Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 7(5); 251-257

ISSN: 0976 822X

Research Article

Chemical Compositions and Antimicrobial Activity of Leaves *Eucalyptus camaldulensis* Essential Oils from Four Syrian Samples

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Available Online: 1st October, 2016

ABSTRACT

Essential oils are highly concentrated substances extracted from medicinal plants. These essential oils are often used for their flavor and their therapeutic or odoriferous properties. Extraction of essential oils is one of the most time and effort consuming processes. This is why it is important to study the chemical composition of the essential oil. Aim of this study is to determine the essential oil content variation and antimicrobial activity of four different *Eucalyptus camaldulensis* samples in Damascus region. *E. camaldulensis* Leaves were collected in May 2015. Fresh leaves of samples were subjected to hydro distillation for 6 hours in a Clevenger-type apparatus. The chemical compositions of essential oils were characterized by GC-MS, and were evaluated for their antimicrobial activities by disc diffusion and macro broth dilution methods against ten bacterial strains. The essential oil yields were (S1: 0.71%, S2: 0.37%, S3: 0.65% and S4: 0.45% v/w). Monoterpene hydrocarbons were a major class of compounds. Among them, dominant compounds were P-cymene (19.21, 20.37, 16.58, and 21.70) and β-phellandrene (11.46, 4.14, 13.39 and 2.97) respectively. The second largest group was oxygenated monoterpenes with 1,8-Cineole (5.67, 7.82, 2.39, 3 and 3.29), cryptone (8.61, 6.81, 6.41 and 14.01), and terpinene-4-ol (3.82, 2.10, 4.35 and 4.23) respectively, as predominant. Besides, high content of sesquiterpene Caryophyllene oxide (9.16, 19.72, 23.57 and 15.39) respectively, was found. The essential oils extracted was tested for antimicrobial activity against ten organisms, four gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Micrococcus luteus*) and six gram-negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Vibrio parahaemolyticus*). The samples essential oil shows a max zone against *Vibrio parahaemolyticus*, and shows no activity detected against the *Pseudomonas aeruginosa* and *Proteus vulgaris*. Minimum inhibitory concentration (MIC) revealed the highest activity against *Vibrio parahaemolyticus* (0.1 mg/mL) and *Staphylococcus aureus* (0.2 mg/mL), while the lowest activity was against *Bacillus subtilis* and *Klebsiella pneumoniae* (1.6 and 3.2 mg/mL). The Minimum bactericidal concentration (MBC) activity ranged from (0.2 mg/mL) to (3.2 mg/mL).

Keywords: *Eucalyptus Camaldulensis*, Essential oil, antibacterial activity, GC-MS, *Myrtaceae*.

INTRODUCTION

Plants are an important source for drug discovery[1]. In recent years, there has been an upsurge of interest for natural substances as phyto medicines has resulted in a more thorough investigation of plant resources. Aromatic plants and their chemical content have been used in memorial time in folk medicine for the preservation of food, as antimicrobial and antioxidant[2]. Essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants, as a complex mixture of biologically active substances. Many volatile compounds naturally found in essential oils have strong antibacterial activities[3]. Essential oils are, therefore, widely used in food production industries for preservation of food because they are generally safer than inorganic chemicals. Interest in essential oils has revived with the popularity of aromatherapy, a form of alternative medicine which uses specific aromas carried by essential oils for healing. The investigation of the essential oil of these plants will help to verify the rationale behind the use of this plant as a cure for these illnesses. In this work, the emphasis is on the widely used *Eucalyptus* essential oil, among many others. *Eucalyptus (Myrtaceae)* is one of the most important and most widely planted genera. Although being Australia's native, more than 700 species wildly grow in many parts of the world[4]. Around 15 *Eucalyptus* species grow in the Mediterranean region. Among them, *Eucalyptus camaldulensis* is the most commonly found in Syria. This species is used in the indigenous system of medicine to cure various human ailments such as diarrhea, chronic dysentery, malaria, infection of upper respiratory tract, and certain skin diseases[5,6]. Essential oils obtained from the *Eucalyptus* leaves are of particular commercial interest[7], in addition, *E. camaldulensis* is also known to contain

Table 1: essential oil content (%) in fresh leaves of *E. camaldulensis* collected from Damascus area.

Sample	S1	S2	S3	S4
Oil Content v/w%	0.71	0.37	0.65	0.45

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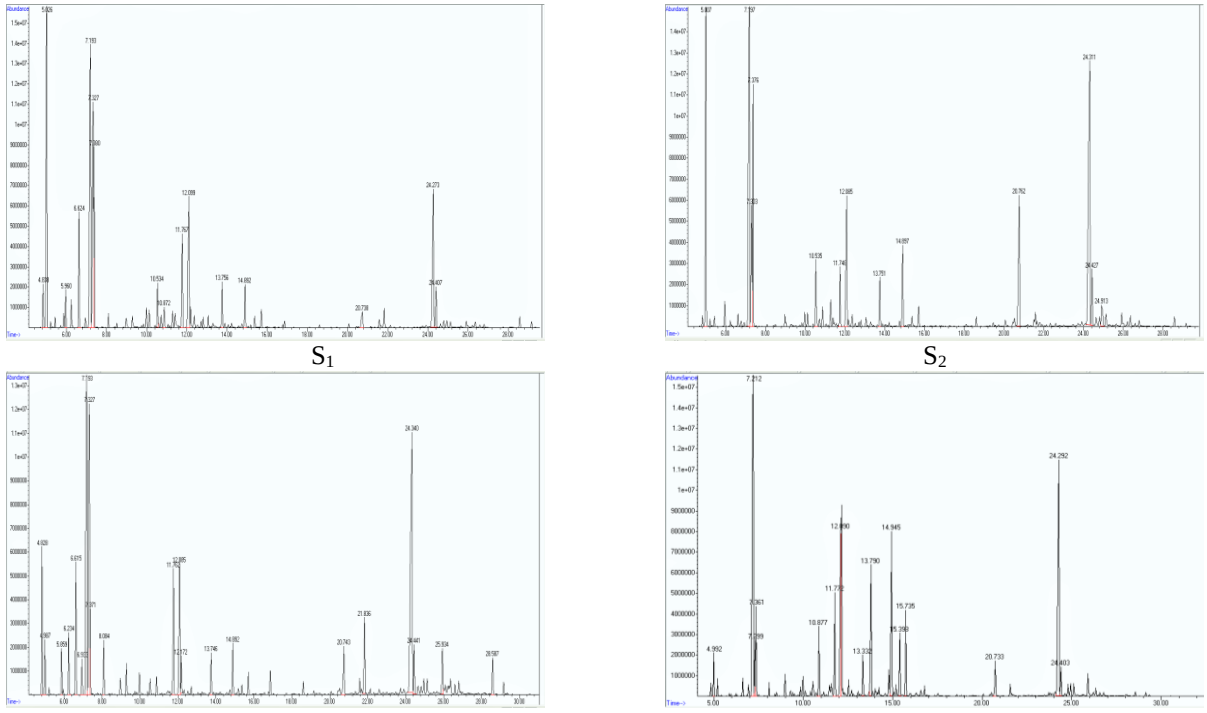

Figure 1: GC/MS Chromatogram of fresh *E. camaldulensis* leaves essential oil For all samples S_1, S_2, S_3, S_4.

bioactive products that display antibacterial[8], antifungal[9], analgesic and anti-inflammatory effects, antioxidative and antiradical activities[10]. Therefore, in this study we analyzed the constituents of leaf essential oils of *E. camaldulensis* samples and studied their antibacterial activity.

MATERIALS AND METHODS

Plant material

Four fresh samples Leaves of *E. Camaldulensis* were collected from several places in Damascus in the southern part of Syria, in May 2015. The plant identity was confirmed in Department of Botany, Faculty of Science - University of Damascus. The samples were collected early in the morning. The leaves were cut out by a pair of scissors, and then taken to the laboratory in a plastic bag. The leaves were cut to smaller pieces by scissors.

Essential oil extraction

Fresh leaves of *E. camaldulensis* samples (200 g) cut into small pieces, were subjected to hydro distillation for 6 hours in a Clevenger-type apparatus[11]. The Light yellow colored essential oil was separated from aqueous layer, dried over anhydrous sodium sulfate and preserved in a sealed sample tube and stored under refrigeration at (4ºC) until analysis.

GC-MS analysis of essential oil

Qualitative analysis was performed using an Agilent 6890 N gas chromatograph (GC) equipped with Agilent 5973 mass selective detector (MSD), Agilent Auto sampler 7683 and Agilent DB-5MS capillary column (30 m, 0.25 i.d., 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA, USA). The MS detector was operated in electron impact (EI) mode at 70eV with interface temperature of 280°C; the scan range was 50–550 amu. The injection port temperature was set at 250°C. GC was performed in split less mode; carrier gas was helium at a constant flow rate of 1 mL/min. The column temperature was programmed as follows: an initial temperature of 60 °C increased to 280°C at rate of 3°C/min. The injection volume was 1.0 µL.

Identification of essential oil compound

Relative percentage amounts were calculated from peaks total area by apparatus software. The identification of individual compounds was based on comparison of their mass spectra with those obtained from the NIST/NBS, Wiley Libraries spectra. Further confirmation was done from Retention Index data generated from a series of alkane's retention indices (relatives to C8-C20 on the DB-5MS column) and (Adams, 2007).

Antimicrobial activity

Bacterial cultures

Ten different bacterial species were collected from Department of Medical Microbiology and Parasitology, Faculty of Medicine, Damascus University, four gram-positive bacteria (*Bacellus subitus, Staphylococcus aureus, Streptococcus pyogenes, Micrococcus luteus)* and six gram-negative bacteria *(Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginose, Klebsellia pneumonia, Proteus vulgaris, Vibrio parahaemolyticus)* were inoculated into the nutrient broth and kept on a rotary shaker overnight for incubation. The stock cultures were maintained at 4ºC in nutrient agar.

Agar disc diffusion

Essential oils were tested for its antibacterial potential by the agar disc diffusion method according to established procedure[12]. The microorganisms were grown overnight at 37ºC in 20 mL of Müeller-Hinton broth. The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 5 standard (1.0×10^8) CFU/ml. 50 mm Petri dishes containing 12 mL of sterilized

Table 2: Percentage content of compounds in essential oils of the *E. camaldulensis* leaves.

S. No.	R.I	Compounds	S_1	S_2	S_3	S_4
		Monoterpene Hydrocarbons	55.87	40.92	43.97	29.82
1.	929	α -Thujene	1.30	0.32	3.27	0.36
2.	937	α-pinene	16.41	14.22	2.17	2.18
3.	946	α-Fenchene	0.45	-	1.02	0.10
4.	979	β –Pinene	1.14	0.69	0.13	0.11
5.	993	β-Myrcene,	0.81	0.16	1.41	0.10
6.	1010	α-Phellandrene	3.79	0.39	3.47	0.58
7.	1019	α -Terpinene	0.36	0.13	0.88	0.42
8.	1028	P-Cymene	19.21	20.37	16.58	21.70
9.	1032	β -phellandrene	11.46	4.14	13.39	2.97
11.	1060	γ -Terpinen	0.43	0.10	1.21	0.40
12.	1091	Terpinolene	0.51	0.40	0.44	0.90
		Oxygenated Monoterpenes	28.34	26.47	19.96	47.03
10.	1035	1,8-Cineole	5.76	7.82	2.39	3.29
13.	1102	cis-Thujone	0.47	0.10	0.84	0.23
14.	1124	α-Campholenal	0.77	0.52	0.64	0.79
15.	1141	Camphor	1.59	2.20	0.48	0.78
16.	1154	Nerol oxide	0.89	0.71	0.47	2.43
17.	1165	Pinocarvone	0.59	0.89	-	0.10
18.	1170	Borneol	0.72	0.28	-	0.58
19.	1174	Terpinen-4-ol,	3.82	2.10	4.35	4.23
20.	1183	Cryptone,	8.61	6.81	6.41	14.01
21.	1190	α-Terpineol	0.62	0.17	0.98	1.48
22.	1229	Nerol	1.70	1.59	1.11	5.60
23.	1242	Carvone	1.61	2.25	1.66	8.11
24.	1277	Carvone oxide - trans	0.48	0.35	-	2.15
25.	1293	Carvacrol	0.71	0.68	0.63	3.25
		Sesquiterpene hydrocarbons	1.84	6.20	4.74	1.40
26.	1463	Alloaromadendren	0.83	0.10	2.98	-
27.	1499	Selinene - α	1.01	6.10	1.76	1.40
		Oxygenated Sesquiterpenes	12.12	24.16	29.20	18.02
28.	1585	Caryophyllene oxide	9.16	19.72	23.57	15.39
29.	1588	Thujopsan-2α-ol	1.68	1.97	1.62	1.13
30.	1606	Cedrol	0.32	1.23	0.58	0.49
31.	1641	Cubenol	0.28	0.48	1.62	1.01
32.	1657	Cadinol - α	0.24	0.39	0.54	-
33.	1739	γ-Costol	0.44	0.37	1.27	-
		Total identified	98.17	97.95	97.87	96.27

Müeller-Hinton agar were inoculated with the microbial suspensions. Sterile Whatman No.1 (6 mm) discs, papers were individually placed on the surface of the seeded agar plates and 20 µL of essential oil in dimethyl sulfoxide (DMSO) was applied to the filter paper disk. The essential oils were added with concentration 25%, 50%, 75%, and 100 % (The various dilutions concentrations of essential oil were made by DMSO). After the plates were kept at room temperature for 30 min to allow the essential oil to diffuse into the agar, they were incubated at 37ºC for 24 h and the diameter of the resulting zones of inhibition (IZ) was measured. All tests were performed in triplicates. DMSO served as negative controls, and amoxicillin as positive control.

Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentrations (MIC) of the essential oils were determined using 96-well micro titer dilution method as described previously[13]. Bacterial cultures were incubated in Müeller-Hinton broth overnight at 37ºC and a 1:1 dilution of each culture in fresh Müeller-Hinton broth was prepared prior to use in the micro dilution assay. Sterile water (100 µL) was pipetted into all wells of the micro titre plate, before transferring 100 µL of essential oil in DMSO. Serial dilutions were made to obtain concentrations ranging from 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 mg/mL. 100 µl of bacterial culture of an approximate inoculum size of 1.0×10^8 CFU/mL was added to all well and incubated at 37ºC for 24 h. MIC is defined as the lowest concentration that produces an almost complete inhibition of visible micro-organism growth in liquid medium. DMSO solution served negative control.

To determine (MBC), broth was taken from the (MIC), where the organisms quantitatively indicate the minimum concentration when no viable organism appears in the culture and inoculated in Mueller Hinton agar for 24 h at 37ºC. The MBC is defined as the lowest

Table 3: Antibacterial activity of the essential oils (IZ in mm, MIC and MBC in mg/ml) of fresh leaves of *E. camaldulensis*.

Organism	*E.* samples	Different concentrations (percentages) of essential oil				amoxicillin
		25%	50%	75%	100%	30mg\mL
gram-positive bacteria						
Bacillus subtilis	S_1	7.70 ± 0.11	8.10 ± 0.10	15.10 ± 0.14	20.50 ± 0.17	40 ± 0.11
	S_2	14.10 ± 0.16	15.2 ± 0.18	19.10 ± 0.11	20.30 ± 0.18	
	S_3	8.30 ± 0.11	8.70 ± 0.14	9.20 ± 0.19	10.30 ± 0.15	
	S_4	12.20 ± 0.17	13.10 ± 0.11	15.20 ± 0.14	16.10 ± 0.12	
Micrococcus luteus	S_1	19.30 ± 0.17	21.50 ± 0.13	22.50 ± 0.16	28.30 ± 0.13	17 ± 0.21
	S_2	20.20 ± 0.18	22.40 ± 0.16	25.30 ± 0.12	28.20 ± 0.11	
	S_3	30.10 ± 0.14	38.50 ± 0.12	40.40 ± 0.17	42.20 ± 0.16	
	S_4	17.50 ± 0.11	23.50± 0.18	30.20 ± 0.14	35.30 ± 0.12	
	S_1	25.50 ± 0.14	29.70 ± 0.12	32.80 ± 0.11	34.50 ± 0.13	NA
Streptococcus	S_2	24.20 ± 0.18	28.40 ± 0.19	35.30 ± 0.12	38.20 ± 0.18	
pyogenes	S_3	19.60 ± 0.12	21.60 ± 0.18	27.50 ± 0.17	35.70 ± 0.17	
	S_4	23.10 ± 0.12	25.40 ± 0.13	30.30 ± 0.14	40.10 ± 0.19	
	S_1	24.70 ± 0.21	26.20 ± 0.31	29.50 ± 0.11	42.20 ± 0.18	45± 0.13
Staphylococcus	S_2	25.50 ± 0.17	29.60 ± 0.14	37.70 ± 0.15	43.50 ± 0.13	
aureus	S_3	22.40 ± 0.17	28.60 ± 0.14	35.30 ± 0.18	40.60 ± 0.15	
	S_4	32.60 ± 0.19	34.50 ± 0.13	40.30 ± 0.15	44.50 ± 0.18	
gram-negative bacteria						
Klebsiella	S_1	11.40 ± 0.19	13.50 ± 0.13	15.50 ± 0.14	16.50 ± 0.12	NA
pneumoniae	S_2	8.20 ± 0.14	9.40 ± 0.19	9.50 ± 0.15	10.20 ± 0.16	
	S_3	9.50 ± 0.15	10.30 ± 0.13	12.40 ± 0.12	15.20 ± 0.17	
	S_4	12.20 ± 0.15	16.30 ± 0.14	17.30 ± 0.11	18.40 ± 0.13	
Vibrio	S_1	30.20 ± 0.14	35.50 ± 0.16	42.70 ± 0.12	45.60 ± 0.15	30± 0.32
parahaemolyticus	S_2	35.50 ± 0.17	40.40 ± 0.15	42.40 ± 0.16	45.50 ± 0.14	
	S_3	32.40 ± 0.12	35.50 ± 0.13	42.30 ± 0.14	46.50 ± 0.16	
	S_4	38.10 ± 0.15	41.20 ± 0.11	44.30 ± 0.16	48.40 ± 0.13	
Proteus vulgaris	$S_{1,2,3,4}$	NA	NA	NA	NA	45± 0.12
Salmonella	S_1	9.70 ± 0.15	13.10 ± 0.10	19.70 ± 0.14	22.50 ± 0.13	16± 0.31
typhimurium	S_2	8.20 ± 0.14	8.70 ± 0.11	9.50 ± 0.18	10.20 ± 0.15	
	S_3	17.40 ± 0.15	18.30 ± 0.16	20.70 ± 0.12	23.40 ± 0.11	
	S_4	8.20 ± 0.15	8.50 ± 0.13	9.30 ± 0.12	10.20 ± 0.15	
Escherichia coli	S_1	13.20 ± 0.12	21.10 ± 0.11	25.10 ± 0.13	35.20 ± 0.14	NA
	S_2	9.60 ± 0.12	10.20 ± 0.14	10.80 ± 1.14	11.60 ± 1.12	
	S_3	12.40 ± 0.13	14.60 ± 0.12	16.50 ± 0.18	18.60 ± 0.15	
	S_4	12.70 ± 0.18	14.70 ± 0.13	21.30 ± 0.12	23.50 ± 0.17	
Pseudomonas aeruginosa	$S_{1,2,3,4}$	NA	NA	NA	NA	NA

Data are mean ± SD of three independent experiments. NA: Not actve.

concentration of the essential oil at which inoculated bacteria was totally killed. DMSO solution served negative control.

RESULTS AND DISCUSSION

Essential Oil Components

The amount of essential oil obtained by hydro distillation from the fresh leaves from four several places of province Damascus, Syria (S_1 – S_4) is presented in (Table 1). All the obtained essential oils were light yellow color and have camphor smell. The total chromatogram of the essential oil is displayed in (Fig. 1). The amounts of the components from the essential oil were determinate by the peak area normalization method. This presence of several overlapping peaks shows the complexity of the mixture. Thirty-three components were identified in the essential oil of (**S1**), this amount to 98.17% and of the total essential oil composition, and represent four different groups of hydrocarbon namely, monoterpene hydrocarbons (55.87%), oxygenated monoterpenes (28.34%), sesquiterpene hydrocarbons (1.84%,) and Oxygenated Sesquiterpenes (12.12%). The major components were, α -pinene (16.41%), P-cymene (19.21%), β -phellandrene, (11.46%), α-Phellandrene (3.79%), 1,8-cineole (5.76%), Cryptone (8.61%), Terpinen-4-ol, (3.82%) and Caryophyllene oxide (9.16%). Thirty-two compounds were identified in the essential oil of (**S2**), this amount to 97.95 % of the total essential oil

Table 4: (MIC) and (MBC) of the *E. camaldulensis* essential oil (Values in mg/mL).

Microorganism	*E.* samples	MIC	MBC
gram-positive bacteria			
Bacillus subtilis	S_1	1.6	1.6
	S_2	1.6	1.6
	S_3	3.2	3.2
	S_4	3.2	3.2
Micrococcus luteus	S_1	0.4	1.6
	S_2	0.4	1.6
	S_3	0.4	0.8
	S_4	0.2	0.4
	S_1	0.8	1.6
Streptococcus	S_2	0.8	0.8
pyogenes	S_3	0.8	0.8
	S_4	0.4	1.6
	S_1	0.2	0.8
Staphylococcus	S_2	0.2	0.8
aureus	S_3	0.2	0.8
	S_4	0.2	0.8
gram-negative bacteria			
Klebsiella	S_1	1.6	3.2
pneumoniae	S_2	3.2	3.2
	S_3	3.2	3.2
	S_4	1.6	3.2
Vibrio	S_1	0.1	0.2
parahaemolyticus	S_2	0.1	0.2
	S_3	0.1	0.2
	S_4	0.1	0.2
Proteus vulgaris	$S_{1,2,3,4}$	NA	NA
Salmonella	S_1	1.6	3.2
typhimurium	S_2	3.2	3.2
	S_3	1.6	3.2
	S_4	1.6	3.2
Escherichia coli	S_1	0.4	0.8
	S_2	3.2	3.2
	S_3	0.8	1.6
	S_4	1.6	1.6
Pseudomonas aeruginosa	$S_{1,2,3,4}$	NA	NA

NA: Not active.

composition, and the groups of hydrocarbon were monoterpene hydrocarbons (40.92%), oxygenated monoterpenes (26.47%), and sesquiterpene hydrocarbons (6.20%), oxygenated sesquiterpenes (24.16%). The main components of this essential oil were α -pinene (14.22%), P-cymene (20.37%), β -phellandrene, (4.14%), 1,8-cineole (7.82%), Cryptone (6.81%), Terpinen-4-ol, (2.10%), α -Selinene (6.10) and Caryophyllene oxide (19.72%). Thirty compounds were characterized in the oil of (**S3**), this amount to 97.87% the total essential oil composition, and the groups of hydrocarbon were: monoterpene hydrocarbons (43.97%), oxygenated monoterpenes (19.96%), sesquiterpene hydrocarbons (4.74%) and Oxygenated Sesquiterpenes (29.20%). The main components were: α -Thujene (3.27), α -pinene (2.17%), P-cymene (16.58%), β-phellandrene, (13.39%), α-Phellandrene (3.47%), 1,8-cineole (2.39%), Terpinen-4-ol, (4.35%), Cryptone (6.41%), Alloaromadendren (2.98), Caryophyllene oxide (23.57%). Also thirty compounds were characterized in the oil of (**S4**), this amount to 96.27% the total essential oil composition, and the groups of hydrocarbon were: monoterpene hydrocarbons (29.82%), oxygenated monoterpenes (47.03%), sesquiterpene hydrocarbons (1.40%) and Oxygenated Sesquiterpenes (18.02%). The main components were: α -pinene (2.18%), P-cymene (21.70%), β-phellandrene, (2.97%), 1,8-cineole (3.29%), Terpinen-4-ol, (4.23%), Cryptone (14.01%), Nerol (5.60), Carvone (8.11%), Carvacrol (3.25), Caryophyllene oxide (15.39%). The chemical composition of the oils can be seen in Table 2.

Antimicrobial activity

The in vitro antibacterial activity of the essential oils of *E. camaldulensis* against the bacterial strains and the activity (MIC) and (MBC) are summarized in (Table 3 and Table 4). The results showed the essential oils were active against eight of the ten bacterial strains organisms tested. The growths of tested bacteria in high concentrations of essential oil leaves were highly inhibited, where it was considered that these organisms were sensitive to the essential oil. The rate of inhibition was greater on bacteria *Vibrio parahaemolyticu* **(S4**: 48.40 ± 0.13), *Staphylococcus aureus* **(S4**: 44.50 ± 0.18), *Micrococcus luteus* **(S3:** 42.20 ± 0.16), and *Streptococcus pyogenes* **(S4**: 40.10 ± 0.19), than that observed on *Escherichia coli* **(S1**: 35.20 ± 0.14), *Bacillus subtilis* **S1** (20.50 ± 0.17), *Klebsiella pneumoniae* **(S4:** 18.40 ± 0.13) and *Salmonella typhimurium* **(S3**: 23.40 ± 0.11). Whereas it showed no activity against the *Pseudomonas aeruginosa* and *Proteus vulgaris*.

DISCUSSION

Essential oils composition, from the *E. camaldulensis* leaves, has been widely studied. By surveying the data reported we found a great diversity of essential oil composition, which was effected by many factors such as geographical origin, tissue explored, date of harvest, genetic factors[14,15] etc. Two groups of *E. camaldulensis* essential oils can be distinguished, those that contain 1,8-cineole as the main compound, which include *E. camaldulensis* from Iran[16] the main compounds were 1,8-cineole (26.1%), p-cymene (14.4%), spathulenol (13.2%), α-pinene (12.6%) and β-phellandrene (9.2%). In Egypt[17], the major constituents of the oil were 1,8-cineole (20.81%) followed by O-Cymene (19.11%), α- Phellandrene (9.21%) and Crypton (9.4%). IN Taiwan[9], the major constituents of the essential oil were 1,8-cineole (29.60%), limonene (15.20%), α-pinene (9.70%), citronellyl acetate (5.20%), globulol (4.70%). In Nigeria[18], 1,8-cineole (32.8%), (Z)-β-ocimene (11.6%), β-pinene (9.0%) and α-pinene (8.8%) were the main compounds. In Mozambique[19], 1,8-cineole (37.10%), P-Cymene (11.6%), γ –Terpinene (10.40%), Globulol (9.60%), Terpinen-4-ol (5.50%). And those that contain spathulenol, p-cymene, β -phellandrene, γ-Terpinene and cryptone as main compounds, and small quantities of 1,8-cineol, which are similar to *E. camaldulensis* from this study, and USA south of Florida[20], the major constituents identified in the essential oil included 1,8-cineole (2.70%), P-Cymene (35.0%),

Cryptone (13.70%), Terpinen-4-ol (5.70%), spathulenol (4.30%), Cuminaldehyde (3.70%). In Malaysia[21] 1,8-cineole (0.46%), O-Cymene (17.63%), γ –Terpinene (71.37%), Terpinen-4-ol (7.01%). In Montenegro[22] the major constituents identified in the essential oils collected from five locations at Montenegro coastline were 1,8-cineole (1.70 – 2.89%), P-Cymene (17.38 – 28.60%), β -phellandrene, (12.35 – 14.47%), Spathulenol (7.83 – 14.15%), α -phellandrene (2.36 - 4.26%), Terpinen-4-ol (2.75 – 4.21%). In Turkey[23], the main compounds were 1,8-cineole (13.92%), P-Cymene (68.43%), 1-(S)-α-pinene (3.45%), R-(+)-limonene (2.84%), 1,4-Terpineole (2.56%). essential oils of herbal origin have diverse biological activities which are caused by the terpenes that are their main components herbal essential oils are composed mostly of monoterpenes, sesquiterpemes and their oxygenic derivatives[24]. The antimicrobial activity of *E. camaldulensis* essential oil is due to the presence of a mixture of monoterpenes and oxygenated monoterpenes, when the most of the antimicrobial activity in the oils has been attributed to the oxygenated monoterpenes. The identification of such compounds with a wide biological activity is critical for the mankind as it helps in the search for chemical structures that should assist in designing new drugs as therapeautic agents against human pathogens. Some recent studies show that *E. camaldulensis* essential oil exhibits a great antimicrobial and repellent activity[25,26]. Also the essential oil had an excellent mosquito larvicidal activity[27]. The essential oil composition was similar to the one examined here, in respect to high cymene, α-pinene and phellandrene portion. The MIC and MBC values were determined by the macro dilution broth assay. In the present study, essential oils (Table 4) showed a high activity against a majority of the selected microorganisms. The Gram-positive strains showed more susceptibility to the tested essential oils than the Gram-negative. On the other hand, no activity was registered against *Proteus vulgaris* and *Pseudomonas aeruginosa* (gram-negative). The results of the MIC values against tested Gram-positive and Gram-negative bacteria varied from 0.1 to 3.2 mg/mL and from 0.2 to 3.2 mg/mL, respectively, with the lowest activity for *Bacillus subtilis* (Gram-positive), and for *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Escherichia coli* (Gram- negative), while the highest activity was observed against *Staphylococcus aureus* (Gram-positive), and *Vibrio parahaemolyticus* (Gram-negative). The results of the MBC values against tested Gram-positive and Gram-negative bacteria ranged from 0.4 to 3.2 mg/mL and from 0.2 to 3.2 mg/mL, respectively, with the lowest activity for *Bacillus subtilis* (Gram-positive), and for *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Escherichia coli* (Gram- negative), while the highest activity was observed against *Staphylococcus aureus* (Gram-positive), and *Vibrio parahaemolyticus* (Gram- negative).

CONCLUSION

These results indicate that the essential oil derived from *E. camaldulensis* trees, can inhibit the growth of Gram-positive and Gram-negative bacteria such as: *Micrococcus luteus*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Vibrio parahaemolyticus*. Therefore, it is possible can be used as antibacterial agents in medical treatment. On other hand, Monoterpene hydrocarbons were a major class of compounds. The major compounds were P-cymene, spathulenol, cryptone and β-phellandrene. It could be concluded from the present studies that *E. camaldulensis* from Syria is a potential source of essential oil. It should be further characterized for various commodities of cosmetics, medicinal and pharmacological attributes. The authors will investigate the essential oils in other seasons to find the best time of harvesting for obtaining the best quantity and quality of the essential oils.

ACKNOWLEDGEMENTS

The authors are thankful to the Head of Chemistry Department, faculty of science, Damascus University Prof. M. J. Alkhateeb for their keen interest in this work.

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