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Antimicrobial and antioxidant Activity of *Leonotis nepetifolia* L. (Lamiaceae) Grown in Sudan

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Abstract
The genus *Leonotis* has 12 species widely distributed in tropics. *Leonotis nepetifolia* is economically important and has many medicinal uses. It is used against kidney diseases, rheumatism, dysmenorrhoea, bronchial asthma, fever and diarrhea. In this study the GC-MS analysis of *Leonotis nepetifolia* oil revealed the presence of 22 components. The major constituents of the oil are: 9-octadecenoic acid (Z)-, methyl ester (35.40%); 6-octadecynoic acid, methyl ester (22.68%); 9, 12-octadecadienoic acid (Z, Z)-, methyl ester (15.59%) and hexadecanoic acid, methyl ester (14.44%). The oil was evaluated for antimicrobial activity via the agar diffusion bioassay against five standard pathogenic microbes. The oil showed significant activity against *Pseudomonas aeruginosa* and partial activity against *Staphylococcus aureus.* In the DPPH assay the oil exhibited moderate free radical scavenging capacity.

Keywords: *leonotis nepetifolia*, oil, gc-ms analysis, antimicrobial, antioxidant activity

1. Introduction
Leonotis nepetifolia L. is a plant in the family Lamiaceae. It grows to a height of 3 meters and has whorls of striking lipped flowers, that are most commonly orange, but can vary to red, white, and purple. *Leonotis nepetifolia* generally grows in patches along roadside or barren unused agriculture waste land during rainy season. The distinct odour is amongst the unique characters of this plant [1, 2]. The genus *Leonotis* has 12 species widely distributed in tropics, It is doubtful whether the herb is indigenous to India [3]. Two varieties of the species are identified: *L.nepetifolia* var.*nepetifolia* (with long orange hairs on corolla) and *L.nepetifolia* var.*africana* [4]. *Leonotis nepetifolia* is economically important and has many medicinal uses. It is used against kidney diseases, rheumatism, dysmenorrhoea, bronchial asthma, fever and diarrhea [5]. The plant is reported as wound healing [6], antibacterial [7], antirheumatic [8], anti-inflammatory [9], analgesic and anticancer [10]. The decoction of the leaves is used to treat coughs, burns and skin ailments. The whole plant is used for menstrual pain. This plant exhibited various pharmacological activities. Phytochemical examination of this plant indicated the presence of alkaloids (leonurine and stachydrene), iridoid glycosides (leonurin and leonuridine), diterpenoids (leocardin), flavonoids (rutin, quercetin, hyperoside, apigenin), volatile oil, tannins and vitamin A [7].

Materials and Methods
Plant material
Leonotis nepetifolia seeds were collected from Alfashir western Sudan. The plant was identified and authenticated by the Medicinal and Aromatic Plants Research Institute, Khartoum, Sudan.

Instruments
A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used for GC-MS analysis**.**

Test organisms
Test organisms used in this study are: *Bacillus subtilis (*G+ve), *Staphylococcus aureus (*G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve) and *Candida albicans* (fungus).

Extraction of oil
Powdered seeds of *Leonotis nepetifolia* (300g) were macerated with n-hexane for 48h.The solvent was removed under reduced pressure giving the oil.

GC-MS analysis
(2ml) of the oil were mixed thoroughly with 7ml of alcoholic sodium hydroxide that was prepared by dissolving 2 g of (Na) in 100 ml methanol. (7 ml) alcoholic sulfuric acid (1ml H_2SO_4 in 100 ml methanol) was added. The mixture was then shaked for 5 minutes. The content of the test tube was left to stand overnight. (1ml) of supersaturated sodium chloride was added and the tube was shaked for 5 min. (2ml) of normal hexane were added and the contents were shaked thoroughly for 5 minutes. (5 µl) of the n-hexane layer were diluted with (5ml) of diethyl ether and dried over anhydrous sodium sulphite. (1µl) of the diluted sample was injected in the GC.MS vial.
The qualitative and quantitative analysis of the sample was carried out by using a Shimadzu machine- model (GC/MS-QP2010-Ultra) The sample was injected under the following chromatographic conditions:column oven temperature

:150.0ºC; injection temperature:300.0ºC; injection mode: split; flow mode: linear velocity; pressure:139KPa; total flow: 50.0ml/min; column flow:1.54ml/sec; linear velocity: 47.2cm/sec; purge flow:3.0 ml/min; split ratio: -1.0. Oven temperature program is presented Table 1.

Table 1: Oven temperature program

Rate Temperature(ºC) Hold Time (min.$^{-1}$)		
-	150.0	1.00
4.00	300.0	0.00

Antimicrobial assay

The paper disc diffusion method was used to screen the antimicrobial activity of the oil and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999) [11] with some minor modifications. Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of test sample. The inoculated plates were incubated at 37 °C for 24 h. The diameters (mm) of the inhibition zones were measured and recorded as average of two replicates.

Results and Discussion

The GC-MS analysis of the studied oil showed 21 constituents (Table 2).The total ion chromatograms is illustrated in Fig.1.

Table 2: Constituents of the oil

No	Name	Ret. Time	Area%	Formula
1	Dodecanoic acid, methyl ester	11.227	0.02	$C_{13}H_{26}O_2$
2	cis-5-Dodecenoic acid, methyl ester	13.264	0.04	$C_{13}H_{24}O_2$
3	Methyl tetradecanoate	13.538	0.14	$C_{15}H_{30}O2$
4	4-Octadecenoic acid, methyl ester	14.347	0.02	$C_{19}H_{36}O_2$
5	Pentadecanoic acid, methyl ester	14.612	0.03	$C_{16}H_{32}O_2$
6	Cyclododecyne	15.341	0.01	$C_{17}H_{32}O_2$
7	7-Hexadecenoic acid, methyl ester, (Z)-	15.399	0.22	$C_{17}H_{32}O_2$
8	9-Hexadecenoic acid, methyl ester, (Z)-	15.444	0.96	$C_{17}H_{32}O_2$
9	Hexadecanoic acid, methyl ester	15.656	14.44	$C_{17}H_{34}O_2$
10	cis-10-Heptadecenoic acid, methyl ester	16.408	0.05	$C_{18}H_{34}O_2$
11	Heptadecanoic acid, methyl ester	16.617	0.09	$C_{18}H_{36}O_2$
12	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.312	15.59	$C_{19}H_{34}O_2$
13	9-Octadecenoic acid (Z)-, methyl ester	17.395	35.40	$C_{19}H_{36}O_2$
14	6-Octadecynoic acid, methyl ester	17.609	22.68	$C_{19}H_{34}O_2$
15	cis-11-Eicosenoic acid, methyl ester	19.087	1.46	$C_{21}H_{40}O_2$
16	Eicosanoic acid, methyl ester	19.305	2.15	$C_21H_{42}O_2$
17	6,9-Octadecadienoic acid, methyl ester	19.441	0.53	$C_{19}H_{34}O_2$
18	Docosanoic acid, methyl ester	20.927	0.69	$C_{21}H_{42}O_2$
19	.gamma.-Sitosterol	21.890	4.40	$C_{29}H_{50}O$
20	Tetracosanoic acid, methyl ester	22.431	0.29	$C_{25}H_{50}O_2$
21	.alpha.-Amyrin	22.704	0.64	$C_{30}H_{50}O$

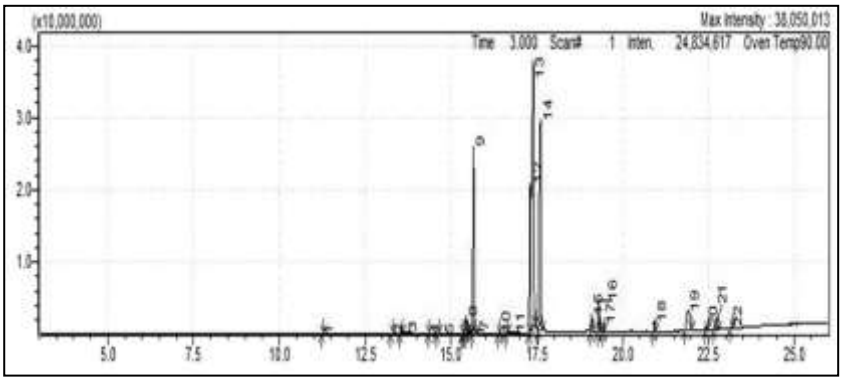

Fig 1: Total ion chromatograms

The mass spectra of the major constituents are discussed below.

The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester (35.40%) is displayed in Fig. 2. The molecular ion M^+ $[C_{19}H_{36}O_2]^+$ appeared at m/z 296 (R.T. 17.395).

Mass spectrum of 6-octadecynoic acid, methyl ester (22.68%) is shown in Fig.3. The peak at m/z 294(RT.17.609) represents M^+ $[C_{19}H_{34}O_2]^+$.

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester (15.59%) is illustrated in Fig. 4. The molecular ion M^+ $[C_{19}H_{34}O_2]^+$ appeared at m/z 294 (*RT*. 17.312)

The mass spectrum of hexadecanoic acid, methyl ester (14.44%) is shown in Fig.5. The molecular ion $[C_{17}H_{34}O_2]^+$ appeared at m/z 270(R.T 15.656).

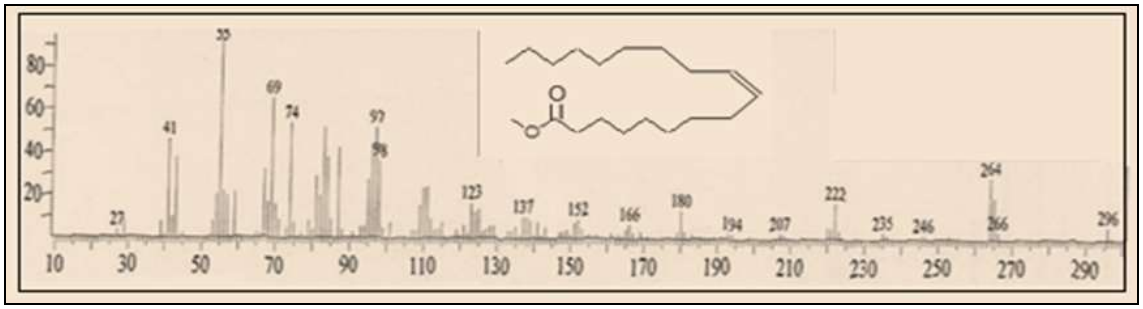

Fig 2: Mass spectrum of 9-octadecenoic acid methyl ester

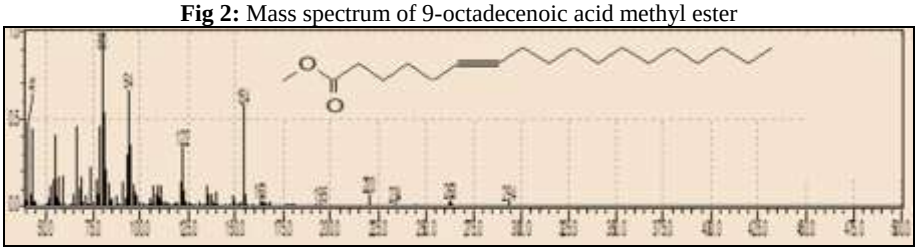

Fig 3: 6-Octadecynoic acid, methyl ester

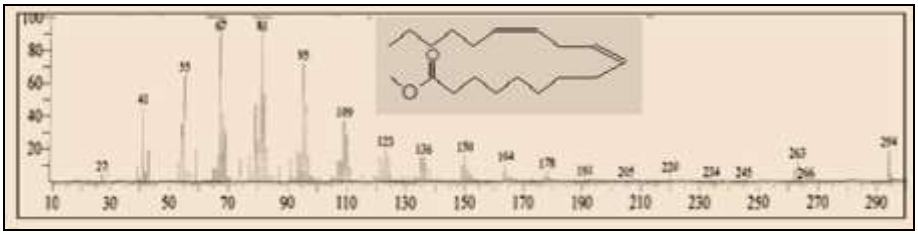

Fig 4: Mass spectrum of 9, 12-octadecanoic acid methyl ester.

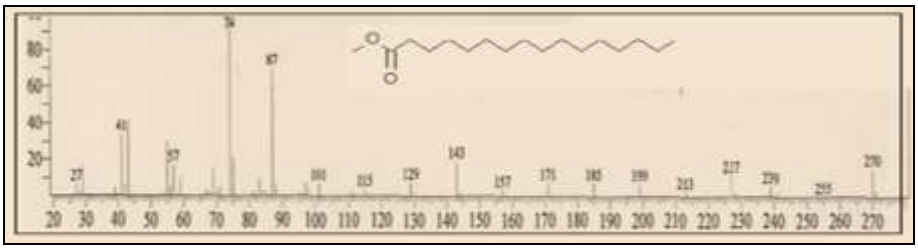

Fig 5: Mass spectrum of hexadecanoic acid methyl ester

Antimicrobial activity

The averages of the diameters of the growth inhibition zones of the studied oil are listed in Table (3).The oil showed significant activity against *Pseudomonas aeroginosa* (G-ve) and partial activity against *Staphylococcus aureus(* G+ve). Ampicilin gentamicin and clotrimazole were used as positive control (Tables 4 and 5).

Antioxidant activity

The antioxidant potential of *Leonotis nepetifolia* oil is depicted in Table 6.The oil showed moderate antioxidant potential.

Table 3: Diameters of inhibition zones (mm)

Sample (100mg/ml)	Ec.	Pa.	Sa.	Bs.	Ca.
Leonotis nepetifolia oil	--	16	12	--	--

Table 4: Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 5: Antifungal activity of clotrimazole: MDIZ (mm)

Drug	Conc. mg/ml	Ca.
Clotrimazole	30	38
	15	31
	7.5	29

Table 6: Free radical scavenging activity of the oil

Sample(100mg/ml)	%RSA± SD(DPPH)
Leonotis nepetifolia oil	34 ± 0.03
Standard(propylgallate)	93. ± 0.01

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