CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1161339

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

ANTIBACTERIAL ACTIVITY AND COMPOSITION OF ESSENTIAL OILS EXTRACTED FROM SOME PLANTS BELONGING TO FAMILY LAMIACEAE AGAINST SOME MULTIDRUG RESISTANT GRAM NEGATIVE BACTERIA

Fatma A. Ahmed[1], Nadia Hafez Salah El-Din Ouda[2], Sherif Moussa Husseiny[3] and Abeer Adel[1]

[1]Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt.
[2]Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University.
[3]Botany Department, Faculty of Women for Art, Science and Education, Ain Shams University.

Abstract:

The aim of this study was to evaluate the antibacterial activity of eight essential oils against some multi-drug resistant Gram negative bacteria (three different isolates of each Acinetobacter baumannii and Klebsiella pneumoniae). The hydrodistilled essential oils of the fresh aerial part of some medicinal plants belonging to family Lamiaceae namely: Origanum majorana L. , Origanum majorana L. , Origanum syriacum L., Thymus capitatus L., Thymus vulgaris L., Salvia fruticosa Mill., Mentha virdis L. and Lavandula officinalis L. were subjected to Gas Chromatography–Mass spectrometry analysis. This revealed 25, 22, 22, 23, 23, 27, 19 and 31 compounds had been identified in the essential oils, respectively. The major components were identified as terpinen-4-ol (21.99%), terpinen-4-ol (23.56%), cymene (27.98%), carvacrol (41.55%), m-thymol (23.97%), eucalyptol (54.84%), carvone (47.79%) and eucalyptol (36.42%), respectively. Antibacterial activity was carried out and the activities were correlated to chemical composition of analyzed essential oils. We found five essential oils were the most effective. The study concluded that, some essential oils of Egyptian medicinal plants belonging to family Lamiaceae can be used to treat infections caused by multi-drug resistant gram negative bacteria.

Key words: *Lamiaceae, Essential oil, Hydrodistillation, Gas Chromatography–Mass spectrometry, multi-drug resistant gram negative bacteria, Antibacterial activity.*

Corresponding author:

Abeer Adel,
Medicinal and Aromatic Plants Department,
Desert Research Center,
Cairo, Egypt.

QR code

Please cite this article in press as Abeer Adel et al., ***Antibacterial Activity and Composition of Essential Oils Extracted From Some Plants Belonging To Family Lamiaceae against Some Multidrug Resistant Gram Negative Bacteria****, Indo Am. J. P. Sci, 2018; 05(01).*

1. INTRODUCTION:

Antimicrobial resistance is a major challenge for current and future medical practice [1]**.** The World Health Organization (WHO) recently reported alarmingly high rates of bacterial resistance across all WHO regions, this resistance is seen in both community and hospital-acquired infections [2]**.** This resistance leads to the emergence of multi-drug resistant gram-negative bacteria (MDRGNB) phenomenon, that is globally widespread and present a major challenge to modern medical practice [3]. The plant family Lamiaceae (Labiatae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide. Many biologically active essential oils (EOs) have been isolated from various members of this family. Lamiaceae species have many medicinal uses as in treatment of many ailments related to the digestive system, especially flatulence and dyspepsia and also for the treatment of some problems such as reconstituent and infection [4]. *Origanum majorana* L. (Sweet Marjoram) essential oil has an antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*, and an antifungal effect against *Aspergillus flavus* and *Aspergillus parasiticus* [5]. Moreover, its EO could act as insecticide [6, 7]**.** *Origanum syriacum* L. has drawn attention for its antioxidant activity and acetylcholinestrase (Alzehimer's disease); antifungal activity (*Alternaria solani*, *Aspergillus niger*, *Cladosporium* spp., *Fusarium oxysporum* and *Pencillium* spp.); antibacterial activity; analgesic activity; antiflogistic activity; antirheumatic; expectorant; sedative; antiparasitic and antihelminthic activities [8]. *Thymus capitatus* L. EOs are important for perfumes, cosmetics, flavoring and pharmaceutical industries. They are used in folk medicine against cold, influenza and throat infection in Turkey [9]. Moreover, they show strong antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Enterococcus faecalis*, and also have antifungal activity against *Candida albicans* [10]. *Thymus vulgaris* L. (Thyme) possesses various beneficial effects as carminative, antiseptic, antimicrobial and antioxidant properties [11]. Essential oil of thyme is known as therapy adjuvants in many diseases especially for its spasmolytic, expectorant, antimicrobial, antioxidant and choleretic properties [12]. *Salvia fruticosa* Mill. is a native species of the eastern Mediterranean basin [13, 14]. It is used as a hypoglycemic herb, against inflammations, hepatitis, tuberculosis and also possesses antifungal activity [15]. The essential oil obtained from *S. fruticosa* possessed antimicrobial activity against foodborne bacteria [16]. *Mentha viridis* L. (Spearmint / green mint), its boiled leaf extract has anti-infectious, anti-flatulence and anti-inflammatory properties and are used to stimulate digestion and in treatment of viral hepatitis, colitis, gastric acidity and aerophagia [17, 18, 19]. Pharmaceutically, *Lavandula officinalis* L. plant and its preparations have been used for carminative, antispasmodic, antidepressant, expectorant, anti-rheumatic, relaxant, sedative, anti-inflammatory and tonic properties. Their leaves and flowers have the highest amount of EOs that were used in aromatherapy, perfume, cosmetic and flavoring industries [20].

2. MATERIALS AND METHODS:

2.1. Collection of plant materials:

The fresh shoot system of each studied plant was collected from their habitats as shown in Table 1. The collected plants were identified by comparison with plant description in the flora of Egypt as well as herbarium specimens at Desert Research Center (DRC), Egypt.

Table 1: Habitats of collected studied medicinal plants.

Sample No.	Plant Name	Abbreviation	Habitat
1	*Origanum majorana* L.	*O. m.*1	Sekem organic farm
2	*Origanum majorana* L.	*O. m.*2	El-Maghara station, DRC, North Sinai
3	*Origanum syriacum* L.	*O. s.*	El-Sheikh Zowied station, DRC
4	*Thymus capitatus* L.	*T. c.*	Marsa Matrouh (Wadi Habbes)
5	*Thymus vulgaris* L.	*T. v.*	Sekem organic farm
6	*Salvia fruticosa* Mill.	*S. f.*	El-Maghara station, DRC, North Sinai
7	*Mentha viridis* L.	*M. v.*	Sekem organic farm
8	*Lavandula officinalis* L.	*L. o.*	Sekem organic farm

2.2. Estimation of volatile oils content:

2.2.1. Preparation of the oil:

Separately, about 50g of shoot system of each plant sample was subjected to hydrodistillation for 5-6

hours, using a modified Clevenger-type glass apparatus to produce essential oil. The obtained oil was dried over anhydrous sodium sulphate (Na_2SO_4) and kept sealed in dark glass vial at -8°C until further investigations [8]. Percentage yield was determined [21]. Diluted essential oil (1/50 in n-heptane, v/v) was used for Gas Chromatography–Mass spectrometry (GC-MS) analyses [22]. GC-MS analyses HP 6890 series Gas Chromatography system with an HP 5973 mass selective detector was used. Column used was TR-FAME (Thermo 260 M142P) (30m, 0.25mm ID, 0.25 µm film), 70% cyanopropyl-polysilphphenylene siloxane, capillary column. Injector temperature was 200°C, temperature transfer line was 250°C and the carrier gas was He_2 (1.5ml/min). The injected volume was 1µl (5µl/1ml solvent). The ionization energy was 70eV.

2.2.2. Identification of the compounds:

The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and mass spectra with NIST library data, Flavor 2 and Adams libraries spectra and literature [23]. Chem Station software (Agilent Technologies) was used for data analysis, and curves used for experimental estimation of Kovats indices which were plotted and drawn using Sci Da Vis software.

2.3. Tested microorganisms:

Six different isolates of multi-drug resistant (MDR) *Acinetobacter baumannii* and *Klebsiella pneumoniae* were used throughout the present work (Table 2). They were isolated from patients with variable nosocomial infections. These clinical isolates were identified phenotypically by using the Microbact ™ Identification Kits GNB 12A-Oxoid [Gram-Negative Identification System-12A for Enterobacteriaceae]. All isolated bacterial strains used throughout the present study were maintained on Mueller Hinton agar slants and stored at 4ºC with regular transfer at monthly intervals [24].

Table 2: Tested microbial isolates

Microbial isolates	Number
Acinetobacter baumannii	3 isolates (A_1, A_2, A_3)
Klebsiella pneumoniae	3 isolates (K_1, K_2, K_3)

2.4. Antibacterial activity of commercially available antibiotics

The microbial isolates were subjected to antibiotic susceptibility test (disc diffusion method) to confirm that they are MDR [24]. The used antibiotics (Oxoid) are shown in Table 3. Petri dishes were prepared with 10ml of sterile Mueller Hinton (MH) agar. For preparation of bacterial inocula, the bacterial density was adjusted to approximately 10^8 colony forming units (CFU) per ml (optical density was adjusted at 0.5 McFarland turbidity). The tested isolates were swabbed on the top of the solidified media and allowed to dry for 10 min. Antibiotic discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 hrs at 37ºC [24]. After incubation, diameters of inhibition zones (IZ) around the standard antibiotic discs were measured.

Table 3: Commercially used antibiotics

Abbreviations	Name and Concentration
AK	Amikacin 30 µg
AMC	20 µg Amoxicillin + 10 µg Clavulanic acid
C	Chloromphenicol 30 µg
CAZ	Ceftazidime 30 µg
CFM	Cefixime 5 µg
CFP	Cefaperazone 75 µg
CIP	Ciprofloxacin 5 µg
CL	Cephalexin 30 µg
CN	Gentamicin 10 µg
CRO	Ceftriaxone 30 µg
CT	Colistin 10 µg
CTX	Cefotaxime 30 µg
ETP	Ertapenem 10 µg
FEP	Cefepime 30 µg
IPM	Imipenem 10 µg
LEV5	Levofloxacin 5 µg
LOM10	Lomefloxacin 10 µg
MEM10	Meropenem 10 µg
OFX5	Ofloxacin 5 µg
SAM20	10 µg Ampicillin + 10 µg Sulbactam
SCF105	Sulbactam / Cepaperazone 105 µg
SXT25	Sulphamethazole / Trimethoprim 25 µg
TGC15	Tigecycline 15 µg
TZP110	100 µg Piperacillin + 10 µg Tazobactam

2.5. Antibacterial activities of EOs:

Under aseptic conditions, the antibacterial activities of 8 EOs under investigations were carried out by the disc diffusion method [25]. For preparation of bacterial inocula, the bacterial density was adjusted to approximately 10^8 colony forming units (CFU) per ml (optical density was adjusted at 0.5 McFarland

turbidity). Bacterial suspension was spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in three directions in order to get a uniform microbial growth [25]. Empty sterile discs of 6 mm diameter of Whatmann paper number No 3 were loaded with 10µl of the tested EOs and then were placed on inoculated MH agar surface. Whenever it was possible, 2 discs of each tested EO were used. All plates were left at 4ºC for 1h-2hrs for better oil diffusion and then incubated at 37ºC for 24hrs. After incubation, average diameters of inhibition zones around the discs with essential oils were measured [26, 27]. From the previous screening step of the 8 EOs, the oils that show antibacterial effect against tested MDR bacterial isolates were subjected for determination of their minimum inhibitory concentration (MIC).

2.6. Critical dilution and disc diffusion determination of MIC:

The minimal inhibitory concentration (MIC) values of effective EOs were determined for all the tested bacterial isolates by combinations of critical dilution method and disc diffusion assay [28]. The experiment was performed in triplicate. The inoculums of the bacterial isolates were adjusted to approximately 10^8 colony forming units (CFU) per ml. The EOs was emulsified using 0.15% agar [29] and then serial two fold dilutions were made in a concentration range from (0.5 to 0.0000305 ml/ml). The empty sterile discs that were used in this experiment were saturated with 15µl of serially diluted EOs. The plates were then incubated at 37ºC for 24-48 hrs.

2.7. Statistical analysis: Values were expressed as means ± standard deviation (SD).

3. RESULTS AND DISCUSSION:

Medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics because of their safety and low cost as well as their impact on a large number of microbes [30]. The antimicrobial action of essential oil components can be classified in the following decreasing order: phenols, aldehydes, ketones, alcohol, ethers (esters) and hydrocarbons [31, 32]. In the present study, the efficacy of EOs of some medicinal plants were determined quantitatively by measuring the diameter of the inhibition zones (IZ) around the discs and MIC of the most effective oils against six different isolates of *Acientobacter baumannii* and *Klebsiella pneumoniae*.

3.1. Chemical composition of EOs.

The fresh shoot system of plant samples of (*O.m.*1, *O.m.* 2, *O.s.*, *T. c.*, *T.v.*, *S.f.*, *M.v.* and *L.o.*) yielded 0.4, 1.62, 0.54, 1.0, 0.3, 1.09, 0.15, and 0.1% v/w essential oils, respectively. Qualitative and quantitative variations in the components of the 8 EOs were complied in Tables 4 to 11. GC/MS analysis of the (*O.m.*1, *O.m.*2, *O.s.*, *T.c.*, *T.v.*, *S.f.*, *M.v.* and *L.o.*) oils revealed the presence of terpinen-4-ol (21.99%), terpinen-4-ol (23.56%), cymene (27.98%), carvacrol (41.55%), m-thymol (23.97%), eucalyptol (54.84%), carvone (47.79%) and eucalyptol (36.42%) as major components, respectively. For *Origanum majorana* species

Table 4: Chemical composition of essential oil of *O.m.*1

Peak No.	RT	%	Constituents
1	3.50	8.33	*β*-Phellandrene
2	4.23	13.60	(+)-4-Carene
3	5.09	16.83	Gamma-Terpinene
4	5.75	5.10	2-Carene
5	6.15	3.74	Benzene, 1-methyl-3-(1-methylethyl)-
6	12.06	2.83	ρ-Mentha-8-en-1-ol, stereoisomer
7	13.33	2.49	1, 6-Octadien-3-ol, 3, 7-dimethyl-
8	14.98	10.44	Cyclohexanol,1-methyl-4-(1-methylethyl)-, cis,
9	16.45	21.99	Terpinene-4-ol
10	16.75	1.81	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, cis,
11	17.01	0.56	*β*-Ocimene
12	17.42	0.70	Piperitol isomer I
13	18.26	0.29	Humulene
14	18.93	5.29	L-*α*-Terpineol
15	19.67	0.97	Terpinene-3-ol
16	20.22	2.63	1, 5, 5-Trimethyl-6-methylene-cyclohexene
17	20.80	0.24	Geranyl acetate
18	22.30	0.19	Geraniol
19	22.61	0.30	D-Carvone
20	29.98	0.10	Ledol
21	30.50	0.29	(-)-Globulol

22	30.88	0.30	Caryophyllene oxide
23	31.45	0.43	Thymol
24	31.65	0.39	(-)-Spathulenol
25	33.48	0.16	Isospathulenol

Table 5: Chemical composition of essential oil of *O.m.*2

Peak No.	RT	%	Constituents
1	3.51	10.98	*β*-Phellandrene
2	4.22	16.77	(+)-2-Carene
3	5.07	19.96	Gamma-Terpinene
4	5.75	6.47	(+)-4-Carene
5	6.16	2.37	*o*-Cymene
6	12.04	0.90	*ρ*-Mentha-8-en-1-ol
7	13.30	0.84	1, 6-Octadien-3-ol, 3, 7-dimethyl-
8	14.83	4.30	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-
9	16.44	23.56	Terpinen-4-ol
10	16.73	1.83	2-Cyclohexen-1-ol,1-methyl-4-(1-methylethyl)-, cis,
11	16.98	1.60	Tricyclo [2.2.1.0 (2,6)] heptanes, 1, 7, 7-trimethyl-
12	17.40	0.66	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis-
13	18.22	0.13	1, 8-Menthadiene-4-ol
14	18.92	6.11	L-alpha-Terpineol
15	19.66	1.23	Cis-Piperitol
16	20.17	1.29	1, 5, 5-Trimethyl-6-methylene-cyclohexene
17	20.79	0.11	Geranyl acetate
18	21.44	0.09	*ρ*-Diethylbenzene
19	22.29	0.12	2, 6-Octadien-1-ol, 3, 7-dimethyl-(z)-
20	22.59	0.46	D-Carvone
21	31.46	0.14	Thymol (3-methyl-4-isopropylphenol)
22	31.64	0.14	(+) Spathulenol

(*O.m.*1. and *O.m.*2), terpinen-4-ol (member of alcohol group) is the major component, with percentages of 21.99% and 23.56%, as indicated in Table 4 and Table 5, respectively, this is in agreement with [5]. For *O.s.* essential oil (Table 6), *ρ*-cymene is its main component (27.98%), this result is in agreement with [33] which demonstrated that, ρ-cymene is the main component (62.18%) in the early spring but in contrast with [34] which demonstrates that, thymol is the main component (23.5%). For *T.c.* essential oil, carvacrol is the main component (41.55%) as indicated in Table 7 confirming that, *T.c.* is a carvacrol chemotype [35, 36].

As regard, *T.v.* essential oil, thymol is the main component (23.97%) as indicated in Table 8. This finding suggests that the essential oil belongs to the thymol chemotype which is in agreement with those previously reported in Romania [37]. In case of *S. f.* essential oil (Table 9), it was found that, eucalyptol is the main component with percentages of 54.84%. These results are in full agreement with [38].

Table 6: Chemical composition of essential oil of *O. s.*

Peak No.	RT	%	Constituents
1	3.51	2.82	Cyclofenchene
2	4.22	13.88	(+)-4-Carene
3	5.07	17.43	Gamma-Terpinene
4	5.70	3.19	2-Carene
5	6.21	27.98	*p*- Cymene = Thymene
6	10.63	0.93	Benzene, 1-methyl-4-(1-methylethenyl)-
7	12.08	0.90	4-Thujanol

8	12.66	0.23	Benzene, (2-methyl-1-propenyl)-
9	13.30	0.59	1, 6-Octadien-3-ol, 3, 7-dimethyl-
10	14.83	1.75	Cyclohexanol,1-methyl-4-(1-methylethyl)-, cis,
11	15.20	0.69	Aromandendrene
12	16.19	11.11	Terpinen-4-ol
13	16.65	0.41	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans,
14	16.98	3.40	Tricyclo [2.2.1.0 (2, 6)] heptane, 1, 7, 7-trimethyl-
15	18.86	4.75	L-alpha-Terpineol
16	22.60	0.20	(-)-Carvone
17	30.20	0.14	Thymol
18	30.52	8.21	Benzene, 2-methoxy-1, 3, 5-trimethyl
19	30.89	0.35	Phenol, 2-ethyl-4, 5-dimethyl-
20	31.44	0.69	3-methyl-4-isopropylphenol-
21	33.66	0.35	Hexadecanoic acid, methyl ester

As regard, *T.v*. essential oil, thymol is the main component (23.97%) as indicated in Table 8. This finding suggests that the essential
oil belongs to the thymol chemotype which is in agreement with those previously reported in Romania [37]. In case of *S. f.* essential oil (Table 9), it was found that, eucalyptol is the main component with percentages of 54.84%. These results are in full agreement with [38].

Table 7: Chemical composition of essential oil of *T. c.*

Peak No.	RT	%	Constituents
1	3.71	1.62	*β*-Myrcene
2	4.28	3.12	2-Carene
3	5.07	11.48	Gamma-Terpinene
4	5.72	7.59	Eucalyptol
5	6.15	12.39	*o*-Cymene
6	9.02	0.35	3-Octanol
7	9.92	1.14	1-Octen-3-ol
8	12.08	0.50	*ρ*-Menth-8-en-1-ol, stereoisomer
9	13.31	2.52	1, 6-Octadien-3-ol, 3, 7-dimethyl-
10	14.94	0.51	Cis-sabinene hydrate = 4-Thujanol
11	16.02	6.29	Caryophyllene
12	17.13	1.06	(+)-2-Bornanone
13	18.23	0.29	Humulene
14	18.65	0.22	Naphthalene, 1, 2, 3, 5, 8a-hexahydro-4, 7-dimethyl-1-1-(1-methylethyl)-, (1S-cis)-
15	19.07	3.84	Endo-Borneol
16	26.58	3.62	Phenol, 2-methyl-5-(1-methylethyl)-, acetate
17	30.22	0.19	3-methyl-4-isopropyl phenol
18	30.51	0.89	Thymol
19	31.78	41.55	Carvacrol
20	33.55	0.40	Phenol, 2-methyl-5-(1-methylethyl)-
21	33.85	0.16	Phenol-2-methyl-5-(1-methylethyl)-
22	34.07	0.12	Benzene, 2-methoxy-1,3,5-trimethyl
23	37.21	0.12	Carvacrol

Table 8: Chemical composition of essential oil of *T. v.*

Peak No.	RT	%	Constituents
1	3.16	0.22	Camphene
2	3.73	0.83	*β*-Pinene
3	4.22	2.36	(+)-4-Carene
4	5.09	18.47	Gamma-Terpinene
5	5.70	0.52	1, 8-Cineole
6	6.30	27.26	Benzene, 1, 2, 3, 4-tetramethyl-
7	9.93	0.85	1-Octen-3-ol
8	12.05	0.63	Cyclohexanol,1-methyl-4-(1-methylethyl)-cis-
9	13.29	2.74	1, 6-Octadien-3-ol, 3, 7-dimethyl-
10	14.83	3.01	Benzene, 2-methoxy-4-methyl-1-1-(1methylethyl)-
11	15.62	1.05	Benzene, 2-methoxy-4-methyl-1-1-(1methylethyl)-
12	15.96	1.54	Caryophyllene
13	16.12	1.64	Terpinen-4-ol
14	17.04	1.46	(+)-2-Bornanone
15	19.04	3.70	Endo-Borneol
16	22.91	0.28	Butanoic acid, 2-methyl-, 3, 7-dimethyl-2, 6-octadienyl ester, (E)-
17	25.60	0.19	Phenol, 5-methyl-2-(1-methylethyl)-, acetate
18	30.20	0.43	3-Methyl-4-isopropylphenol-
19	30.59	23.97	*m*-Thymol
20	30.93	2.24	Phenol, 2-ethyl-4, 5-dimethyl-
21	31.32	0.28	Phenol-2-methyl-5-(1-methylethyl)-
22	31.46	6.12	2, 5-Diethyl phenol
23	37.20	0.21	Alloaromadendrene

For *M.v.* essential oil (Table 10), carvone is the main component (47.79%) as reported by other studies [39].

Table 9: Chemical composition of essential oil of *S. f.*

Peak No.	RT	%	Constituents
1	3.25	2.45	*β*-Pinene
2	3.63	1.79	Bicyclo [3.1.1] heptane, 6-6-dimethyl-2-methylene-, (1S)-
3	4.27	1.33	D-Limonene
4	5.81	54.84	Eucalyptol
5	13.50	3.11	Bicyclo [3.1.0] hexan-3-one, 4-methyl-1-1-(1-methylethyl)-
6	14.00	1.45	Thujone
7	14.81	0.50	Trans-sabinene hydrate
8	15.18	0.42	Aromandendrene
9	16.08	6.07	Caryophyllene
10	16.43	0.49	Bicyclo [3.1.1] heptan-3-one, 2, 6, 6-trimethyl-
11	17.07	8.82	(+)-2-Bornanone
12	18.25	1.47	Humulene
13	18.46	2.06	Delta-Terpineol
14	18.86	5.00	L-alpha-Terpineol
15	19.04	0.88	Endo-Borneol
16	19.35	2.10	2-Carene
17	25.06	0.21	Coumarin-6-ol
18	30.00	1.84	Aromandendrene
19	30.52	0.24	(-)-Globulol
20	30.89	1.69	Caryophyllene oxide
21	31.45	0.29	Thymol
22	31.65	1.29	(-)-Spathulenol
23	32.40	0.50	12-Oxabicyclo [9.1.0] dodeca-3,7-diene,1, 5, 5, 8-tetramethyl-
24	35.52	0.13	10, 10-Dimethyl-2, 6-dimethylene bicyclo [2.2.0] undecan-5-beta-ol
25	36.23	0.19	3-[(Trimethylsilyl) ethynyl] benzaldehyde
26	37.19	0.23	Patchoulene
27	43.67	0.60	1-Naphthalenepropanol, *α*-ethenyldecahydro-*α*, 5, 5, 8-a tetramethyl-Z-methylene-, [1S-[1*α*, 4aB, 8a*α*]]-

In case of *L.o*. essential oil (Table 11), it was found that, 1, 8-cineole (Eucalyptol) was the main component (36.42%). This finding is in agreement with the study [40].

Table 10: Chemical composition of essential oil of *M.v.*

Peak No.	RT	%	Constituents
1	3.66	1.27	*α*-Pinene
2	3.75	1.67	*β*-Myrcene
3	4.33	27.63	D-Limonene
4	5.67	3.11	Eucalyptol
5	12.71	1.87	(-)-*β*-Bourbonene
6	16.09	3.22	Caryophyllene
7	17.85	0.72	(+)-Epi-Bicyclosesqu-iphellandrene
8	19.31	4.35	Neodihydrocarveol
9	19.62	2.03	Cyclohexanone, 2-methyl-5-(1-methylethyl)-, trans-
10	20.40	1.53	Cyclohexanol, 2-methyl-5-(1-methylethyl)-
11	21.87	2.67	Trans-Carveyl acetate
12	23.14	47.79	Carvone
13	23.65	0.33	2-Cyclohexane-1-one, 3-methyl-6-(1-methyl-ethyl)-
14	27.29	0.22	Carvone oxide, cis-
15	28.54	0.21	Butanoic acid, 2-methyl-, 2-phenyl ethyl ester
16	29.18	0.53	Carotol
17	29.44	0.23	2-Cyclopenten-1-one, 3-methyl-2-(2-pentenyl)-, (Z)-
18	30.88	0.28	Caryophyllene oxide
19	33.56	0.34	*α*-Cadinol

3.2. Antibacterial Activity

In vitro antibacterial activities of the studied 8 EOs were estimated by the diameter of inhibition that varied according to the active ingredients of each essential oil. *L.o.* and *S. f.* were ineffective against all tested bacterial isolates (no clear zones), while *M.v.* was effective only against 3 isolates of *Acinetobacter baumannii* with small clear zones and was ineffective against the 3 isolates of *Klebsiella pneumoniae* (no clear zones) as indicated in table (12). In contrast, the other five essential oils (*T.c.*, *T.v.*, *O.s.*, *O.m.*1, *O.m.*2) were effective against all tested bacterial strains with different inhibition zone diameters. *Thymus capitatus* was the most effective essential oil with the largest inhibition zones against all tested bacterial isolates, except A_3 against which *T.v.* was more effective (clear zone = 3.33cm). From the obtained results, the more effective five EOs (*T.c.*, *T.v.*, *O.m.*1, *O.m.*2 and *O.s.*) were selected to determine their MIC values. The bacteriostatic effectiveness of these essential oils was estimated by MIC and revealed that, *T.c.* shows the highest antibacterial activity against all bacterial isolates. *P*-cymene is the precursor of carvacrol and thymol, so *O.s.* has antibacterial effect but lower than *T.c.*, *T.v.*, and higher than other EOs (*O.m.*1, *O.m.*2 and *M.v.*). Its MIC values ranged from 0.004 to 0.063 mg/ml. This result is in the same context with the finding of [41], which revealed that, *O.s.* EO showed antibacterial activity against some GNB: *Proteus* spp., *Klebsiella pneumoniae*, *Yersinia enterocolitica* and *Escherichia coli* O157: H7.

Table 11: Chemical composition of essential oil of *L.o.*

Peak No.	RT	%	Constituents
1	3.43	0.69	3-Carene
2	4.29	2.81	D-Limonene
3	4.91	8.31	*β*-Phellandrene
4	5.74	36.42	Eucalyptol = 1, 8- cineole
5	6.13	5.45	*o*-Cymene
6	12.55	0.80	1H-Cycloprop [e] azulene, 1a, 2, 3, 4, 4a, 5, 6, 7b-octahydro 1, 1, 7, 4-tetramethyl-, [1aR-(1a-alpha., 4.alpha., 4a.beta., 7b.alpha.)]-
7	14.92	0.53	Campholaldehyde
8	16.17	3.06	Terpineol-4-ol
9	17.14	18.71	(+)-2-Bornanone
10	17.83	0.85	2-Cyclohexen-1-one, 4-(2-oxopropyl)-
11	18.47	0.31	*α*-Terpineol
12	19.05	3.73	Endo-Borneol
13	19.63	0.74	Myrtenal
14	22.27	0.42	2-Cyclohexan-1-ol, 2-methyl-5-(1-methylethyl)-, cis,
15	22.50	1.07	2-Cyclohexen-1-one, 4-(1-methylethyl)
16	22.62	3.07	D-Carvone
17	23.20	0.56	Benzenemethanol, *α, α*, 4-trimethyl-
18	23.37	2.20	Propanol, 2-methyl-3-phenyl-
19	23.56	0.40	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethyl)-
20	29.21	0.53	Copaene
21	29.63	0.26	Selina-3, 7(11)-diene
22	30.03	0.31	*ρ*-Cymen-7-ol
23	30.51	0.50	Thymol
24	30.89	1.58	Caryophyllene oxide
25	31.45	0.69	3-Methyl-4-isopropyl-phenol
26	31.89	3.61	*α*-Copaene
27	33.05	0.23	1H-3a, 7-Methanoazulene, 2, 3, 4, 7, 8, 8a-hexahydro-3, 6, 8, 8-tetramethyl-[3R-(3*α*, 3a*β*, 7*β*, 8a*α*)]-
28	33.56	0.46	*α*-Cadinol
29	37.20	0.92	Viridiflorol
30	38.98	0.32	Cadina-4, 10(15)-dien-3-one
31	40.92	0.45	N-(2'-Thiazolyl)-2, 5-dimethylpyrrole-3-carbaldehyde

Table 12: Antibacterial activity of the studied essential oils against MDRGNB using agar disc diffusion method.

MDR-GNB	IZ (cm ± SD) in cm							
	T.c.	***T.v.***	***O.m.*1**	***O.m.*2**	***O.s.***	***M.v.***	***L.o.***	***S.f.***
A_1	4.43±0.05	4.43±0.30	1.13±0.04	1.46±0.04	2.53±0.04	1.1±0	0	0
A_2	5.63±0.20	5.06±0.10	1.06±0.04	1.86±0.04	2.63±0.04	1.83±0.04	0	0
A_3	3.13±0.04	3.33±0.10	1.56±0.04	1.5±0	2.6±0	1.23±0.04	0	0
K_1	1.93±0.04	1.2±0.10	1.0±0	1.0±0	1.06±0.04	0	0	0
K_2	2.63±0.04	2.26±0.04	1.3±0	1.23±0.04	1.8±0	0	0	0
K_3	2.1±0	1.13±0.04	1.3±0	1.2±0	1.4±0.08	0	0	0

IZ = Inhibition zone ± SD = Standard deviation

This study reported that, the antibacterial activity of *T.c.* was the highest when compared to other EOs activities, its MIC values ranged from 0.001 to 0.008 mg/ml (Table 13). This result is in the same context with [42] that demonstrated the strong antibacterial activity of essential oil of *T.c.* and its significant potential to reduce pathogen colonization in colon epithelium. According to the composition of *T.v*. and *T.c.* oils, antibacterial activity of thymol is less than that of carvacrol, with MIC values of thymol ranged from 0.008 to 0.125 mg/ml (Table 13). This finding is in agreement with the study done [43] which demonstrated that, essential oil of *T.v*. possessed strong antimicrobial properties against 7 common bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis, Salmonella typhimurium, Escherichia coli and Staphylococcus aureus*) and *Candida albicans*. Several studies have

demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug resistant pathogens to powerful antioxidant properties [44]. Thyme plant is used since ancient times to achieve healing, antiseptic fumigation, food preservation and other useful effects [45]. The MIC values of *O.m.*1 ranged from 0.031 to 0.166 mg/ml, while the MIC values of *O.m.*2 ranged from 0.052 to 0.125 mg/ml. The variation of MIC values between the 2 species of *Origanum majorana* is due to the difference in chemical composition of the oil. This result is in agreement with [5] which promoted an inhibitory effect of essential oil of *Origanum majorana* against *Staphylococci aureus* and *E. coli*. Oils containing carvones are used in aromatherapy and alternative medicine [46]. The oxygenated monoterpenes carvone and dihydrocarvone are potential inhibitors of bacterial, fungal growth, as well as prospective insect repellents [47]. Carvone is capable of disrupting the pH gradient and membrane potential of cells. With increasing amount of carvone, it was observed a decrease in the growth rate of *E. coli*, *Streptococcus thermophilus* and *Lactobacillus lactis* and that might be due that carvone act by disturbing the metabolic energy status of cells [48]. Our results revealed that, *M.v.* has weak effect on the 3 different isolates of *Acientobacter baumannii* and no effect on the 3 isolates of *Klebsiella pneumonia*. This is in agreement with [39] as *Klebsiella pneumonia* was inhibited by *M.v.* while *E. coli* was least susceptible. Essential oils of *L.o.* showed no activity against all bacterial isolates of *Acinetobacter baumannii* and *Klebsiella pneumoniae*, which is in agreement [49] as essential oil of *L.o.* had no antibacterial activity against GNB (*Pseudomonas fluorescens*). Eucalyptol is one of the most important monoterpene oxides. It is expectorant widely used in commercial cough lozenges. The characteristic aroma of eucalyptol can be described as eucalyptus, mint and camphoraceous [50]. Essential oils of *S. f.* showed no activity against all bacterial isolates of *Acinetobacter baumannii* and *Klebsiella pneumonia*, this result was in contrast with [51] where essential oil of *S. f.* showed antibacterial activity against Gram +ve bacteria (*Bacillus cereus, Micrococcus flavus, Staphylococcus aureus* and *Listeria monocytogenes*) and Gram -ve bacteria (*Escherichia coli, Pseudomonas aeruginosa, proteus mirabilis* and *Salmonella typhimurium*).

Table 13: MIC (mg/ml) values of the most effective essential oils against MDRGNB using critical dilution method and disc diffusion assay.

MDRGNB	MIC (mg/ml ± SD)				
	T.c.	*T.v.*	*O.m.*1	*O.m.*2	*O.s.*
A_1	0.001±0	0.016±0	0.031±0	0.063±0	0.016±0
A_2	0.001±0	0.063±0	0.125±0	0.063±0	0.063±0
A_3	0.008±0	0.063±0	0.031±0	0.125±0	0.031±0
K_1	0.008±0	0.125±0	0.034±0.02	0.125±0	0.063±0
K_2	0.008±0	0.016±0	0.166±0.05	0.125±0	0.031±0
K_3	0.001±0	0.008±0	0.052±0.01	0.052±0.01	0.004±0

4. CONCLUSION

Some EOs of Egyptian medicinal plants belonging to family Lamiaceae can be considered as potential source of new antibiotics against multi-drug resistant gram negative microorganisms.

5. RECOMMENDATION

Further and more specific studies are recommended to determine the efficacy of these EOs in treatment of multi-drug infections caused by Gram negative microorganisms.

6. REFERENCES:

1.Looke D.F.; Gottlieb T.; Jones C.A. and Paterson D.L., Gram-negative resistance: can we combat the coming of a new "Red Plague"?. Med. J. Aust., 2013;198 (5),: 243-244.

2.Doi Y.; Park Y.S.; Rivera J.I.; Adams-Haduch J.M.; Hingwe A.; Sordillo E.M.; Lewis J.S.; Howard W.J.; Johnson L.E.; Polsky B.; Jorgensen J.H.; Richter S.S.; Shutt K.A. and Paterson, D.L.,. Community-associated extended-spectrum *β*-lactamase-producing *Escherichia coli* infection in the United States. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America,2013; 56 (5): 641-648. doi:10.1093/cid/cis942.

3.Harris P.; Paterson D. and Rogers B., 2015. Facing the challenge of multidrug-resistant gram-negative bacilli in Australia. Med. J. Aust., 2015;202 (5): 243-247.

4.Naghibi F.; Mosaddegh M.; Mohammadi Motamed M. and Ghorbani A., Labiatae Family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian Journal of Pharmaceutical Research,2005; 4 (2): 63-79.

5.Freire J.M.; Cardoso M.G.; Batista L.R. and Andrade Milene A., Essential oil of *Origanum majorana* L., *Illicium verum* Hook. F. and *Cinnamomum zeylanicum* Blume: chemical and antimicrobial characterization. Revista Brasileira de Plantas Medicinais, 2011;13 (2): 209-214.

6.Jang Y.S.; Yang Y.C.; Choi D.S. and Ahn Y.J., Vapor phase toxicity of marjoram oil compounds and their related monoterpenoids to *Blattella germanica* (Orthoptera: Blattellidae). J. Agric. Food Chem., 2005;53 (20): 7892-7898.

7.Yang Y.C.; Lee S.H.; Clark J.M. and Ahn Y.J., Ovicidal and adulticidal activities of *Origanum majorana* essential oil constituents against insecticide - susceptible and pyrethroid / malathion-resistant *Pediculus humanus capitis* (*Anoplora: Pediculidae*). J. Agric. Food Chem., 2009;57 (6): 2282-2287.

8.Farhat M.; Tóth J.; Héthelyi B.É.; Szarka Sz and Czigle Sz., 2012. Anaysis of the essential oil compounds of *Origanum syriacum* L. Acta Fac. Pharm. Univ. Comen. Tomus LIX, 2, 6-12.

9.Tabata M.; Honda G. and Sezik E., 1988. A Report on Traditional Medicine and Medicinal Plants in Turkey. Faculty of Pharmaceutical Sciences, Kyoto University, 105 pp.

10.Gorena A.C.; Bilsela G.; Bilsela M.; Demira H. and Kocabas E.E., Analysis of essential oil of *Coridothymus capitatus* (L.) and its antibacterial and antifungal activity. Zeitschrift für Naturforschung C., 2014;58 (9-10): 687-690.

11.Baranauskiene R.; Venskutonis P.R.; Viskelis P. and Diambrauskiene E., 2003. Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). Journal of Agricultural and Food Chemistry, 2003;51 (26): 7751-7758.

12.Lakis Z.; Mihele D.; Nicorescu I.; Vulturescu V. and Udeanui D.I., The antimicrobial activity of *Thymus vulgaris* and *Origanum syriacum* essential oils on *Staphylococcus aureus, Streptococcus pneumonia* and *Candida albicans*. Farmacia, 2012;60 (6): 857-865.

13.Ali-Shtayeh M.S.; Yaniv Z. and Mahajna J., 2000. Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. J. Ethnopharmacol. 2000;73 (1-2): 221-232.

14.Carmona M.D., Llorach R., Obon C. and Rivera D., 2005. "Zahraa", a Unani multicomponent herbal tea widely consumed in Syria: components of drug mixtures and alleged medicinal properties. J. Ethnopharmacol.,2005; 102 (3): 344-350.

15.Pitarokili D.; Tzakou O.; Loukis A. and Harvala C., Volatile metabolites from *Salvia fruticosa* as antifungal agents in soilborne pathogens. J. Agric. Food Chem.,2003; 51 (11): 3294-3301.
16.Delamare A. P. L.; Moschen-Pistorello I. T.; Artico L.; Atti-Serafini Luciana. and Echeverrigaray S., Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia tribola* L. cultivated in South Brazil. Food Chem.,2007; 100 (2): 603-608.
17.Saleem M.; Alam A. and Sultana S.,. Attenuation of benzoyl peroxide-mediated cutaneous oxidative stress and hyperperproliferative response by the prophylactic treatment of mice with spearmint (*Mentha spicata*). Food and Chemical Toxicology, 2000;38 (10): 939-948.
18.Kouhila M.; Belghit A.; Daguenet M. and Boutaleb B.C., Experimental determination of the sorption isotherms of mint (*Mentha viridis*), sage (*Salvia officinalis*) and verbena (*Lippia citriodora*). Journal of Food Engineering;2001; 47 (4): 281-287.
19.Kumar A. and Chattopadhyay S., 2007. DNA damage protecting activity and antioxidant potential of pudina extract. Food Chemistry,2007; 100 (4): 1377-1384.
20.Hassanpouraghdam M.B.; Hassani A.; Vojodi Lamia; Asl B.H. and Rostami A., Essential oil constituents of *Lavandula officinalis* Chaix. From Northwest Iran. CHEMIJA, 2011;22 (3), 167-171.
21.Egyptian Pharmacopoeia (1984). General Organization for Governmental Printing Affairs. Cairo. p. 31.
22.Milovanović I.Lj.; Misăn Aleksandra Č.; Sakač Marijana B.; Čabarapa Ivana S.; Šarić Bojana M.; Matić Jovana J. and Jovanov Pavle T., Evaluation of GC-MS method for the analysis of *Oregano* essential oil composition. Food Processing, Quality and Safety,2009; 36 (3-4): 75-89.
23. AdamsR.P.,2007. "Indentification of Essential Oil Components by Gas Chromatography/Mass spectroscopy". 4th Ed. Allurad Publishing Corporation., Carol Stream, USA. 804 pp. ISBN 978193263321 4. **Record Number:** 20083116584
24.Khatib A.; Olama Z. and Holeil H., Antibacterial effect of some Lebanese plant oils against some multi drug-resistant bacteria. Global Advanced Research Journal of Microbiology, 2013;2 (2): 35-43.
25.Kon Kateryna and Rai M., Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with other essential oils. Nusantara Bioscience, 2012;4 (2), 50-56.
26.Çetin B.; Çakmakçi S. and Çakmakçi R., The investigation of antimicrobial activity of thyme and oregano essential oils. Turk J. Agric. For., 2011;35: 145-154.
27.Miladi Hanene; Ben Slama Rihab; Mili Donia; Zouari S.; Bakhrouf Amina and Ammar Emna, Essential oil of *Thymus vulgaris* L. and *Rosmarinus officinalis* L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens. Natural Science,2013; 5 (6): 729-739.
28.Yamamoto Y.; Togawa Y.; Shimosaka M. and Okazaki, M., Purification and characterization of a novel bacteriocin produced by *Enterococcus faecalis* strain RJ-11. Appl. Environ. Microbiol.,2003; 69 (10): 5746-5753.
29.Budzyńska A.; Wieckowska-Szakiel M.; Kalemba D.; Sadowska B. and Rózalska B., The optimization of methods utilized for testing the antibacterial activity of essential oils. Med. Dosw Mikrobiol., 2009;61(3), 281-287.
30.Hassawi D. and Kharma A., Antimicrobial activity of some medicinal plants against *Candida albicans*. J. Biol. Sci.,2006; 6 (1): 109-114.
31.Kalemba D. and Kunicka A., 2003. Antibacterial and antifungal properties of essential oils. Curr. Med. Chem., 10 (10), 813-829.
32.Nowak A.; Kalemba D.; Krala L.; Piotrowska M. and Czyzowska A., The effects of thyme (*Thymus vulgaris*) and rosemary (*Rosmarinus officinalis*) essential oils on *Brochothrix thermosphacta* and on the shelf life of beef packaged in high-oxygen modified atmosphere. Food Microbiology, 2012;32 (1), 212-216.
33.Toncer O.; Karaman S. and Diraz E., An annual variation in essential oil composition of *Origanum syriacum* from southeast Anatolia of Turkey. Journal of Medicinal Plants Research, 2010;4 (11): 1059-1064.
34.Hűsnű Can Başer K.; Mine Kűrkçűoğlu; Betűl Demirci and Temel Özek, The essential oil of *Origanum syriacum* L. var. *sinaicum* (Bioss.) letswaart. Flavour Fragr. J.,2003; 18: 98-99.
35.Ruberto G.; Biondi D. and Piatelli M., The essential oil of Sicilian *Thymus capitatus* (L.) Hoffmanns. et Link. J. Essen. Oil Res., 1992;4 (4): 417-418.
36.Mkaddem M.G.; Romdhane M.; Ibrahim H.; Ennajar M.; Lebrihi A. Mathieu F. and Bouajila J., Essential oil of *Thymus capitatus* Hoff. Et Link. from Matmata, Tunisia: gas chromatography-mass spectrometry analysis and antimicrobial and antioxidant activities. J. Med. Food, 2010;13 (6): 1500-1504.
37.Grigore A.; Paraschi I.; Colceru-Mihul S.; Bubueanu C.; Draghici E. and Ichium M., Chemical composition and antioxidant activity of *Thymus vulgaris* L. volatile oil obtained by two different methods. Romanian Biotechnological Letters, 2010;15 (4): 5436-5443.
38.Skoula M.; Abbes J.E. and Johnson C.B., Genetic

variation of voltailes and rosmarinic acid in populations of *Salvia fruticosa* Mill growing in Crete. Biochem. Syst. Ecol., 2000;28 (6): 551-561.
39.Mkaddem M.G., Bouajila J., Ennajar M., Lebrihi A., Mathieu F. and Romdhane M., Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and *viridis*) essential oils. J. Food Sci.,2009; 74 (7): 358-363.
40.Manteghi T.A.; Abdossi V. and Delkhosh B., Essential oil of the aerial parts of *Lavandula officinalis* from Iran. Intl. Res. J. Appl. Basic. Sci., 2015;9 (9): 1479-1481.
41.Al-Mariri A. and Safi M., *In vitro* antibacterial activity of several plant extracts and oils against some Gram-negative bacteria. Iran J. Med. Sci., 2014;39 (1): 36-43.
42.Džamić A.M.; Nikolić B.J.; Giweli A.A.; Mitić-Ćulafić D.S.; Soković M.D.; Ristić M.S.; Knežević-Vukčević J.B. and Marin P.D. Libyan *Thymus capitatus* essential oil: antioxidant, antimicrobial, cytotoxic and colon pathogen adhesion-inhibition properties. J. Appl. Microbiol.,2015; 119 (2): 389-399.
43.Borugă O., Jianu C., Mişcă C., Goleţ I., Gruia A.T. and Horhat F.G., *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. Journal of Medicine and Life,*2014;* 7 (3): 56-60.
44.Mezzoug N.; Elhadri A.; Dallouh A.; Amkiss S.; Skali N.S.; Abrini J.; Zhiri A.; Baudoux D.; Diallo B.; El Jaziri M. and Idaomar M., Investigation of the mutagenic and antimutagenic effects of *Origanum compactum* essential oil and some of its constituents. Mutation Research Genetic Toxicology and Environmental Mutagenesis,2007; 629 (2): 100-110.
45.Stahl-Biskup Elisabeth and Francisco Sáez, 2002. Thyme: The genus *Thymus*. Taylor and Francis. CRC Press, 352 Pages.
46.de Carvalho Carla C.C.R and da Fonseca M. Manuela R., Carvone: Why and how should one bother to produce this terpene. Food Chemistry, 2006;95 (3): 413-422.
47.Porto Carla; Stüker Caroline Z.; Anderson S. Mallmann; Euclesio Simionatto ; Adriana Flach; Tais do Canto-Dorow; Ubiratan F. da Silva; Ionara I. Dalcol & Ademir F. Morel, (R)-(-)-carvone and (1R,4R)-trans-(+)-dihydrocarvone from *Poiretia latifolia* Vogel. Journal of the Brazilian Chemical Society,2010; 21 (5): 782-786.
48.Oosterhaven K.; Poolman B. and Smid E.J., 1995. S-carvone as a natural potato sprout inhibiting, fungistatic and bacteristatic compound. Ind. Crops Prod.,1995; 4 (1): 23-31.
49.Marín I.; Sayas-Barberá E.; Viuda-Martos M.; Navarro C.; Sendra E., Chemical composition, antioxidant and antimicrobial activity of essential oils from organic fennel, parsley, and lavender from Spain. *Foods, 2016;5(1):* 18.
50.Capone D.L.; Van Leeuwen K.; Taylor D.K.; Jeffery D.W., Pardon K.H.; Elsey G.M. and Sefton M.A., Evolution and occurrence of 1, 8-cineole (Eucalyptol) in Australian wine. J. Agric. Food Chem.,2011; 59 (3):, 953-959.
51.Abdulhamid A. Giweli; Džamić Ana M.; Soković Marina; Ristić M.S.; Janaćković P. and Marin P.D., The chemical composition, antimicrobial and antioxidant activities of the essential oil of *Salvia fruticosa* growing wild in Libya. Arch. Biol. Sc. Belgrad. 2013;65 (1): 321-329.