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ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL SCREENING OF SOME IRANIAN HERBAL PLANTS ON *Pectobacterium carotovorum*

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ABSTRACT

In recent years, there has been growing concern regarding undesirable side effects of synthetic antimicrobial drugs used for food preservation or in medicine and an explosive global spreading of multidrug resistant microbes are considered as a substantial global health threat. This necessitates the searching for new classes of safe and more effective antimicrobial agent by acting with different mechanisms. This study aimed to determine the antimicrobial activity of essential oils of some herbs which are endemic Iranian plants using minimum inhibitory (MIC) concentration of their essential oils. Results obtained from minimum inhibitory concentration showed that the essential oil of *Thymus vulgaris*, compared to other extracts, possess the best inhibitory effect in the lowest concentration. The extracts of *Artemisia kermanensis*, *Lavandula officinalis*, *Rosemarinous officinalis* and *Eucalyptus caesia* are reported to have inhibitory effect on *Pectobacterium carotovorum*.

Keywords: *Pectobacterium carotovorum*, essential oil, minimum inhibitory concentration.

Introduction

In recent years, there has been growing concern regarding undesirable side effects of synthetic antimicrobial drugs/chemicals used for food preservation or in medicine and an explosive global spreading of multidrug resistant microbes are considered as a substantial global health threat (Telci *et al.*, 2006). This necessitates the searching for new classes of safe and more effective antimicrobial agent by acting with different mechanisms. A number of plants containing secondary compounds could possess some of these ideal preservative characteristics mainly due to their antioxidant, antimicrobial and other biological potentials (Bakkali *et al.*, 2008). In this regard, an increasing body of research was conducted on many herbal and culinary species in order to seek new natural bioactive compounds with

special aims. Many pharmaceutical characteristics of aromatic plants are partially attributed to essential oils. Essential oils used in this study, including *Thymus vulgaris*, *Artemisia kermanensis*, *Eucalyptus caesia* Benth, *Lavandula officinalis and Rosemarinous officinalis* have been evaluated for their antimicrobial activities. Al-Bayati (Al-Bayati, 2008) claimed that thyme oil and methanolic extract had promising antibacterial activities against most pathogens. Kazemi and his coworkers investigated antimicrobial and antioxidant activities of the essential oil of *Artemisia kermanensis* against *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter* spp, *Klebsiellapneu moniae*and *Escherichia coli*. They reported that *Artemisia kermanensis* oil has maximum inhibition effect against *Klebsiella pneumonia*. In addition to these herbs, other investigated plants showed the same antimicrobial activity (Al-Bayati, 2008, Delaquis *et al.*, 2002, Ghalem and Mohamed, 2008, Kazemi *et al.*, 2011, Orhan *et al.*, 2012). In many cases, it is reported that the yield and composition of the essential oil for each species have been affected by different factors such as physiological variations, environmental conditions, geographic variations and genetic factors (Di Pasqua *et al.*, 2007, Takahashi *et al.*, 2004). *Pectobacterium carotovorum* is a bacterium of the family Enterobacteriaceae; it formerly was a member of the genus Erwinia. The species is a plant pathogen with a diverse host ranges including potato, African violet, and other agriculturally and scientifically important plant species. It causes soft rot and blackleg of potato and vegetables, as well as slime flux on many different tree species. Echis is more frequent in subtropical and tropical climates and has a host range that includes carnation, leopoldlily, maize, pineapple, potato and African violet (Saintpaulia ionantha).The soft rot erwinias are found on plant surfaces and in soil where they may enter the plant via wound sites or through natural openings on the plant surface, e.g. lenticels. As this bacterium causes diseases in lots of different plants, so it is important to find safe and effective antibacterial agent for fighting with this bacterium. In these research different concentrations of *Artemisia kermanensis* Podl, *Eucalyptus caesia*, *thymus vulgaris*, *Lavandula officinalis* and *Rosemarinous officinalis* oils have been used against *Pectobacterium carotovorum* and the aim is to find the best oil and concentration that inhibit bacteria growth.

Methodology

Plant materials and isolation of essential oils

In this study we used fresh aerial parts of the herbs *T. vulgaris*, *A. kermanensis*, *E. caesia* Benth, *L. officinalis* and *R. officinalis* which were collected from Lorestan and Chaharmahal provinces (Iran) in 2012. The herbs were then dried at room temperature (25 °C) for 3 days. The dried herb samples (500 g) were ground and subjected to hydro distillation using a Clevenger-type apparatus. The oils were dried over anhydrous Na_2SO_4 and stored at 4 °C in a sealed amber vials until use (These vials can be used for one month).

Oil analysis procedure

Analysis was performed using GC-mass chromatograph with a HP-5MS column (30 m × 0.25 mm, film thickness 0.25 m). The carrier gas was helium at flow rate of 0.8 ml min^{-1}. The column temperature was kept at 50 °C for 2 minutes and it was programmed to 200 °C at a rate of 3 °C min^{-1} and kept constant at 200 °C for 10 minutes. Helium gas was used as carrier gas. The injection was performed in split mode with ratio of 50:1 at 250 °C. The compounds were identified by comparison of RRI (relative retention indices) with those reported in the literature and also by comparison of their mass spectra with published mass spectra (Adams, 2005; Sparkman, 1997). The retention indices for all the components were determined according to the Van Den Dool method using n-alkanes as standards (Van Den Dool and Kratz, 1963).

Preparing bacterial strain and Dextrose Agar

The bacterial strain was provided from Iranian Research Organization of Science and Technology. In this experiment Potato Dextrose Broth (PDB) and Potato Dextrose Agar (PDA) were taken from Merck Company.

Determination of inhibition of zone (IZ)

In order to study the antimicrobial effect, Disk diffusion method was used. After 18 h of culture, liquid containing bacteria with standard density (1×10^6 CFU ml^{-1}) of 0.5 Mac Farland in Potato Dextrose Broth (PDB) was prepared and 500 µl of the liquid was transferred to Potato Dextrose Agar (PDA). The liquid was gently distributed on the surface of PDA using sterile loop. There have been blank disks with 6mm in diameter containing 30µl with concentrations 25000, 50000, 300000, 100000 and 400000 ppm on PDA. Disk containing antibiotics Gentamicin and Chloramphenicol was used as positive control and also disk containing 30µl of DMSO was used as negative control. The diameter of inhibition of zone was measured using caliper after 24 h of incubation at 37 °C at the times 24. 48 and 72 h in triplicate.

Determination of MIC using dilution of wells

Firstly in order to determine the minimum inhibitory concentration (MIC) of *Pectobacterium carotovorum*, the suspension of bacterial strain was prepared from liquid culture with standard darkness of 0.5 Mac Farland. The essential oil which is diluted 10 % with ethanol with primary concentration of 500µg ml^{-1} was prepared and different dilutions (6 dilutions) were added to the pipes containing 10 ml of liquid culture medium. MIC of essential oils was performed using Microwell method against bacterial strain (Sahin *et al.*, 2004).Then the *96*-well plate was used for determination of MIC. 95µl of Potato Dextrose Broth (PDB) and 5µl of microbial suspension was added to every well. 100µl of the essential oil with concentration of 500µg ml^{-1} was added to the first well. Then 100µl was taken from the first well and it was transferred to the next well. This process went on to the 6th well. The last well was contained 195µl of PDB culture medium and also 5µl of microbial suspension without any essential oil. This well was considered as negative control. In the next step, the ingredients of every well were mixed using Rotary Shaker for 20 min. Then it was put in an incubator for 24 h in an appropriate temperature (37 °C). The microbial growth was measured by spectrophotometer at 600nm (Gavanji *et al.*, 2011). In this study the effect of each essential oil was determined on *Pectobacterium carotovorum* separately with 3 replicates.

Statistical analysis

Before any statistical analysis, the normality of data and homogeneity of variances were evaluated. A factorial experimental technique has been used to investigate the types of plants, concentration of essential oil, interaction between types of plants and also concentration of essential oil. At second analysis, higher concentration of each plant was compared to two important antibiotics, CHEL and GEN through one way ANOVA (Minitab 16). Means of treatments were compared by Tukey's multiple comparison test. Differences were considered as significant at $P<0.05$.

Results and Discussion

According to the results given in Table1, at three times of incubation, *thymus vulgaris* showed antimicrobial activity in a dose dependent manner and the most antimicrobial activity was observed at 300000ppm (21.74 mm inhibition of zone). There was no significant difference at different times of incubation ($p>0.10$). *A. kermanensis* is another plant which was not effective at low doses (6.56 and 8.94 mm for 25000 and 50000 ppm respectively), but moderate dose of the oil (10000 ppm) resulted in a considerable antimicrobial activity (16.24 mm) and the highest dose of inclusion showed the most effective antimicrobial property. The same antimicrobial property was observed for *E. caesia*, but the concentration 300000 ppm was the most effective and increasing the concentration to 400000 ppm led to exert lower antimicrobial activity (19.20 versus 18.03; $p=0.06$). At the concentration 50000 ppm, two essential oils *L. officinalis* and *R. officinalis* showed a mild antimicrobial activity and increasing dose of the oil had improved this response and there was no difference between two higher doses ($p>0,10$).

Results obtained from MIC showed that the essential oils of *T. vulgaris* is equal to 145 µg ml^{-1} which has the best inhibitory effect with lowest concentration compared to other extracts. The extracts of *A. kermanensis*, *L. officinalis*, *R.officinalis* and *E. caesia* are reported to have inhibitory effect on *Pectobacterium carotovorum* respectively (Figure1).

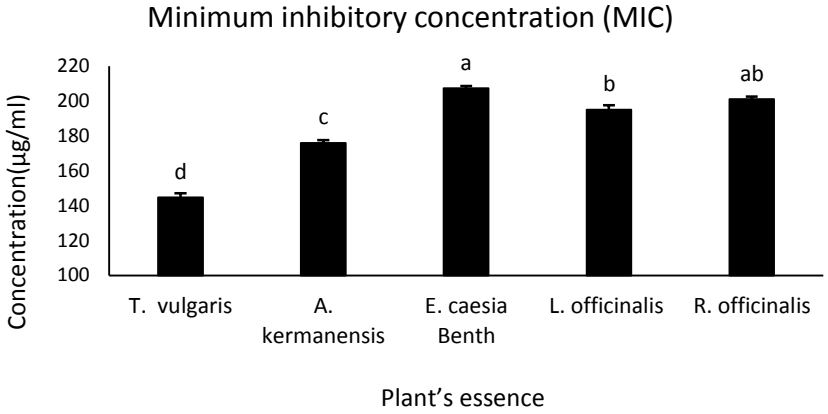

Figure 1: Minimum inhibitory concentration (MIC) of extracts of herbal plants. Means with different letters show significantly different between treatment groups (p< 0.0001).

Table1. Antibacterial activity at different concentrations of some Iranian herbal plants.

		Time(h)		
Plant's essence	**Con (ppm)**	**24**	**48**	**72**
Thymus vulgaris	25000	7.05^{ghi}	7.09^{ghi}	7.14^{ghi}
	50000	13.71^{f}	13.92^{e}	13.92^{de}
	100000	17.96^{cd}	18.16^{bc}	18.36^{bc}
	300000	21.47^{ab}	22.07^{a}	22.11^{a}
	400000	22.41^{a}	22.47^{a}	22.48^{a}
Artemisia kermanensis	25000	6.56^{hi}	6.60^{hi}	6.73^{ghi}
	50000	8.94^{gh}	8.94^{fgh}	8.94^{fgh}
	100000	16.24^{de}	16.43^{cd}	16.41^{cd}
	300000	16.86^{cd}	16.90^{c}	16.89^{c}
	400000	18.72^{c}	18.84^{bc}	18.84^{bc}
Eucalyptus caesia Benth	25000	6.40^{i}	6.40^{i}	6.40^{i}
	50000	6.61^{hi}	6.61^{hi}	6.61^{hi}
	100000	13.57^{f}	13.58^{e}	13.80^{e}
	300000	19.20^{bc}	19.42^{b}	19.45^{b}
	400000	18.03^{cd}	18.22^{bc}	18.23^{bc}
Lavandula officinalis	25000	6.42^{i}	6.43^{i}	6.43^{hi}
	50000	9.16^{g}	9.18^{fg}	9.22^{fg}
	100000	12.34^{f}	12.38^{e}	12.40^{e}
	300000	17.84^{cd}	17.97^{bc}	17.98^{bc}
	400000	18.35^{cd}	18.49^{bc}	18.50^{bc}
Rosemarinous officinalis	25000	6.67^{hi}	6.67^{hi}	6.67^{hi}
	50000	9.42^{g}	9.70^{f}	9.75^{f}
	100000	13.87^{ef}	14.22^{de}	14.32^{de}
	300000	17.85^{cd}	17.92^{bc}	17.97^{bc}
	400000	18.10^{cd}	18.18^{bc}	18.28^{bc}
SEM		0.44	0.45	0.46
P values		<0.0001	<0.0001	<0.0001
R-Square		0.99	0.98	0.98
CV (%)		5.54	5.67	5.76

Means with the same letters in each column are not significantly different at $p<0.05$.

Con: Concentration of plant's essence

Table2. Compositions of *Thymus vulgari*

No	Compositions	%	RI	Sn+1	Sn	RT	Cn+1	Cn
1	alpha-thujene	0.05	930	5.349	3.39	3.97	10	9
2	ALPHA-PINENE	3.19	937	5.349	3.39	4.11	10	9
3	Verbenene	0.03	956	5.349	3.39	4.488	10	9
4	Sabinene	0.51	975	5.349	3.39	4.86	10	9
5	BETA-PINENE	0.12	979	5.349	3.39	4.94	10	9
6	Heptanol	0.08	988	5.349	3.39	5.117	10	9
7	BETA-MYRCENE	0.18	992	5.349	3.39	5.197	10	9
8	gama-terpinene	0.33	1006	7.991	5.349	5.518	11	10
9	alpha-terpinen	0.07	1017	7.991	5.349	5.81	11	10
10	p-Cymene	0.21	1025	7.991	5.349	6.004	11	10
11	LINALOOL	20.2	1032	7.991	5.349	6.187	11	10
12	Ocimene	0.7	1036	7.991	5.349	6.313	11	10
13	Dimethylstyrene	0.12	1087	7.991	5.349	7.658	11	10
14	LINALOOL	0.57	1099	7.991	5.349	7.961	11	10
15	BETA.-THUJONE	0.06	1106	11.075	7.991	8.161	12	11
16	alpha.-Campholen	0.21	1124	11.075	7.991	8.734	12	11
17	Mentha-2-en-1-ol	0.59	1132	11.075	7.991	8.985	12	11
18	Trans Limonene Oxide	0.39	1135	11.075	7.991	9.071	12	11
19	Camphor	0.37	1142	11.075	7.991	9.283	12	11
20	Menthol	0.13	1170	11.075	7.991	10.15	12	11
21	Terpene-4-ol	0.5	1174	11.075	7.991	10.26	12	11
22	Naphtalene	0.12	1177	11.075	7.991	10.38	12	11
23	alpha.-Terpineol	0.66	1191	11.075	7.991	10.80	12	11
24	Carvacrol	43.4	1209	14.282	11.075	11.37	13	12
25	trans-Carveol	1.73	1216	14.282	11.075	11.59	13	12
26	Neral	1	1238	14.282	11.075	12.28	13	12
27	Carvone	1.45	1240	14.282	11.075	12.35	13	12
28	Geraniol	0.4	1250	14.282	11.075	12.67	13	12
29	2.6-Octadiena	1.51	1266	14.282	11.075	13.19	13	12
30	METHYLBENZOATE	0.18	1268	14.282	11.075	13.27	13	12
31	Isomenthone	4.92	1286	14.282	11.075	13.83	13	12
32	LimonelAlchol	1.9	1292	14.282	11.075	14.02	13	12
33	gamma. 1-cadinene	0.08	1343	17.378	14.282	15.61	14	13
34	Eugenol	0.08	1351	17.378	14.282	15.85	14	13
35	.alpha.-Copaene	0.32	1369	17.378	14.282	16.42	14	13
36	LINALYL ACETATE	0.5	1379	17.378	14.282	16.72	14	13
37	CisJasmone	0.18	1393	17.378	14.282	17.15	14	13
38	trans-Caryophyllene	0.27	1412	20.409	17.378	17.75	15	14
39	GERMACRENE-D	0.27	1474	20.409	17.378	19.61	15	14
40	delta.-Cadinene	0.18	1516	23.333	20.409	20.87	16	15
41	Caryophyllene oxide	0.15	1574	23.333	20.409	22.57	16	15
42	alpha-thujene	0.05	930	5.349	3.39	3.97	10	9
43	ALPHA-PINENE	3.19	937	5.349	3.39	4.11	10	9
44	Verbenene	0.03	956	5.349	3.39	4.488	10	9
45	Sabinene	0.51	975	5.349	3.39	4.86	10	9
46	BETA-PINENE	0.12	979	5.349	3.39	4.94	10	9
47	Heptanol	0.08	988	5.349	3.39	5.117	10	9
48	BETA-MYRCENE	0.18	992	5.349	3.39	5.197	10	9
49	gama-terpinene	0.33	1006	7.991	5.349	5.518	11	10
50	alpha-terpinen	0.07	1017	7.991	5.349	5.81	11	10
51	p-Cymene	0.21	1025	7.991	5.349	6.004	11	10
52	LINALOOL	20.2	1032	7.991	5.349	6.187	11	10
	Total	**87.83**						

Table3. Compositions of *Artemisia kermanensis*

No	Compositions	%	RI	Sn+1	Sn	RT	Cn+1	Cn
1	artemisiatriene	0.41	926	5.349	3.39	3.893	10	9
2	ALPHA-PINENE	0.54	934	5.349	3.39	4.064	10	9
3	Camphene	0.93	949	5.349	3.39	4.345	10	9
4	Verbenene	1.88	954	5.349	3.39	4.448	10	9
5	Benzaldehyde	0.11	960	5.349	3.39	4.562	10	9
6	BETA-PINENE	0.08	977	5.349	3.39	4.9	10	9
7	p-menthatriene	0.57	993	5.349	3.39	5.203	10	9
8	yomogi alcohol	2.67	1001	7.991	5.349	5.375	11	10
9	alpha.-Terpinene	0.2	1016	7.991	5.349	5.77	11	10
10	PARA CYMENE	1.88	1024	7.991	5.349	5.97	11	10
11	1,8-Cineole	1.82	1030	7.991	5.349	6.142	11	10
12	Artemisia Ketone,	0.11	1032	7.991	5.349	6.204	11	10
13	trans-Carane	0.13	1050	7.991	5.349	6.674	11	10
14	gama-terpinene	0.41	1056	7.991	5.349	6.828	11	10
15	Artemesia alcohol	1.48	1082	7.991	5.349	7.526	11	10
16	Styrene,	0.82	1087	7.991	5.349	7.658	11	10
17	alpha.-Thujone	13.83	1108	11.075	7.991	8.253	12	11
18	Beta-Thujone	6.23	1117	11.075	7.991	8.522	12	11
19	trans-Pinocarveol	1.39	1138	11.075	7.991	9.163	12	11
20	Camphor	4.13	1142	11,075	7.991	9.289	12	11
21	Camphore	10.23	1144	11.075	7.991	9.363	12	11
22	p-Menth-1,5-dien-8-ol	2.04	1147	11.075	7.991	9.455	12	11
23	1-Menthene	0.49	1156	11.075	7.991	9.712	12	11
24	Pinocarvone	1.37	1160	11.075	7.991	9.838	12	11
25	Borneol	1.97	1164	11.075	7.991	9.952	12	11
26	p-Mentha-1,5-dien-8-ol	4.38	1166	11.075	7.991	10.021	12	11
27	Terpinene-4-ol	1.01	1175	11.075	7.991	10.307	12	11
28	Naphthalene	0.73	1178	11.075	7.991	10.393	12	11
29	p-Cymen-3-ol	1.26	1182	11.075	7.991	10.519	12	11
30	alpha.-Terpineol	0.72	1188	11.075	7.991	10.691	12	11
31	Verbenone	1.53	1206	14.282	11.075	11.274	13	12
32	Norbornane	0.36	1215	14.282	11.075	11.543	13	12
33	Cuminic aldehyde	1.1	1235	14.282	11.075	12.19	13	12
34	(+)-Carvone	0.48	1239	14.282	11.075	12.321	13	12
35	Carvotanacetone	0.28	1243	14.282	11.075	12.441	13	12
36	CIS-MYRTANOL	0.15	1247	14.282	11.075	12.579	13	12
37	Carvenone	0.12	1253	14.282	11.075	12.762	13	12
38	Chrysanthenyl Acetate	1	1256	14.282	11.075	12.882	13	12
39	Cinnamic aldehyde-E	0.16	1264	14.282	11.075	13.134	13	12
40	Bornyl acetate	2.3	1280	17.378	14.282	13.654	14	13
41	Thymol	1.29	1286	17.378	14.282	13.86	14	13
42	Carvacrol	1.78	1297	17.378	14.282	14.175	14	13
43	alpha.-Copaene	0.23	1368	20.409	17.378	16.412	15	14
44	Methyl cinnamate	0.15	1375.7	20.409	17.378	16.641	15	14
45	(Z)-Jasmone	0.22	1393.1	20.409	17.378	17.168	15	14
46	Methyleugenol	0.15	1399.3	20.409	17.378	17.357	15	14
47	trans-Caryophyllene	0.3	1395.6	23.333	20.409	17.746	16	15
48	Alpha.-Curcumen	0.15	1475.4	23.333	20.409	19.691	16	15
49	Spathulenol	0.25	1569	23.333	20.409	22.426	16	15
50	Caryophyllene	0.07	1644.5	26.141	23.333	24.583	17	16
	Total	**75.84**						

Table 4. Compositions of *Lavandula officinalis*

No	Compositions	%	RI	Sn+1	Sn	RT	Cn+1	Cn
1	ALPHA-PINENE	7.58	938	5.349	3.39	4.139	10	9
2	CAMPHENE	4.51	952	5.349	3.39	4.408	10	9
3	Verbenene	0.64	956	5.349	3.39	4.488	10	9
4	1,3,5-Cycloheptatriene	0.03	972	5.349	3.39	4.808	10	9
5	BETA-PINENE	0.49	979	5.349	3.39	4.94	10	9
6	3-OCTANONE	2.19	988	5.349	3.39	5.123	10	9
7	BETA-MYRCENE	1.18	993	5.349	3.39	5.209	10	9
8	3 OCTANOL	0.36	997	5.349	3.39	5.295	10	9
9	AlphaPhellandrene	0.05	1007	7.991	5.349	5.523	11	10
10	o-Isopropenyltoluene	0.08	1014	7.991	5.349	5.712	11	10
11	AlphaTerpinene	0.12	1017	7.991	5.349	5.81	11	10
12	p-Cymene	2.96	1025	7.991	5.349	6.01	11	10
13	1,8-Cineol	12.01	1033	7.991	5.349	6.222	11	10
14	gamma.-Terpinene	0.08	1057	7.991	5.349	6.851	11	10
15	Linalool Oxide	0.06	1072	7.991	5.349	7.24	11	10
16	Methyl banzoate	0.99	1088	7.991	5.349	7.669	11	10
17	Linalool	2.45	1100	11.075	7.991	8.001	12	11
18	Thujancis	0.81	1103	11.075	7.991	8.098	12	11
19	D-Fenchyl alcohol	0.28	1112	11.075	7.991	8.373	12	11
20	Pinocarveol	0.12	1138	11.075	7.991	9.157	12	11
21	Camphore	9.16	1144	11.075	7.991	9.346	12	11
22	Isopinocamphone	1.72	1158	11.075	7.991	9.775	12	11
23	Pinocarvone	0.13	1160	11.075	7.991	9.832	12	11
24	Pinocamphone	0.39	1171	11.075	7.991	10.193	12	11
25	Terpene-4-ol	1.27	1174	11.075	7.991	10.284	12	11
26	naphtalene	0.08	1177	11.075	7.991	10.378	12	11
27	p-Cymen-8-ol	0.23	1183	11.075	7.991	10.553	12	11
28	AlphaTerpineol	2.31	1188	11.075	7.991	10.702	12	11
29	Myrtenol	0.35	1194	11.075	7.991	10.896	12	11
30	no pol (terpene)	1.11	1203	14.282	11.075	11.182	13	12
31	Verbenone	8.47	1209	14.282	11.075	11.366	13	12
32	trans-Carveol	0.13	1215	14.282	11.075	11.554	13	12
33	beta.-Citronellol	0.1	1225	14.282	11.075	11.869	13	12
34	Pulegone	0.09	1235	14.282	11.075	12.195	13	12
35	Piperitone	0.04	1250	14.282	11.075	12.67	13	12
36	Cinnamaldehyde	0.05	1265	14.282	11.075	13.145	13	12
37	Borneol acetate	2.41	1281	14.282	11.075	13.66	13	12
38	Thymol	6.23	1288	14.282	11.075	13.906	13	12
39	2-Hydroxy-4-Isopropyl-1-Methylbenzene	0.17	1290	14.282	11.075	13.975	13	12
40	Carvacrol	4.14	1297	14.282	11.075	14.192	13	12
41	alpha.-Terpinene	0.15	1329	17.378	14.282	15.194	14	13
42	PIPERITENONE	0.37	1335	17.378	14.282	15.36	14	13
43	alpha.-Cubebene	0.06	1343	17.378	14.282	15.617	14	13
44	Thymyl acetate	0.06	1349	17.378	14.282	15.789	14	13
45	alpha.-Copaene	0.43	1369	17.378	14.282	16.424	14	13
46	trans-Caryophyllene	0.47	1412	20.409	17.378	17.751	15	14
47	alpha.-Humulene	0.22	1446	20.409	17.378	18.781	15	14
48	Farnesene	0.08	1451	20.409	17.378	18.93	15	14
49	eta.-Acoradiene	0.09	1460	20.409	17.378	19.182	15	14
50	gamma.-Cadinene	0.16	1473	20.409	17.378	19.599	15	14
51	Zingiberene	0.1	1488	20.409	17.378	20.034	15	14

52	.beta.-Himachalene	0.28	1492	20.409	17.378	20.166	15	14
53	delta.-Cadinene	0.27	1516	23.333	20.409	20.881	16	15
54	Alpha-Cedrene	0.15	1524	23.333	20.409	21.116	16	15
55	Germacrene B	0.06	1548	23.333	20.409	21.814	16	15
56	spathulenol	0.26	1567	23.333	20.409	22.375	16	15
57	Caryophyllene oxide	0.29	1572	23.333	20.409	22.518	16	15
58	AlphaFarnesene	0.06	1587	23.333	20.409	22.941	16	15
59	ButlidenePhthalide	0.15	1642	26.141	23.333	24.515	17	16
60	3N ButylPhthalide	4.62	1687	26.141	23.333	25.773	17	16
61	ButylideneDihydro-Phthalide	0.09	1720	28.733	26.141	26.66	18	17
62	ALPHA-PINENE	7.58	938	5.349	3.39	4.139	10	9
63	CAMPHENE	4.51	952	5.349	3.39	4.408	10	9
64	Verbenene	0.64	956	5.349	3.39	4.488	10	9
65	1,3,5-Cycloheptatriene	0.03	972	5.349	3.39	4.808	10	9
66	BETA-PINENE	0.49	979	5.349	3.39	4.94	10	9
67	3-OCTANONE	2.19	988	5.349	3.39	5.123	10	9
68	BETA-MYRCENE	1.18	993	5.349	3.39	5.209	10	9
69	3 OCTANOL	0.36	997	5.349	3.39	5.295	10	9
	Total	**83.99**						

Table 5. Compositions of *Eucalyptus caesia*

No	Compositions	%	RI	Sn+1	Sn	RT	Cn+1	Cn
1	ALPHA-Thujan	0.3	929	5.349	3.39	3.967	10	9
2	ALPHA-PINENE	7.7	937	5.349	3.39	4.116	10	9
3	CAMPHENE	0.09	951	5.349	3.39	4.385	10	9
4	Sabinene	0.08	975	5.349	3.39	4.86	10	9
5	BETA-PINENE	0.7	979	5.349	3.39	4.94	10	9
6	BETA-MYRCENE	0.63	992	5.349	3.39	5.197	10	9
7	ALPHA. TERPINENE	0.2	1018	7.991	5.349	5.821	11	10
8	p-Cymene	14.11	1029	7.991	5.349	6.107	11	10
9	1,8-CINEOL	40.18	1034	7.991	5.349	6.244	11	10
10	gamma.-Terpinene	12.43	1059	7.991	5.349	6.92	11	10
11	ALPHA. TERPINENOL	1.74	1087	7.991	5.349	7.652	11	10
12	LINALOOL	0.13	1099	11.075	7.991	7.967	12	11
13	FENCHYL ALCOHOL	0.07	1112	11.075	7.991	8.361	12	11
14	trans-Pinocarveol	0.62	1136	11.075	7.991	9.088	12	11
15	MENTHOFURAN	0.16	1159	11.075	7.991	9.815	12	11
16	BORNEOL	0.13	1162	11.075	7.991	9.901	12	11
17	Terpinene-4-ol	5.62	1174	11.075	7.991	10.284	12	11
18	p-Cymen-8-ol	0.72	1181	11.075	7.991	10.502	12	11
19	MENTHOL	1.07	1184	11.075	7.991	10.576	12	11
20	ALPHA. TERPINEOL	1.53	1187	11.075	7.991	10.679	12	11
21	trans-Carveol	0.77	1214	14.282	11.075	11.526	13	12
22	cis-Carveol	0.41	1226	14.282	11.075	11.898	13	12
23	Carvone	0.25	1239	14.282	11.075	12.31	13	12
24	GERANIOL	0.52	1249	14.282	11.075	12.653	13	12
25	Thymol	0.51	1280	14.282	11.075	13.637	13	12
26	Carvacrol ETHYL ETHER	0.52	1288	14.282	11.075	13.912	13	12
27	Carvacrol	0.41	1295	14.282	11.075	14.112	13	12
	Total 91.6							

Table 6. Compositions of *Rosemarinus officinalis*

No	Compositions	%	RI	Sn+1	Sn	RT	Cn+1	Cn
1	alpha-pinene	23.93	942.318	5.349	3.39	4.219	10	9
2	Camphen	8.7	955.436	5.349	3.39	4.476	10	9
3	Vernenen	1.3	959.826	5.349	3.39	4.562	10	9
4	3-Octanone	5.63	991.679	5.349	3.39	5.186	10	9
5	P-cymene	7.48	1026.95	7.991	5.349	6.061	11	10
6	Limonene	2.99	1031.3	7.991	5.349	6.176	11	10
7	P- cymenene	1.13	1089.33	7.991	5.349	7.709	11	10
8	Camphor	10.97	1144.39	11.075	7.991	9.36	12	11
9	Naphtalene	0.32	1178.44	11.075	7.991	10.41	12	11
10	p-Cymen-8-ol	0.36	1182.72	11.075	7.991	10.542	12	11
11	Verbenon	15.44	1208.57	14.282	11.075	11.35	13	12
	Total 78.25							

Comparing various essential oils revealed that at 24 h post incubation, the lowest dose of essential oils showed the same inhibition zone ($p > 0.10$), but increasing essential oil to 50000 ppm has resulted in highest antimicrobial activity for *thymus vulgaris* followed by *Rosemarinus Officinalis* and *Lavandula Officinalis* (13,71, 9,42 and 6.16 mm) respectively. As it is shown in Figure 2, at highest concentration of essential oils (400000), *Thymus vulgaris* was as effective as two positive controls gentamicin and Chloramphenicol in inhibiting microbial growth. Other essential oils had lower antimicrobial activity and at 48 and 72 h post incubation, the same response was observed.

Historically, many plant oils and extracts have been used as topical antiseptics and reported to have antimicrobial properties. This specific characteristics, beside of other health benefit properties of herbs, make them suitable candidate for use in the pharmacological usage. In recent years, many researchers investigated antimicrobial activity of many herbal plants, or their bioactive compounds as possible alternatives to chemically synthetic antimicrobial drugs to which many pathogenic microorganisms have become resistant. Various researches have documented the antimicrobial activity of essential oils and plant extracts including *Artemisia kermanensis* Podl, *Eucalyptus caesia*, *thymus vulgaris*, *Lavandula officinalis*and *Rosmarinus officinalis* (Bayoub *et al.*, 2010, Figueiredo *et al.*, 2008, Fu *et al.*, 2007, Ghalem and Mohamed, 2008, Stojanović-Radić *et al.*, 2010). The medicinal plants have been long used against the growth of bacteria and several studies have been conducted on the effects of these plants (Gavanji *et al.,* 2012a). The results of *T. vulgaris* analysis showed that more than 42 chemical compounds have been identified (Table 2) that 87.83% of which constitute the essential components. The major components of the essential oil are made by Carvacrol (43.42%) and other major compounds are ALPHA-PINENE (3.19%), LINALOOL (20,22%), Carvone (1.45%) and Isomenthone (4.92%). Carvacrol and Thymol are two phenolic compounds found in *T. vulgaris* which their strong antimicrobial effect has been revealed by researchers in several studies (Vernozy-Rozand *et al.*, 2002, Özkan *et al.*, 2003).

Studies in 2003 showed that the *T. vulgaris* essential oil using Disc Diffusion method at the concentration of 0.4% has antimicrobial activity on *E.coli*. Also Burt and his coworker in 2003 showed that the *T. vulgaris* essential oil in low concentrations (0.12% and 0.25%) possess Bacteriostatic and bactericidal effects respectively (Burt and Reinders, 2003). As the oil possess higher degree of phenolic material, it has stronger antimicrobial properties. These materials include Carvacrol, Eugenol and Thymol (Burt, 2004). Also it has been proven that essential components interact with each other and play an important role in determining the antimicrobial effect of the plant. Carvacrol and Thymol have Synergistic effects (Didry *et al.*, 1994). *R. officinalis* is another plant studied in this experiment. The chemical analysis of rosemary essential oil by GC has identified 11 compounds (Table 6) which they make 78.25% rosemary essential oil. Major components of the essential oil include alpha-pinene (23.93%), Camphen (8.7%), Camphor (10.97%), Verbenon (15.44%), P-cymene (7.48%) and 3-Octanone (5.63%). Usually essential oils which are rich in phenolic compounds show significant antimicrobial properties. In fact the phenolic compounds presented in the essential oils create the most effective

antimicrobial properties. These compounds can penetrate into the cell membrane and the cell contents could have been involved in clotting (Gavanji *et al.*, 2012b, Minnunni *et al.*, 1992). In general, terpenes may have other different antimicrobial mechanisms. The investigations have also revealed that the essential oils may disrupt energy production and synthesis of structural components of the yeast enzyme activity system (Conner and Beuchat, 1984). Results obtained from *A.kermanensis* showed more than 50 chemical compounds (Table 3) in which 75.84% is components of essential oil. The major compounds of the essential oil are p-Menth-1, 5-dien-8-ol (4.38%), Camphore (14.36%) and Beta-Thujone (6.23%). The essential oil of various species of *Artemisia kermanensis* have effective antibacterial activities against bacteria such as *oureus*, *Staphylococcus*, *E. coli* and *Staphylococcus epidermidis* as well as against yeast *Crypto coccus*, *Candida albicans* and dermatophytes fungi such as *Canis*, *Microsporum*, *Microsporum gypseum*, *Fonsecaea pedrosoi* and*Trichophyton rubrum* (Lopez- Lutz *et al.*, 2008). According to some studies, the amount of 1,8-Cineole in specie of *Eucalyptusglobuls* in Uruguay country was reported 64.5% (Dellacasa *et al.*, 1990). Also this component in Cuba country was reported between 75 to 77% (Magraner Hernandez *et al.*, 1988). Also in California it is equal to 86.67% (Nishimura and Calvin, 1979), in Morocco is between 58 to 82% (Zrira and Benjilali, 1996) and in South Africa it is reported 48.7% (Thilivahalt *et al.*, 1986). The overall quality and quantity of the essential oil of particular species vary according to season, geographical location and the location of plants. In some species, the essence is well made in warm and sunny season. Climate and soil conditions can affect the composition of the oil (Arnold *et al.*, 1997). Based on results obtained from *E. caesia* Benth in Khuzestan province (Iran), the analysis of 1,8-CINEOL by GC system was reported 69.4%.

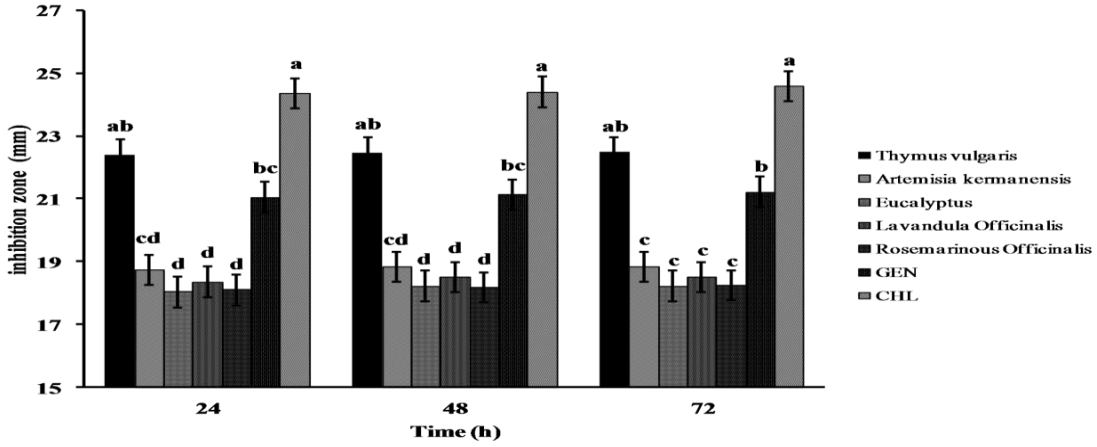

Figure 2. The highest concentration of essential oils of treatment(400000 ppm) in comparison to two positive control antimicrobial agent gentamicin (GEN) and Chloramphenicol (CHL) in inhibiting microbial growth. Same letters in each column show no significantly different existed at $p< 0.05$.

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