ISSN 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OF EGYPTIAN *OCIMUM BASILICUM* L.

Mohamed H. M. Abd El-Azim[1*], Ahmed A.M. Abdelgawad[2,3], MohamedEl-Gerby[1], Sherin Ali[1] and Amani M. D. El-Mesallamy[1].

1. Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.
2. Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, 11753, Egypt
3. Department of Chemistry, Faculty of Science, Jazan University, Jizan, 2097, Saudi Arabia

Abstract:

This study aimed to determine the chemical composition and antimicrobial activity of the volatile oil of Egyptian Ocimum Basilicum L. Forty two volatile compounds were identified by using gas chromatography-mass spectroscopy (GC-MS). The abundant chemical Constituents were Linalool (33.9%), Eugenol (8.31%) and 2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene (8.04%). Antimicrobial activity were studied at two different concentrations of oil extract (100 µL) and (200 µL) against three bacterial strains Escherichia coli, Staphylococcus aureous and Bacillus cereus, and three fungal strains, Aspergillus niger, Candida albicans and Saccharomyces cerevisial. The volatile oil showed good inhibitory effect for all bacterial strains especially E. coli and weak activity against fungal species.

Keywords: *Ocimum Basilicum, Volatile oil, Chemical composition, GC-MS, Antimicrobial activity.*

Corresponding Author:

Mohamed H. M. Abd El-Azim,
Department of Chemistry,
Faculty of Science, Zagazig University,
Egypt.
Email: mhmsm01213@yahoo.com

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Please cite this article in press as ***Mohamed H. M. Abd El-Azim*** *et al.* ***Chemical Composition and Antimicrobial Activity of Essential Oil of Egyptian Ocimum Basilicum L.,*** *Indo American J of Pharm Sci, 2015:2(4):837-842.*

INTRODUCTION:

Ocimum Basilicum L. (basil) is an annual, herbaceous, white to purple flowering plant, 20–60 cm tall, that originated in Iran and India [1-2]. The taxonomy of *Ocimum* is complex due to interspecific hybridization and polyploidy of the species in the genus [3], recognized more than 150 species; however, Paton [4] proposed that *Ocimum* had only 65 species and other attributions should be considered as synonyms. Among the species of the genus, *Ocimum Basilicum* L. (basil) is the major essential oil crop around the world and cultured commercially in many countries. Basil (*Ocimum Basilicum* L.) is aromatic herb that is used extensively to add a distinctive aroma and flavor to food. The leaves can be used fresh or dried for use as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in food, pharmaceuticals, and cosmetics [5]. Indian basil (*Ocimum Basilicum*), is an economical essential oil producing industrial crop used in flavor and fragrance industries [6]. The essential oil had a significant toxic effect against late third-stage larvae of *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus* with LC_{50} values of 14.01, 11.97 and 9.75 ppm and LC_{90} values of 23.44, 21.17 and 18.56 ppm, respectively [7]. Sweet basil (*Ocimum Basilicum* L.), one of the most popular aromatic plants, shows great variation in both morphology and essential oil components. The composition of 18 Turkish basil essential oils by gas chromatography (GC) and gas chromatography mass spectrometry (GC-MS) were studied [8].

The essential oil of basil (*Ocimum Basilicum* L.) investigated, exhibited good antioxidant activity as measurements by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging ability, bleaching *β*-carotene in linoleic acid system and inhibition of linoleic acid oxidation [9].

The comparison of chemical composition of volatile aglycones with the chemical composition of essential oil reveals four common compounds: eugenol, chavicol, linalool and α-terpineol [10]. Six samples of essential oils from four *Ocimum* species (*O. Basilicum, O. Kilim and Scharicum, O. Lamiifolium,* and *O. Suave*) were analyzed by GC and GC-MS. The major compounds were either phenyl propane derivatives or terpenoids, including methyl eugenol, 1,8-cineole, camphor, bornyl acetate, germacrene-D, E-myroxide, germacrene-B, caryophylene oxide and *p*-cymene [11-12]. In continuation of our studies, [13-15] we report herethe chemical composition and antimicrobial activity of the volatile oil of Egyptian *Ocimum Basilicum* L., from the family *Lamiaceae*.

MATERIALS AND METHODS:

Plant Material:

The plant was collected from Aswan city (south Egypt) in 2012 and dried in closed room in shadowthen was identified by Botany Department, Faculty of Science, Zagazig University (Zagazig, Egypt).

Chemicals:

Diethyl ether, dimethyl sulfoxide (DMF) and anhydrous sodium sulfate were supplied from El-Gomheria Company for chemicals (Egypt).

Microbial Strains:

The bacterial and fungal strains were obtained from the microbiology Laboratory, micro analytical center, Faculty of Science, Cairo University. The tested bacterial strains were *Escherichia coli* (KQ 103), *Staphylococcus aureus* (LC 405) and *Basillus cereus* (Laboratory collection strains), and fungal strains were *Saccharomyces Cerevisial, Aspergillus niger* and *Candida albicans* (Laboratory collection strains).

Isolation of the Volatile Oils:

The dried plant about 0.5 kg was subjected to steam distillation for three hours using the method described by Marcus and Lichtenstein and Weaver *et al.*, [16-17]. The volatile oils were obtained by diethyl ether from aqueous solution and dried over anhydrous sodium sulfate and stored under N_2 atmosphere in amber vials at 4ºC until they were analyzed.

GC-MS analysis:

For qualification, the essential oil was analyzed on Gas Chromatography Mass Spectrometry HP 6890 Series A (Agilent) by using A Thermo Scientific (TR-5MS), (5% Phenyl Polysil Phenylene Siloxane) capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness). Helium (He), having a flow rate of 1.00 ml/min, was used as carrier gas. The GC oven temperature was kept at 50ºC for 5 minutes and programmed to 250ºC. The injector temperature was 250°C. The amount of injection was 1µL.

Retention indices for all compounds were determined according to the Van Den Dool method [18]**.** While, Identification of the components was based on comparison of their mass spectra with those of internal (computer) library, Wiley7n.1 and PMW_Tox3.1 libraries and some reference compounds.

Antibacterial Activity:

The susceptibility test of the crude extract of the plant was determined using the agar disc diffusion method as described by Penduka *et al*., and Jayanthi and

Lalitha [19-20], with some modifications. A 6 mm sterile Whatt-mann filter paper was impregnated 3 times with 100 µL and 200 µL of the bio-active extracts dissolved in Dimethylsulfoxide (DMSO) and then dried up. One ml of 24 hours old bacterial suspension was mixed with 30 ml of 45 - 50ºC, sterile melted Nutrient agar and poured plate in sterile Petri dish. The plate was allowed tosolidify and the filter paper containing the extract was placed in the center of the plate. Inhibition zone around the paper disk was measured after incubation for 18-24 hours at 37ºC.

Antifungal activity:

Antifungal activity of the crude extract was determined usingagar well diffusion method as described by Jin *et al*., [21]. In brief 100µL and 200 µL of the plant extract were transferred to two pores in Czapek's agar medium previously seeded with the tested fungi. Inhibition zones diameter were measured after 2-4 days of incubation at 28ºC.

RESULTS:

Chemical Composition of the Volatile Oil:

Forty two compounds were characterized and identified by using GC-MS for the oil of *Ocimum Basilicum* L. Cultivated in Egypt. The most predominant compounds were Linalool (33.9%), Eugenol (8.31%) and 2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo [3.1.1]hept-2-ene (8.04%) of the oil constituents (Table 1 represents the chemical composition of the oil).

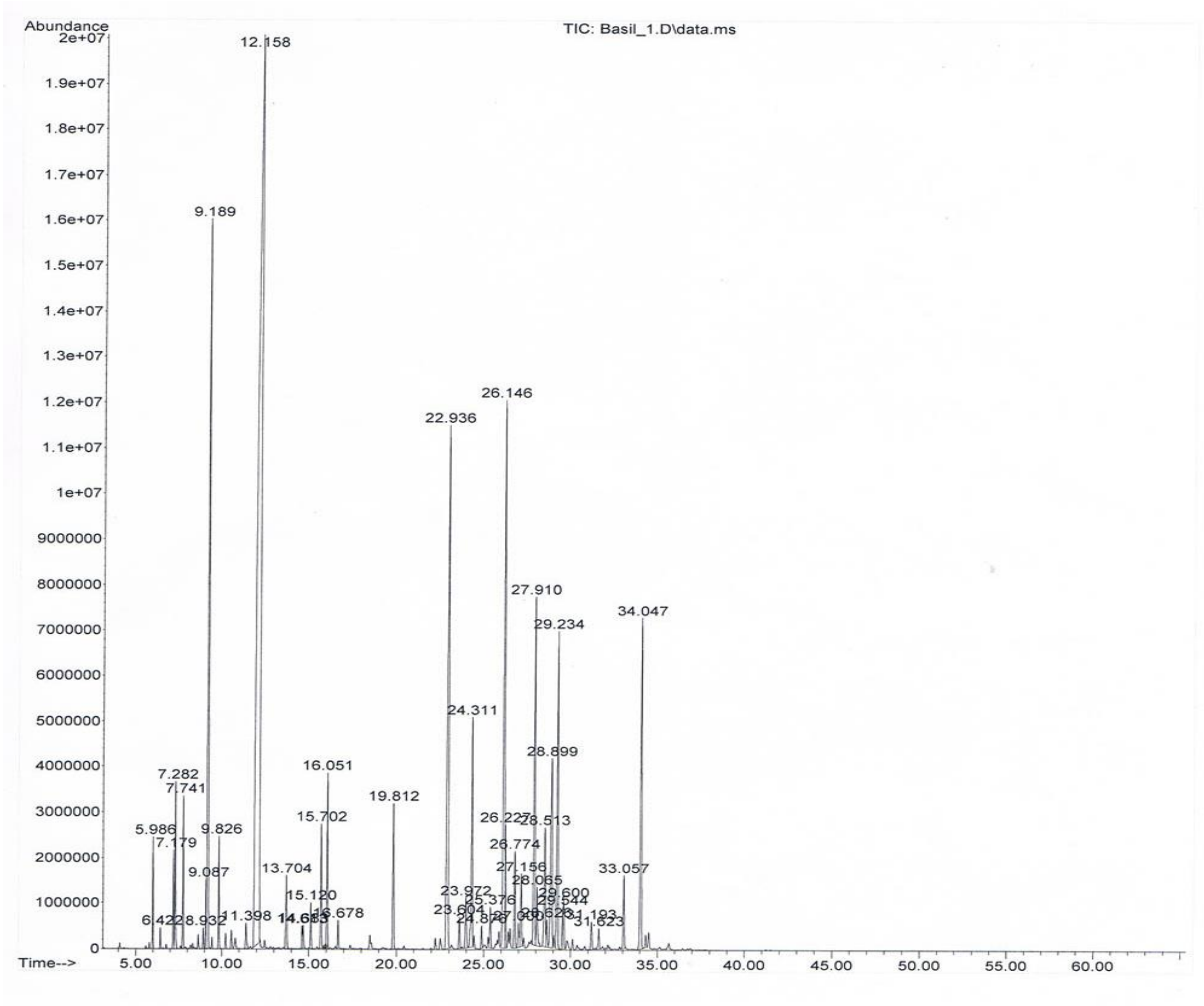

Fig 1: Gas Chromatography-Mass Spectroscopy (GC-MS) of the Volatile Oil of *Ocimum Basilicum* L.

Table 1: The Volatile Constituents Identified From the Volatile Oil of *O. Basilium* by Using GC-MS

No	Compound name	Retention time in min.	Peak area %
1	α-Pinene	5.98	0.75
2	Camphene	6.42	0.45
3	Sabinene	7.17	0.71
4	β-Pinene	7.28	1.33
5	β-Myrcene	7.73	1.16
6	O-Cymene	8.92	0.17
7	dl-Limonene	9.08	0.54
8	1.8-Cineole	9.18	8.4
9	β-Ocimene	8.82	0.92
10	α-Terpinolene	11.39	0.29
11	Linalool	12.15	33.9
12	2-Bornanone	13.7	0.86
13	Borenol	14.61	0.21
14	3-Cyclohexene-1-methanol	14.68	0.24
15	Terpinen-4-ol	15.12	0.44
16	β-Fenchyl alcohol or α-Terpineol	15.7	1.25
17	Chavicol	16.05	1.71
18	Caprylyl acetate	16.67	0.26
19	Bornyl acetate	19.81	1.57
20	Eugenol	22.93	8.31
21	α-Copaene	33.60	0.34
22	β-Bourbonene	23.97	0.66
23	β-Elemene	24.31	2.79
24	Methyl Eugenol	24.87	0.22
25	Trans-Caryophyllene	25.37	0.47
26	2,6-Dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene	26.14	8.04
27	α-Guaiene	26.22	1.11
28	α-Humulene	26.77	1.09
29	Cis-β-Farnesene	26.99	0.34
30	Germacene D	27.15	0.86
31	β-Cubebene	27.90	4.33
32	Trans-β-Farnesene	28.06	0.79
33	Bicyclo germacene	28.51	1.54
34	α-Gurjuene	28.62	0.82
35	α-Bulnesene	28.89	3.31
36	α-Amorphene	29.23	3.95
37	Cis-Calamenene	29.54	0.41
38	δ-Cadinene	29.59	0.57
39	Aromadendrene	31.19	0.37
40	Spathulenol	31.62	0.25
41	α-Cubebene or Cadina-1,4-diene	33.05	0.84
42	α-7-H-Eudesma-3,5-diene	34.04	4.24

Table2: Antibacterial Activity of Volatile Oil of *O. Basilicum* Using 2 Different Concentrations.

Bacterial Species	Inhibition zone diameter in mm for 100µl and 200µl	
	For 100µl concentrations	For 200µl concentrations
Escherichia coli	34	54
Staphyllococcus aureus	24	44
Bacillus cereus	24	44

Table 3: Antifungal Activity of Volatile Oil of *O. Basilicum* Using 2 Different Concentrations

Fungal Species	Inhibition zone diameter in mm for 100µl and 200µl	
	For 100µl concentrations	For 200µl concentrations
Saccharomyces cerevisial	4	6
Aspergillus niger	2	3
Candida albicans	3	4

Antimicrobial Activity:

The results of antimicrobial activity of the volatile oil of the *Ocimum basilicum* L. Cultivated in Egypt against the tested strains were shown in table 2 and 3. High activity was noted against *E. coli and Saccharomyces cerevisial*

DISCUSSION:

Basil is used as a medicinal herb in medical treatments of many conditions such as for headache, cough, diarrhea, parasitic worms, and kidney malfunctions. Basil essential oil has been utilized extensively in the food industry as a flavoring agent, and in perfumery and medical industries [22]. The predominant components of the Egyptian *Ocimum Basilicum* were Linalool and Eugenol; this finding is in agreement with the findings of Duman *et al*., and Stefan *et al*., [23-24], who stated that Linalool and Eugenol were the common chemical composition of the Basil, although the compositions were varies in their order and concentration, this variation might be due toseasonal changes [9], variation in the site and environment of cultivation and collection, or variation in the extraction methods.

The oil of the *Ocimum Basilicum* has a good antimicrobial activity against all tested organisms with noted high activity against *Escherichia coli*, and was attributed mainly to its eugenol and linalool content. Eugenol has stronger antibacterial activity than linalool according to Bassolé *et al*., [25-26].

CONCLUSION:

Forty two compounds were characterized and identified by using GC/MS from the oil of *Ocimum Basilicum* L. cultivated in Egypt. Also it has antimicrobial activities toward the tested microorganism with large zone against bacterial strains especially *E. coli* and small zone against fungi.

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