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Research Article

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Constituents, Antioxidant and Antimicrobial Activity of *Salvia officinalis* Marketed in Sudan

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Abstract *Salvia officinalis* is distributed in southern Asia, central and south America and around the Mediterranean region [3]. The plant has been used traditionally for centuries against sore throat, liver disorders, indigestion and gastroenteritis. *Salvia officinalis* is also used to improve regularity of menstrual cycle and to improve memory. In this study *Salvia officinalis* essential oil was analyzed by GC-MS which revealed 42 constituents. The major constituents of the oil are: eucalyptol (30.36%) and 2-bornanone (13.02%) at a concentration of 100mg/ml the oil showed significant activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and the fungal species *Candida albicans*. It also exhibited significant activity against *Pseudomonas aeruginosa* at a concentration of 50mg/ml. Except for *Staphylococcus aureus* the oil showed a dose-dependent antimicrobial activity. In the DPPH assay, *Salvia officinals* oil showed weak free radical scavenging capacity.

Keywords *Salvia officinalis*, Oil, Constituents, Antimicrobial Activity, Antioxidant activity

Introduction

Salvia officinalis L. belongs to the family Lmaiaceae [1]. The genus Salvia is the largest genus in its family comprising around 1000 species [2]. *Salvia officinalis* is distributed in southern Asia, central and south America and around the Mediterranean region [3]. The plant has been used traditionally for centuries against sore throat, liver disorders, indigestion and gastroenteritis. *Salvia officinalis* is also used to improve regularity of menstrual cycle and to improve memory [4-5]. It has been reported that *Salvia officinalis* possesses antidiabetic [6], antioxidant and gastroprotective properties [7]. The anti-inflammatory [8] and antispasmodic [9] effects have been documented. It has been shown that *Salvia officinalis* possesses fungicidal and bacteridicidal properties [10-12]. The anticancerogenc activity of this herb has been reported [13].

Materials and Methods

Plant Material

Salvia officinals seeds were purchased from the local market- Khartoum(Sudan) and authenticated by direct comparison with a reference herbarium sample.

Instruments

Salvia officinals oil was studied by gas chromatography – mass spectrometry using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m , length ; 0.25mm diameter ; 0.25 μm, thickness).

Microorganism

The antimicrobial assay was performed using the following standard microorganisms: *Bacillus subtilis (*G+ve), *Staphylococcus aureus (*G+ve), *Pseudomonas aeroginosa* (G-ve), *Escherichia coli* (G-ve), *Aspergillus nige*r (fungus) and *Candida albicans* (fungus).

Extraction of oil

Salvia officinals seeds (300g) were exhaustively extracted with n-hexane at room temperature for 72 hr. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further work.

GC-MS analysis

The constituents of *Salvia officinals* oil were investigated by GC-MS .Chromatographic conditions are as follows: column oven temperature: 150.0 ^{o}C; injection temperature: 300.0 ^{o}C; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow: 50.0ml/min; column flow: 1.54ml/sec.; linear velocity: 47.2 cm/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 (^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

The antimicrobial screening was performed by using the cup plate agar diffusion assay .Bacterial culture was maintained in nutrient agar while fungal culture was performed on Sabouraud dextrose agar. Wells (6 mm in diameter) were made in the seeded agar using sterile cork borer (No. 4).Test samples were added into wells of the seeded medium and then incubated for 24 hrs.(at 37^{0}C)-for bacteria- and for 72 hrs at25^{0}C for fungal species. The diameters of inhibition zones were measured as average of two replicates.

Results and Discussion

Salvia officinals oil was analyzed by GC-MS. The analysis showed 42 constituents (Table 6). Total ions chromatograms is displayed in Figure 1. The mass spectra of the major constituents of the oil: eucalyptol (30.36%) and 2-bornanone (13.02%) are shown in figures 2 and 3 respectively. The mass spectrum of eucalyptol gave m/z 154 corresponding to the molecular ion M^{+} $[C_{10}H_{18}O]^{+}$. The mass spectrum of the other major component- 2-bornanone - showed m/z152 which accounts for: M^{+} $[C_{10}H_{16}O]^{+}$.

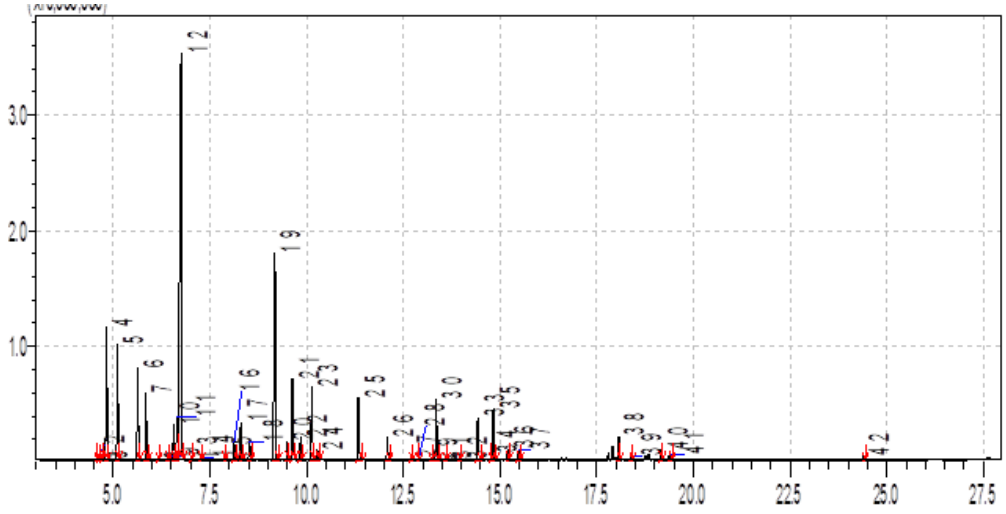
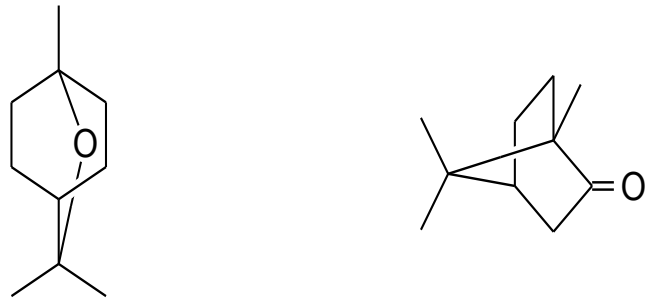


Figure 1: Total ions chromatograms of the oil



Eucalyptol 2-Bornanone

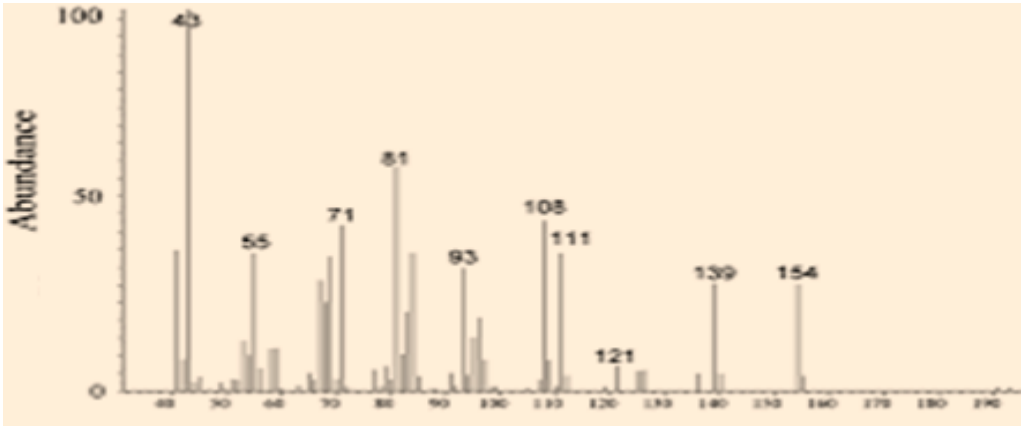


Figure 2: Mass spectrum of eucalyptol

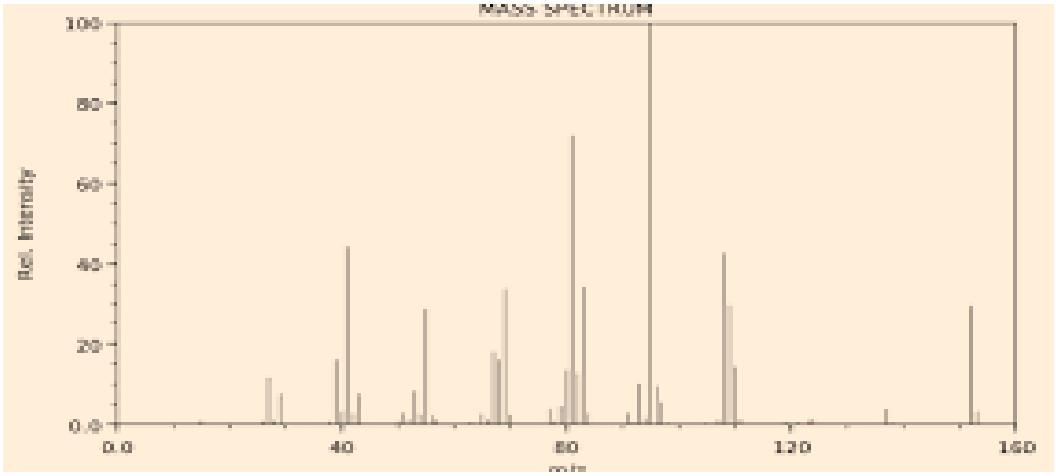


Figure 3: Mass spectrum of 2-bornanone

Antimicrobial Activity

The studied oil was assessed for antimicrobial activity via the cup plate agar diffusion bioassay using 6 standard human pathogens. The average of the diameters of the growth of inhibition zones are shown in Table 2. The results were interpreted in terms of the commonly used terms (<9mm: inative; 9-12mm: partially active; 13-18mm: active ; >18mm: very active). Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

At a concentration of 100mg/ml the oil showed significant activity against *Pseudomonas aeruginosa Bacillus subtilis Staphylococcus aureus* and the fungal species *Candida albicans*. It also exhibited significant activity against

Pseudomonas aeruginosa at a concentration of 50mg/ml. Except for *Staphylococcus aureus* the oil showed a dose-dependent antimicrobial activity.

Table 2: Inhibition diameters (mm) of the oil

Sample	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	Sn
Salvia officinals oil	100	15	30	20	30	26	14
	50	14	20	14	15	16	12
	25	12	15	14	12	15	10
	12.5	--	--	11	--	13	--
	6.15	--	--	--	--	--	00

Table 3: 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	-	-
Gentamicin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4: Antifungal activity of standard chemotherapeutic agent

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillus niger*
- *Bs.: Bacillus subtilis*
- Ca.: *Candida albicans*

Antioxidant activity

In the DPPH assay, *Salvia officinals* oil showed weak free radical scavenging capacity (Table 5).

Table 5: Antioxidant activity of the oil

Sample	%SRA ± SD (DPPH)
Salvia officinals oil	22.9 ± 0.01
Standard (Propyl gallate)	90.6 ± 0.08

Table 6: Constituents of the oil

Peak#	R.Time	Area	Area%	Name
1	4.561	252453	0.07	.beta.-Pinene
2	4.619	922242	0.25	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimeth
3	4.680	236090	0.06	.alpha.-Phellandrene
4	4.820	23115107	6.29	.alpha.-Pinene
5	5.104	20081114	5.47	Camphene
6	5.629	15612766	4.25	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-meth
7	5.840	10340926	2.81	.beta.-Myrcene
8	6.142	318339	0.09	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-e
9	6.389	672783	0.18	(+)-2-Carene
10	6.559	7166739	1.95	p-Cymene
11	6.643	9274186	2.52	D-Limonene
12	6.748	111553677	30.36	Eucalyptol
13	6.998	235374	0.06	.beta.-Ocimene
14	7.256	480828	0.13	.gamma.-Terpinene
15	7.887	263673	0.07	Cyclohexene, 1-methyl-4-(1-methylethylide
16	8.117	3307220	0.90	1,6-Octadien-3-ol, 3,7-dimethyl-
17	8.291	6484754	1.77	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-m
18	8.520	3505022	0.95	Thujone
19	9.175	47831240	13.02	(+)-2-Bornanone
20	9.492	3243164	0.88	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl
21	9.630	16187977	4.41	Isoborneol
22	9.840	4408419	1.20	3-Cyclohexen-1-ol, 4-methyl-1-(1-methyleth
23	10.132	13917371	3.79	.alpha.-Terpineol
24	10.272	2122517	0.58	(-)-Myrtenol
25	11.330	13404054	3.65	2-Cyclohexen-1-one, 2-methyl-5-(1-methyle
26	12.085	4438563	1.21	Bornyl acetate
27	12.699	635510	0.17	3-Cyclohexene-1-methanol, .alpha.,.alpha.,
28	12.879	170956	0.05	Myrtenyl acetate
29	13.207	208931	0.06	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimeth
30	13.348	11370949	3.09	3-Cyclohexene-1-methanol, .alpha.,.alpha.,
31	13.571	382553	0.10	4-Cyclopentene-1,3-dione, 4-(3-methyl-2-bu
32	13.936	434723	0.12	.beta.-Bisabolene
33	14.424	8985960	2.45	Methyleugenol
34	14.763	1220480	0.33	Benzene, 2-(1,1-dimethylethyl)-1,4-dimethe
35	14.824	9796676	2.67	Caryophyllene
36	15.198	2207952	0.60	Alloaromadendrene
37	15.480	2302556	0.63	Humulene
38	18.069	4727236	1.29	Epiglobulol
39	18.371	1043840	0.28	3,5-Dimethylcyclohex-1-ene-4-carboxaldeh
40	19.169	2130999	0.58	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.a
41	19.386	1618783	0.44	Andrographolide
42	24.399	785818	0.21	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-tri
		367400520	100.00	

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