National Institute for Interdisciplinary Science and Technology (NIIST), Thiruvananthapuram, Kerala, India. [3]Department of Chemistry, College of Sciences, Federal University of Petroleum Resources, Effurum, Delta State, Nigeria. [4]Department of Chemistry, Faculty of Science, Federal University Lokoja, Lokoja, Kogi State, Nigeria.

Received: 17 March 2023 Accepted: 4 June 2023
Published online: 14 June 2023

References

1. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W (2016) Essential oils' chemical characterization and investigation of some biological activities: a critical review. Medicines 3(25):1–16. https://doi.org/10.3390/medicines3040025
2. Sell CS (2006) The chemistry of fragrance from perfumer to consumer, 2nd edn. The Royal Society of Chemistry, Cambridge, p 329
3. Vainstein A, Lewinsohn E, Pichersky E, Weiss D (2001) Floral Fragrance. new inroads into an old commodity. Plant Physiol 27:1383–1389. https://doi.org/10.1104/pp.010706
4. Pophof B, Stange G, Abrell L (2005) Volatile organic compounds as signals in a plant—herbivore system: electrophysiological responses in Olfactory Sensilla of the Moth *Cactoblastis cactorum*. Chem Senses 30:1–9. https://doi.org/10.1093/chemse/bji024
5. Shelef LA (1983) Antimicrobial effects of spices. J Food Saf 6:29–44. https://doi.org/10.1111/j.1745-4565.1984.tb00477.x
6. Nychas GJE (1995) Natural antimicrobials from plants. In: Gould GW (ed) New methods of food preservation, 1st edn. Blackie Academic & Professional, London, pp 58–89. https://doi.org/10.1007/978-1-4615-2105-1_4
7. Sikkema J, de Bont JAM, Poolman B (1994) Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 269:8022–8028
8. Koh KJ, Pearce AL, Marshman G, Finlay-Jones JJ, Hart PH (2002) Tea tree oil reduces histamine-induced skin inflammation. Br J Dermatol 147:1212–2121. https://doi.org/10.1046/j.1365-2133.2002.05034.x
9. Burkill HM (1994) The useful plants of West Tropical Africa, 2nd edn. Royal Botanic Gardens, London, pp 99–100
10. Oben JE, Assi SE, Agbor GA, Musoro DF (2006) Effect of *Eremomastax speciosa* on experimental diarrhea. Afr J Tradit Complenet Altern Med 3:95–100. https://doi.org/10.4314/AJTCAM.V3I1.31144
11. Siwe GT, Maharjan R, Amang AP, Mezui C, Zondegoumba EN, Akhtar SS, Choudhary MI, Tan PV (2021) *Eremomastax speciosa* (Hochst.) Cufod. (Acanthaceae) leaves aqueous extract eradicates *Helicobacter pylori* infection in mice. J Pharm Pharmacogn Res. 8(2):135–145. https://doi.org/10.1016/j.jep.2021.114374
12. Siwe TG, Zondegoumba EN, Maharjan R, Amang AP, Mezui C, Choudhary MI, Tan PV (2019) Comparative GC-MS analysis of two crude extracts from *Eremomastax speciosa* (Acanthaceae) leaves. J Med Plant Stud 7(2):25–29. https://doi.org/10.1016/j.jep.2021.114374
13. Tagne RS, Telefo PB, Njina SN, Bala B, Goka SMC, Yemele DM, Lienou LL, Mbemya GT, Donfack NJ, Kamdje AHN, Moundipa AF (2014) In vivo anti-androgenic, anti-estrogenic and antioxidant activities of the aqueous extract of *Eremomastax speciosa*. Asian Pac J Trop Dis 4:952–956. https://doi.org/10.1016/S2222-1808(14)60765-9
14. Siwe TG, Enow-Orock GE, Amang AP, Mezui C, Dongmo AB, Tan PV (2015) Acute and subacute toxicological assessment of the leaf aqueous extract

of *Eremomastax speciosa* (Acanthaceae) in Wistar rats. J Adv Med Pharm Sci 4(1):1–13. https://doi.org/10.9734/JAMPS/2015/18361
15. Mbemya GT, Goka MSC, Lienou LL, Sylvain NN, Donfack JN, Guerreiro DD, Njimou JR, Rodrigues APR, Telefo PB (2020) *Eremomastax speciosa* potentializes the PMSG-inducing effect on some physiological and biochemical parameters in PMSG-primed immature rats. Zygote. https://doi.org/10.1017/S0967199420000350
16. Okokon JE, Antia BS, Udoh AE, Akpan MM (2007) Antianaemic and antimicrobial activity of *Eremomsastax speciosa*. J Pharmacol Toxicol 2(2):196–199. https://doi.org/10.3923/ipt.2007.196.199
17. Tan PV, Nditafon NG, Yewah MP, Dimo T, Ayafor FJ (1996) *Eremomastax speciosa*: effects of leaf aqueous extract on ulcer formation and gastric secretion in rats. J Ethnopharmacol 54:139–142. https://doi.org/10.1016/s0378-8741(96)01461-4
18. Amang AP, Mezui C, Siwe GT, Zondengoumba EN, Enow-Orock GE, Tan PV (2017) Prophylactic and healing activities of the leaves aqueous extract of *Eremomastax speciosa* on gastric ulcers in rats. J Adv Biol Biotech 12(3):1–13. https://doi.org/10.9734/JABB/2017/32652
19. Amang AP, Mezui C, Siwe TG, Emakoua J, Mbah G, Nkwengoua EZ, Enow-orock GE, Tan PV (2017b) Healing and antisecretory effects of aqueous extract of *Eremomastax speciosa* (Acanthaceae) on unhealed gastric ulcers. Biomed Res Int. https://doi.org/10.1155/2017/1924320
20. Amang AP, Tan PV, Nkwengoua E, Nyasse B (2014a) Antisecretory action of the extract of the aerial parts of *Eremomastax speciosa* (Acanthaceae) occurs through antihistaminic and anticholinergic pathways. Adv Pharmacol Sci. https://doi.org/10.1155/2014/323470
21. Amang PA, Tan PV, Patamaken SA, Mefe MN (2014) Cytoprotective and antioxidant effects of the methanol extract of *Eremomastax speciosa* in rats. Afr J Trad Compl Altern Med 11(1):165–171. https://doi.org/10.4314/ajtcam.v11i1.26
22. Siwe GT, Maharjan R, Amang AP, Mezui C, Zondegoumba EN, Akhtar SS, Choudhary MI, Tan PV (2020) *Eremomastax speciosa* (Hochst.) Cufod. (Acanthaceae) leaves aqueous extract eradicates *Helicobacter pylori* infection in mice. J 595 Pharm Pharmacogn Res 8(2):135–145. https://doi.org/10.1016/j.jep.2021.114374
23. Siwe GT, Maharjan R, Amang AP, Mezui C, Zondegoumba EN, Akhtar SS, Choudhary MI, Tan PV (2021) *Eremomastax speciosa* (Hochst.) Cufod. counteracts the delaying 2 effect of indomethacin on Helicobacter pylori-associated chronic 3 gastric ulcers healing. https://www.researchgate.net/publication/352771181
24. Ndem JI, Otitoju O, Akpaniabiatu MI, Uboh FE, Uwah AF, Edet OA (2013) Haematoprotective property of *Eremomastax Speciosa* (Hochst.) on experimentally induced anaemic wistar rats. Ann Biol Res 4(6):356–360
25. MeSH (Medical Subject Headings) is the U.S. National Library of Medicine's controlled vocabulary thesaurus used for indexing articles for PubMed. URL: https://www.ncbi.nlm.nih.gov/mesh/68011280
26. Dehpour AA, Yousefian M, Jafary Kelarijani SA, Koshmoo M, Mirzanegad S, Mahdavi V, Mousavi SE, Shirzad E, Afzali M, Javad Bayani MJ, Olyaei juybari E, Yahyapor MK, (2012) Antibacterial activity and composition of essential oils of flower *Allium rotundum*. Adv Environ Biol 6(3):1020–1025
27. Parchin RA, Ebadollahi A (2016) Biological activities of *Hypericum perforatum* L. essential oil against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J Entomol 13(3):91–97. https://doi.org/10.3923/je.2016.91.97
28. Hickey SM, Ashton TD, White JM, Li J, Nation RL, Yu HY, Elliott AG, Butler MS, Huang JX, Cooper MA, Pfeffer FM (2015) Synthesis of norbornane bisether antibiotics via silver mediated alkylation. RSC Adv 5:28582–28596. https://doi.org/10.1039/C5RA03321G
29. Ramos RS, Rodrigues BL, Farias ALF, Simoes RC, Pinheiro MT, Ferreira RMA, Barbosa MC, Souto RNP, Fernandes JB, Santoes LS, Amelda SSM (2007) Chemical composition and in vitro antioxidant, cytotoxic, antimicrobial, and larvicidal activities of the essential oil of *Mentha piperita* L. (Lamiaceae). Sci World J. https://doi.org/10.1155/2017/4927214
30. Odeja O, Ibok MG, Okpala E, Akpaeva U (2020) Chemical composition, antimicrobial and antioxidant activities of root essential oil of Nigerian specie of *Asparagus flagellaris* (Kunth) Baker. Int J Innov Res Dev 9(5):1–6
31. Odeja O, Ibok MG, Okpala E (2021) Composition and biological assays of the leaf essential oil of *Asparagus flagellaris*. Clin Phytosci 7(12):1–8. https://doi.org/10.1186/s40816-020-00245-1
32. Wurzenberger M, Grosch W (1984) The formation of 1-octen-3-ol from the 10-hydroperoxide isomer of linoleic acid by a hydroperoxide lyase in mushrooms *Psalliota bispora*. Biochim Biophys Acta. https://doi.org/10.1016/0005-2760(84)90293-5
33. Xiong C, Li Q, Li S, Chen C, Chen Z, Huang W (2007) In vitro antimicrobial activities and mechanism of 1-Octen-3-ol against food-related bacteria and pathogenic fungi. J Oleo Sci. https://doi.org/10.5650/jos.ess16196
34. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free-radical method to evaluate antioxidant activity. Food Sci Technol 28:25–30
35. Sanchez-Moreno C, Larrauri JA, Saura-Calixto F (1998) A Procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agri 79:270–276
36. Olaoluwa OO, James DA, Adigun OA (2018) Volatile oil analysis of aerial parts of *Boerhavia coccinea* (Mill.). Nat Prod Res 32(8):959–962
37. Stobiecka A (2015) Comparative study on the free radical scavenging mechanism exerted by geraniol and geranylacetone using the combined experimental and theoretical approach: anti-radical activity of geraniol and geranylacetone. Flavour Fragr J 30(5):1–11. https://doi.org/10.1002/ffj.3256
38. Adams R. (2017) Identification of essential oil components by gas chromatography/mass spectrometry., Carol Stream, IL. 5th edn. (Texensis Publishing Gruver, TX UDA,), ISBN 978-0-9981557-2-2; 2017: 150–200
39. British Standards Institute. BS EN ISO 7937: 2004. Microbiology of Food and Animal feeding Stuffs – Horizontal Method for the Enumeration of Clostridium perfringens – Colony-coun Technique; http://img.21food.cn/img/biaozhun/20100108/181/11285427.pdf. Accessed 05 June 2022
40. Rosa OPS, Torres SA, Ferreira CM, Ferreira FBA (2002) In vitro effect of intracanal medicaments on strict anaerobes by means of the broth dilution method. Pesqui Odontol Bras 16:31–36. https://doi.org/10.1590/s1517-74912002000100006
41. Clinical and Laboratory Standards Institute. Methods for Dilution AntimicrobialSusceptibility Tests for Bacteria that grow aerobically; Approved Standard. Seventh ed. Wayne: CLSI; 2006.http://isoforlab.com/phocadownload/csli/M7-A7.pdf. Accessed 05 June 2022
42. Radaellia M, da Silvaa BP, Weidlicha L, Hoehneb L, Flachc A, Mendonc LA, da Costac A, Ethur EM (2016) Antimicrobial activities of six essential oils commonly used as condiments in Brazil against *Clostridium perfringens*. Braz J Microbiol 47:424–430. https://doi.org/10.1016/j.bjm.2015.10.001
43. Eloff JN (1998) A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med 64:711–713. https://doi.org/10.1055/s-2006-957563

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com