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GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SAUDI *OCIMUM BASILICUM* (LAMIACEAE.) ESSENTIAL OIL

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ABSTRACT

Ocimum basilicum, which has been used for centuries in traditional medicine, is a potential medicinal plant. It is a medium size herb in the family Lamiaceae. The plant contains bioactive constituents like tannins, saponins and cardiac glycosides. *Ocimum basilicum* essential oils has been used traditionally against, nervous disorders and digestive troubles. This study was designed to investigate the constituents of *Ocimum basilicum* essential oil and to screen its antimicrobial potential. *Ocimum basilicum* was investigated by GC-MS analysis. The GC-MS analysis revealed the following major constituents: 9,12-octadecadienoic acid methyl ester(40.77%); 9,12,15-octadecatrienoic acid methyl ester(26.56%); hexadecanoic acid methyl ester(14.74%) and methyl stearate (9.83%). *Ocimum basilicum* oil was investigated for antimicrobial activity against five standard microbial isolates. However, the oil failed to give antimicrobial potential against the following microbial isolates: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Candida albicans*

KEYWORDS: *Ocimum basilicum*, Essential oil, GC-MS analysis.

INTRODUCTION

Recently, the screening of medicinal plants for bioactive molecules and pharmacological activity has become a worldwide active field of research. Plants represent a rich source of phytochemicals that could serve as leads for drug design and drug development.

Ocimum basilicum, which has been used for centuries in traditional medicine, is a herb of many attributes. It is a medium size herb in the family Lamiaceae. The plant contains bioactive constituents like tannins, saponins and cardiac glycosides.[1] *Ocimum basilicum* essential oils has been used traditionally against, nervous disorders and digestive troubles. The plant is claimed to be cardioprotective, stomachic, antipyretic and anthelmintic.[2] The antifungal, antiviral, antinociceptic and larvicidal properties of *Ocimum basilicum* oil has been documented.[3-5] The oil has also been used in ethnomedicine against fever, achne, snake bite, nausea, migraine, abdominal cramps, gonorrhea, inflammation, dysentery, headache, piles, cough, colic pain, paralysis and nervous temperament.[6,7]

The immunomodulatory properties of leave extract has been reported.[8] The ethanol extract and the essential oil of *Ocimum basilicum* exhibited free radical scavenging capacity.[9-11] The in vivo antihyperglycemic and hypolipidemic properties of *Ocimum basilicum* extracts has been reported.[12,13] In the paw edema model, *Ocimum basilicum* seeds showed antiinflammatory activity.[14,15] The in vivo hepatoprotective potential of leave extract has also been demonstrated.[16]

MATERIALS AND METHODS

Plant material

Ocimum basilicum seeds were purchased from the local market, Ryiad Saudi Arabia. The plant was identified and athenticated by direct comparison with reference herbarium sample.

Instruments

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

Methods

Extraction of oil

Powdered seeds of *Ocimum basilicum* (300g) were macerated with n-hexane for 72hr. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

Ocimum basilicum oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument under the following chromatographic conditions:

Table 1: Chromatographic conditions.

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial assay

Microbial strains

The antimicrobial activity was screened using two G+ve strains: *Staphylococcus aureus, Bacillus subtilis;* two G-ve stains: *Escherichia coli, Pseudomonas aeruginosa* and the fungal species: *Candida albicans*.

Inoculum preparation

For bacteria, each of the bacterial strain was cultured in Mueller Hinton agar slants at 35°C. Fungal strain was cultured in Sabouraud dextrose agar at 37°C .The microbial growth was harvested using sterile saline solution (5ml) and diluted to a viable cell count of 10^7CFU/ml.

Antibacterial activity

The disc diffusion assay was used to screen the antibacterial activity. As basal layer, ten ml of Mueller Hinton agar was poured into sterile Petri dishes followed by 15 ml of seeded medium previously inoculated with bacterial suspension. Sterile filter paper discs (6mm) loaded with the test oil (100mg/ml) were placed onto the top of the Mueller Hinton agar plates. The plates were incubated at 35°C for 24h. After incubation inhibition zones were measured as indicator of antibacterial activity.

For antifungal activity, the same procedure was adopted but instead of Mueller Hinton agar Sabouraud dextrose agar was used and incubation continued here for 72h at 37°C.

RESULTS AND DISCUSSION

The oil extracted from *Ocimum basilicum* was investigated by GC-MS analysis. Identification of oil constituents was based on retention times and the observed fragmentation pattern. Fifty six components were detected in total ion chromatogram. The typical total ion chromatogram (TIC) is presented in Fig. (1). The constituents of the oil are outlined in Table 2. The GC-MS analysis revealed the following major constituents.

1. 9,12-Octadecadienoic acid methyl ester(40.77%)
2. 9,12,15-Octadecatrienoic acid methyl ester(26.56%)
3. Hexadecanoic acid methyl ester (14.74%)
4. Methyl stearate (9.83%).

Fig. 1: Total ion chromatograms.

Table 2: Constituents of the oil.

No.	Name	Ret. Time	Area%
1.	.beta.-Pinene	4.162	0.01
2.	o-Cymene	4.772	0.01
3.	D-Limonene	4.829	0.22
4.	Eucalyptol	4.885	0.10
5.	Gamma.-Terpinene	5.244	0.02
6.	Alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	5.451	0.00
7.	3,3,6-Trimethyl-1,4-heptadien-6-ol	5.524	0.00
8.	Spiro[4.5]decane	5.583	0.01
9.	1,6-Octadien-3-ol, 3,7-dimethyl-	5.817	0.04
10.	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	6.593	0.03
11.	Benzene, pentyl-	6.696	0.01
12.	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	6.823	0.00
13.	Alpha.-Terpineol	7.255	0.01
14.	4-Isopropyl-5-methylhexa-2,4-dien-1-ol	7.384	0.01
15.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	7.590	0.01

16.	Acetaldehyde, (3,3-dimethylcyclohexylidene)-, (E)-	7.861	0.08
17.	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	8.058	0.03
18.	2-Cyclohexen-1-one, 5,5-dimethyl-3-(1-methylethyl)-	8.746	0.01
19.	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, acetate	9.486	0.17
20.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	10.122	0.03
21.	Caryophyllene	10.576	0.01
22.	4,5-di-epi-aristolochene	11.242	0.04
23.	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	11.289	0.02
24.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	11.344	0.07
25.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha., 7.alpha.,8a.beta.)]-	11.487	0.83
26.	Ledol	12.044	0.19
27.	Methyl tetradecanoate	14.189	0.09
28.	Pentadecanoic acid, methyl ester	15.320	0.04
29.	7-Hexadecenoic acid, methyl ester, (Z)-	16.156	0.06
30.	9-Hexadecenoic acid, methyl ester, (Z)-	16.201	0.35
31.	Hexadecanoic acid, methyl ester	16.435	14.74
32.	Hexadecanoic acid, 14-methyl-, methyl ester	17.147	0.54
33.	Cis-10-Heptadecenoic acid, methyl ester	17.217	0.09

Table 2: Contd.

34	Heptadecanoic acid, methyl ester	17.432	0.19
35	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.305	40.77
36	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.387	26.56
37	Methyl stearate	18.473	9.83
38	Cis-10-Nonadecenoic acid, methyl ester	18.889	0.11
39	Octadecanoic acid, 17-methyl-, methyl ester	19.112	0.36
40	Nonadecanoic acid, methyl ester	19.362	0.03
41	Gamma.-Linolenic acid, methyl ester	19.933	0.41
42	7-Tetradecenal, (Z)-	19.961	0.31
43	Cis-11-Eicosenoic acid, methyl ester	20.062	0.43
44	Eicosanoic acid, methyl ester	20.265	1.18
45	Methyl 18-methylicosanoate	20.903	0.17
46	Heneicosanoic acid, methyl ester	21.132	0.04
47	Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.291	0.05
48	13-Docosenoic acid, methyl ester, (Z)-	21.790	0.03
49	Docosanoic acid, methyl ester	21.966	0.25
50	Methyl 20-methyl-docosanoate	22.563	0.08
51	Tricosanoic acid, methyl ester	22.771	0.12
52	1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-, (all-E)-	23.322	0.29
53	Tetracosanoic acid, methyl ester	23.543	0.16
54	Gamma.-Sitosterol	23.985	0.55
55	Squalene	24.336	0.14
56	Gamma.-Tocopherol	26.545	0.07

The characterization of the major components of the oil is briefly discussed below.

i-9,12-Octadecadienoic acid methyl ester(40.77%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is depicted in Fig.2. The signal which was observed at m/z294 (R.T. 18.305) is due to M+[$C_{19}H_{34}O_2$]+, while the signal at m/z263 corresponds to loss of a methoxyl.

ii-9,12,15-Octadecatrienoic acid methyl ester(26.56%)

Mass spectrum of 9,12,15-octadecatrienoic acid,methyl ester is depicted in Fig. 3.The peak at m/z292,which appeared at R.T.18.387 corresponds to M+ [C_{19} $H_{44}O_2$]$^+$, while the peak at m\z261 is attributed to loss of methoxyl.

iii-Hexadecanoic acid methyl ester(14.74%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.4. The peak at m/z 270 (R.T. 16.435) accounts for M^+ [$C_{17}H_{34}O_2$] $^+$. The signal at m/z 239 is due to loss of methoxyl.

iv-Methyl stearate (9.83%)

The mass spectrum of methyl stearate is shown in Fig.5. The peak at m/z 298 (R.T.18.473) is due to M^+ $[C_{19}H_{38}O_2]^+$, while the signal at m/z 267 corresponds to loss of a methoxyl.

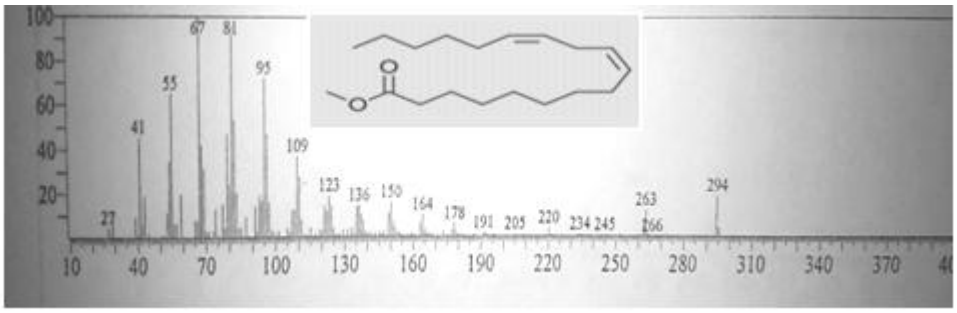

Fig. 2: Mass spectrum of 9,12-octadecadienoic acid (Z,Z)-methyl ester.

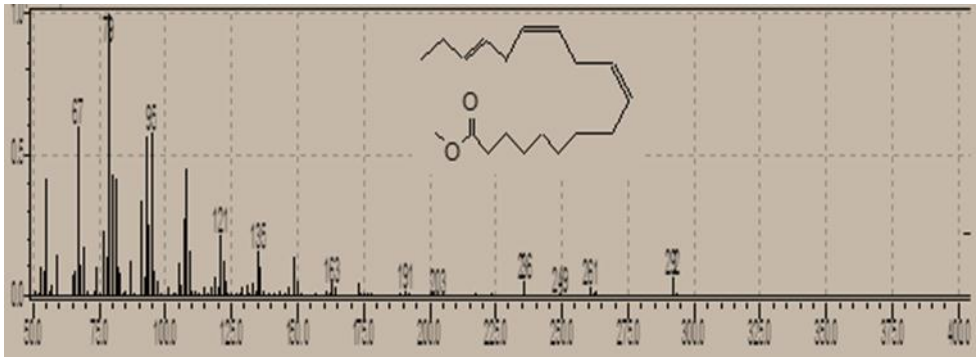

Fig. 3: Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester.

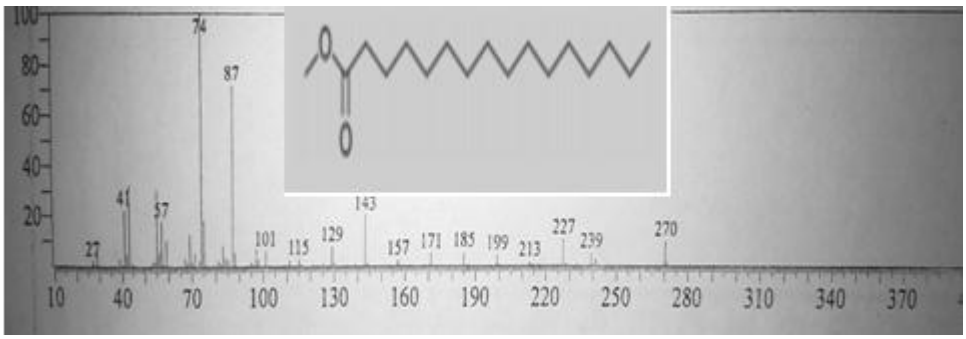

Fig. 4: mass spectrum of hexadecanoic acid, methyl ester.

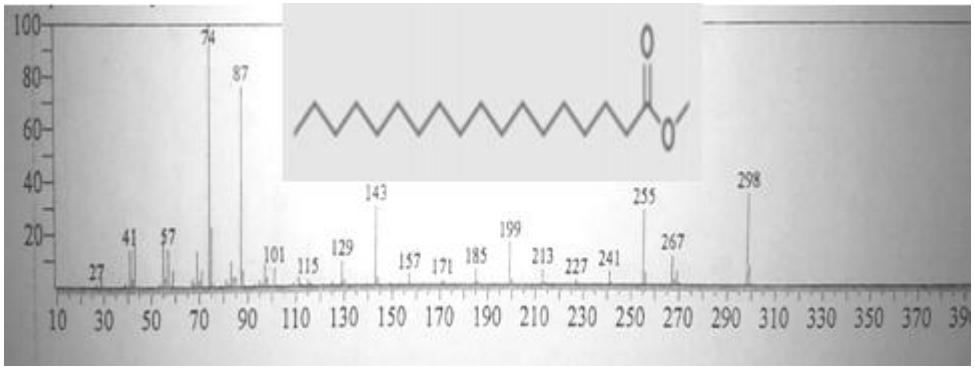

Fig. 5: mass spectrum of methyl stearate.

Antimicrobial assay

Ocimum basilicum oil was investigated for antimicrobial activity against five standard microbial isolates. However, the oil failed to give antimicrobial potential against the following microbial isolates: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans.*

REFERENCES

1. Daniel, V.N.; Daniang, I.E.; Nimyel, N.D. Phytochemical Analysis and Mineral Elements Composition of *Ocimum basilicum* Obtained in Jos Metropolis, Plateau State, Nigeria, *International Journal of Engineering & Technology*, 2011; 11(6): 161-165.
2. Bunrathep, S.; Palanuvej, C.; Ruangrungsi, N. Chemical Compositions and Antioxidative Activities of Essential Oils from Four Ocimum Species Endemic to Thailand, *J. Health Res*, 2007; 3: 201-206.
3. Kashyap, C.P.; Ranjeet, K.; Vikrant, A.; Vipin, K. Therapeutic Potency of *Ocimum Kilimandscharicum Guerke* - A Review, *Global Journal of Pharmacology*, 2011; 5(3): 191-200.
4. Shafique, M.; Khan, J.S.; Khan, H.N., Study of Antioxidant and Antimicrobial Activity of Sweet Basil (*Ocimum basilicum*) Essential Oil, *Pharmacolagyonline*, 2011; 1: 105-111.
5. Hanif, A.M.; Al-Maskari, Y.M.; Al-Maskari, A.; Al-Shukaili, A.; Al-Maskari, Y.A.; Al-Sabahi, N.J., Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil, *Journal of Medicinal Plants Research*, 2011; 5(5): 751-757.
6. Saganuwan, A.S. Some Medicinal Plants of Arabian Pennisula, J. Med. Plants Res, 2010; 4(9): 766-788.
7. Marwat, K.S.; Khan, A.M.; Akbari, H.A.; Shoaib, M.; Shah, A.M., Interpretation and Medicinal Potential of Ar-*Rehan* (*Ocimum basilicum* L)-A *Review*, *American-Eurasian J. Agric. & Environ. Sci*, 2011; 10(4): 478-484.
8. Jeba, C.R.; Vaidyanathan, R.; Rameshkumar, G., Efficacy of *Ocimum basilicum* for Immunomodulatory Activity in Wistar Albino Rats, International *Journal of Pharmacy and Pharmaceutical Sciences*, 2011; 3(4): 199-203.
9. Sarfraz, Z.; Anjum, M.F.; Khan, I.M.; Arshad, S.M.; Nadeem, M. Characterization of Basil (*Ocimum basilicum* L.) parts for antioxidant potential, *African Journal of Food Science and Technology*, 2011; 2(9): 204-213.
10. Sekarl, K.; Thangaraj, S.; Babu, S.S.; Harisaranraj, R.; Suresh, K., Phytochemical Constituent and Antioxidant Activity of Extract from the Leaves of *Ocimum basilicum*, *J. Phytol*, 2009; 1(6): 408-413.
11. Durga, R.K.; Karthikumar, S.; Jegatheesan, K., Isolation of Potential Antibacterial and Antioxidant Compounds from *Acalyphha indica* and *Ocimum basilicum*, *Journal of Medicinal Plants Research*, 2009; 3(10): 703-706.
12. Umar, I.A.; Mohammad, A.; Dawud, F.A.; Kabir, A.M.; Sai, J.V.; Muhammad, F.S.; Okalor, M.E. The hypolipidemic and antioxidant actions of aqueous extracts of *Ocimum basilicum* and *Ocimum suave* in high fat fed Rats, *J. Chem. Bio. Phy. Sci*, 2012; 2(1): 298-301.
13. Zeggevagh, A.N.; Sulpice, T.; Eddouks, M., Anti-hyperglycaemic and Hypolipidemic Effects of *Ocimum basilicum* Aqueous Extract in Diabetic Rats, *American Journal of Pharmacology and Toxicology*, 2007; 2(3): 123-129.
14. Rakha, P.; Sharma, S.; Parle, M., Anti - inflammatory potential of the seeds of *Ocimum basilicum* Linn. in rats, *Asian Journal of Bio Science*, 2010; 5(1): 16-18.
15. Selvakkumar, C.; Gayathri, B.; Vinaykumar, S.K.; Lakshmi, S.; Balakrishnan, A., Potential Anti-inflammatory Properties of Crude Alcoholic Extract of *Ocimum basilicum* L. in Human Peripheral Blood Mononuclear Cells, *Journal of Health Science*, 2007; 53(4): 500-505.
16. Meera, R.; Devi, P.; Kameswari, B.; Mahumita, B.; Merlin, J.N., Antioxidant and hepatoprotective activities of *Ocimum basilicum* Linn. and *Trigonella foenum-graecum* Linn. Against H2O2 and CCl4 induced hepatoyoxicity in goat liver, *Indian Journal of Experimental Biology*, 2009; 47: 584-590.