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Constituents and antimicrobial activity of Sudanese *Mentha Viridis* (Lamiaceae) essential oil

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Abstract
Information on the constituent of medicinal plants used in Sudanese ethnomedicine is very scarce. This study was aimed to investigate the chemical constituents of the medicinally important *Mentha viridis* essential oil and to evaluate its antimicrobial activity. 52 components were detected by GC-MS Analysis. Major constituents are: D-carvone (39.87%) and D-limonene (22.36%). The antimicrobial activity of the oil was evaluated via disc diffusion method against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonasa aeruginosa* and the fungal species *Candida albicans*)**.** The oil showed significant activity against *Pseudomonasa aeruginosa* and *Bacillus subtilis* beside moderate activity against *Staphylococcus aureus*. However it was inactive against other test organisms.

Keywords: mentha viridis, volatile oil, GC-MS, antimicrobial activity

Introduction
Mentha is a genus of plants in the family Lamiaceae. About 18 species exist in this genus and the exact distinction between species is still unclear. Many species of the family Lamiaceae have important uses in pharmaceutical industries [1].
Mentha Viridis is a potential medicinal plant widely used in traditional systems of medicine. It is mainly used to cure gastrointestinal disorders [2], stomach ache and cold [3]. The plant is also used traditionally for treating irritable bowel syndrome [3].
Mentha Viridis essential oil is used in aromatherapy [3, 5]. It is also used as an insecticide [6]. Besides being used in many consumer products, Mentha Viridis may cause allergic reactions in some people including diarrhea, headache, heartburn, contact dermatitis [3, 7].

Materials and methods
Plant material
Mentha Viridis was purchased from the local market - Khartoum, Sudan. The plant was authenticated by the Aromatic and Medicinal Plants Research Institute– Khartoum, Sudan.

Test organisms
Mentha Viridis oil was screened for antimicrobial activity using the standard microorganisms shown in Table (1)

Table 1: Test organisms

No	Microorganism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylcococcus aureus*	G+e
3	*Pseudomonas aeroginesa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	fungi

Methods
Extraction of oil
Hydrodistillation was used for extraction of Mentha Viridis volatile oil.

GC-MS analysis
Mentha Viridis volatile oil was analyzed by gas chromatography - mass spectrometry. A Shimadzo GC-MS-QP2010 ultra instrument with a RTY-5MS column (30m, length; 0.25ml diameter; 0.25µm, thickness) was used. Helium (purity; 99.99%) was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are displayed in Table 3.

Table 2: Oven Temperature Program

Rate	Temperature (ºC)	hold time ($min.^{-1}$)
-	50.0	0.00
7.00	180.0	0.00
10.00	300.0	0.00

Table 3: Chromatographic conditions

Column oven temperature	$50.0.^{0}$c
Injection temperature	300.00^{0}c
Injection mode	Split
Flow control mode	Pressure
Pressure	100.0 KPa
Total flow	50.0 ml/min
Column flow	1.69 ml/min
Linear velocity	47.2 cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Antimicrobial assay
Preparation of bacterial suspensions
One ml aliquots of a 24 hour broth culture of the test organism were aseptically distributed onto nutrient agar slopes and incubated at 37o C for 24 hours. The bacterial

Growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 〖10〗^8-〖10〗^9. C.F.U/ml. The suspension was stored in the refrigerator at 4^0C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solutions and 0.02 ml volumes of the appropriate dilution were transferred onto the surface of dried nutrient agar plates. The plated were allowed to stand for two hours at room temperature for the drops to dry, and then incubated at 〖37〗^0C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimented conditions were maintained constant so that suspension with very close viable counts would be obtained.

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 〖25〗^0C for 72h. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing of antimicrobial susceptibility

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of the oil and performed by using Mueller Hinton agar (MHA). Bacterial suspension was diluted with sterile physiological solution to 〖10〗^8 cfu/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.1.6mm in diameter) were placed on the surface of the MHA and soaked with 100mg/ml of a solution of test sample. The inoculated plates were incubated at 〖37〗^0C for 24 hours. The diameters (mm) of the inhabitation zones-average of two replicates - were recorded. The same method was adopted for antifungal activity.

Results and Discussion

GC-MS Analysis

GC-MS analysis of the studied oil showed the presence of 52 components (Table 4) the typical total chromatograms (TIC) is shown in Fig 1.

Table 4: constituents of Mentha Viridis essential oil

No.	Name	Ret. Time	Area%
1.	Furan, 2,5-diethyltetrahydro-	4.190	0.07
2.	.beta.-Pinene	4.580	0.14
3.	.alpha.-Phellandrene	4.700	0.04
4.	.alpha.-Pinene	4.837	1.28
5.	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	5.567	0.99
6.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	5.648	1.85
7.	.beta.-Myrcene	5.857	2.91
8.	3-Octanol	5.966	0.19
9.	.beta.-Ocimene	6.158	0.15
10.	(+)-2-Carene	6.406	0.04
11.	o-Cymene	6.574	0.10
12.	D-Limonene	6.670	22.36
13.	Eucalyptol	6.733	4.08
14.	trans-.beta.-Ocimene	7.014	0.06
15.	.gamma.-Terpinene	7.272	0.10
16.	2-Carene	7.902	0.11
17.	Benzene, 1-methyl-4-(1-methylethenyl)-	7.940	0.07
18.	1,6-Octadien-3-ol, 3,7-dimethyl-	8.121	0.09
19.	3-Octanol, acetate	8.579	0.20
20.	p-Mentha-1(7),8-dien-2-ol	8.931	0.03
21.	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-	8.977	0.08
22.	Spiro[2.4]heptane, 4-methylene-	9.186	0.04
23.	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-	9.571	0.14
24.	Cyclohexanol, 2-(2-hydroxy-2-propyl)-5-methyl-	9.633	0.26
25.	Terpinen-4-ol	9.849	0.56
26.	.alpha.-Terpineol	10.136	0.29
27.	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-	10.217	3.33
28.	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-	10.263	2.61
29.	3-Hexadecyne	10.436	0.35
30.	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-	10.747	1.29
31.	Carveol	11.014	0.73
32.	Cyclohexanone, 5-methyl-2-(1-methylethylidene)-	11.172	0.37
33.	D-Carvone	11.322	39.84
34.	2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2.alpha.,4a.alpha.,8a.alpha.)-	12.192	0.34
35.	Isopulegol acetate	12.638	0.12

36.	trans-Shisool	12.924	3.61
37.	trans-Carveyl acetate	13.618	2.13
38.	(-)-.beta.-Bourbonene	14.148	1.55
39.	Caryophyllene	14.845	1.89
40.	.gamma.-Muurolene	15.011	0.25
41.	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	15.307	0.17
42.	(E)-.beta.-Famesene	15.373	0.52
43.	Humulene	15.500	0.14
44.	.beta.-ylangene	16.010	0.88
45.	Bicyclogermacrene	16.305	0.13
46.	Cyclohexane, 1,2-diethenyl-4-(1-methylethylidene)-, cis-	17.928	0.53
47.	3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde	18.391	1.44
48.	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	18.849	0.20
49.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	19.180	0.42
50.	Andrographolide	19.398	0.18
51.	Longifolene-(V4)	19.535	0.55
52.	2H-Cyclopropa[a]naphthalen-2-one, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a.alpha.,7.alpha.,7a.alpha.,7b.alpha.)	19.862	0.20

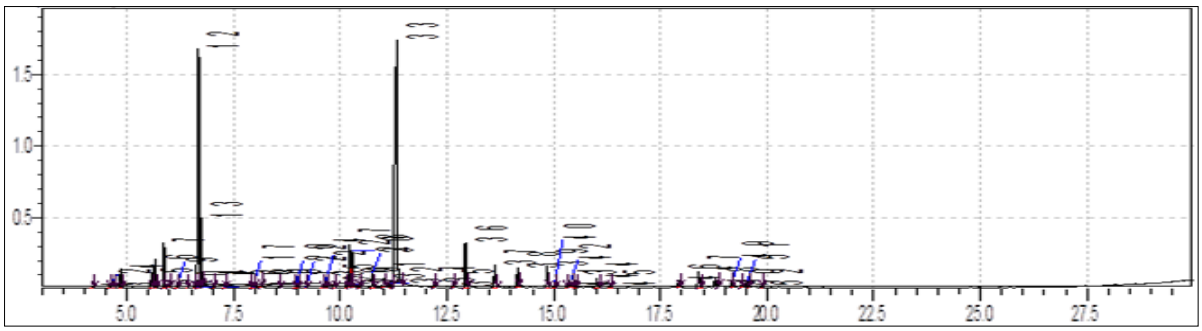
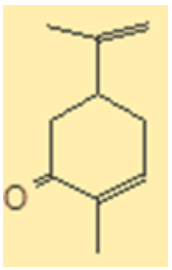


Fig 1: Typical total ion chromatograms (TIC)

Some important constituents are discussed below.
(i) D-Carvone (39.84%)



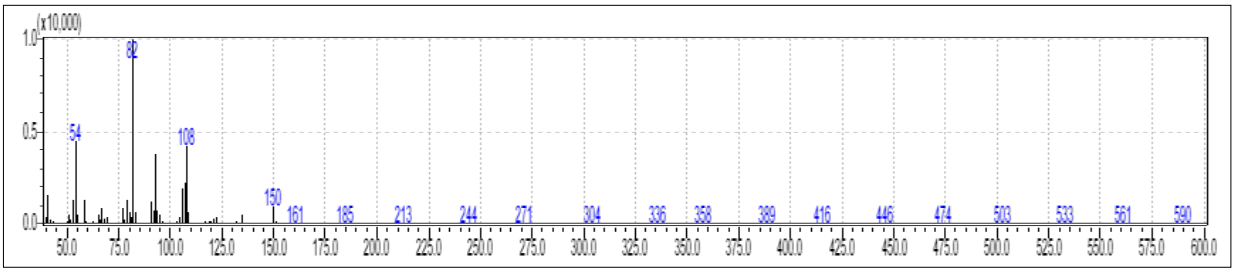


Fig 2: Mass spectrum of D-carvone

The mass spectrum of D-carvone is presented in Fig. 2. The peak at m/z150 (RT.11.322) is due to the molecular ion M+ [C10H14O] +.
Carvone is a terpenoid found naturally in many essential oils. However, it is most abundant in the oils from seeds of Carum carvi and Mentha spicata. Carvone is used in aromatherapy and food industry. It is also used in air fresheners for its pleasant smell. Carvone has several therapeutic effects including the treatment of cough, bronchitis and asthma [8].

D-Limonene (22.36%)

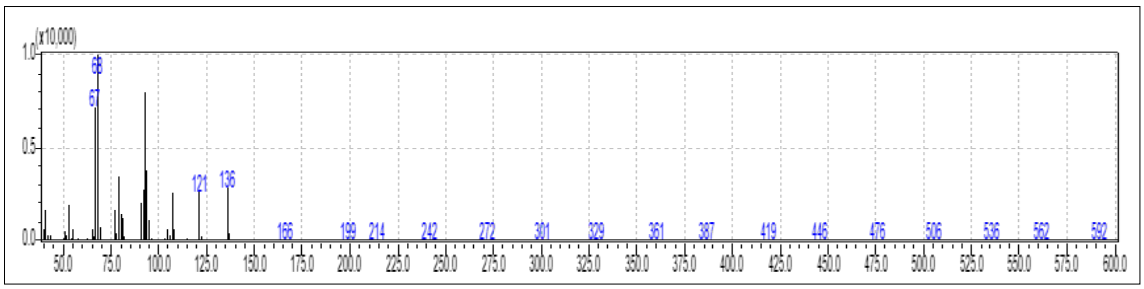


Fig 3: Mass spectrum of D-limonene

In Fig. 3, the signal at m/z136 (RT.6.670) corresponds the molecular ion M+ [C10H16] +. Limonene (1-methyl-4-prop-1-en-2-ylcyclohexene). Is a cyclic monoterpene. The D-limonene is used as a flavoring agent in food manufacturing. It is also used in chemical synthesis as a precursor to carvone. D-limonene is also utilized in pharmeceutical industry [9].

Antimicrobial assay

In disc diffusion bioassay, the studied oil was screened for antimicrobial activity against five standard human pathogens, the average of the diameters of the growth of inhibition zones are depicted in Table (5). The result were interpreted in terms of the commonly used terms (<9mm: inactive, 9-12 mm: partially active; 13-18mm: active ;> 18mm: very active.)

Table 5: antimicrobial activity the oil

Type	Ec	Ps	Sa	Bs	Ca
Oil 100mg/ml	-	26	14	20	-

Ec: *Escherichs coli.*
Ps: *Pseudomonas aeruginosa.*
SA: *Staphylococcus aureus.*
Bs: *Bacillus subtitis.*
Ca: *Candida albicans*.

The oil showed significant activity against Pseudomonasa aeruginosa and Bacillus subtilis and moderate activity against staphylococcus aureus. However, the oil failed to give any anticandidal activity.

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