academicJournals

Vol. 8(3), pp. 186-194, 17 January, 2014
DOI: 10.5897/JMPR12.1298
ISSN 1996-0875 ©2014 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro antibacterial activity and phytochemical analysis of some medicinal plants

Mohammadi Abolfazl[1], Amrollahi Hadi[2], Malek Frhad[3] and Nazari Hossein[1]*

[1]Department of Biochemistry, Faculty of Medicine, Semnan University of Medical Science, Semnan, Iran.
[2]Department of Microbiology, Faculty of Medicine, Semnan University of Medical Science, Semnan, Iran.
[3]Department of Internal Medicine, Faculty of Medicine, Semnan University of Medical Science, Semnan, Iran.

Accepted 19 November, 2013

Essential oils of medicinal plants have been used traditionally against pathogenic bacteria that caused infectious disease in human and microbial spoilage of food and have been used safely in herbal medicine as antibacterial compounds. In the present study, the antibacterial activities of the oils were evaluated against human and animals pathogenic bacteria. In this assay, the selective plants reported ethnobotanical uses traditionally and also were referenced in some herbal medicine text. The essential oil of *Stachys pubescens*, *Mentha piperita, Clinopodium vulgare* and *Satureja hortensis* were prepared by hydrodistillation and were analyzed by gas chromatography/mass spectrometry (GC/MS). The number 23, 22, 21 and 21 components were identified in *S. pubescens*, *S. hortensis*, *M. piperita* and *C. vulgare*, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of oils were determined with broth microdilution and agar diffusion method on bacterial strains. Results from the antibacterial testing indicated that *S. pubescens, M. piperita* and *C. vulgare* essential oils showed high activities and inhibited the growth of all the selected bacteria. While the essential oil of *S. hortensis* displayed the moderate potential activity. Our finding supported the notion that plant essential oils composition or total extract may have a role as pharmaceuticals and preservatives effects as safely and effective drugs with low resistance against microorganisms. Therefore, these essential oils could be used for management of these pathogens as a potential source of sustainable eco-friendly botanical bactericides.

Key words: Antibacterial, *Stachys pubescens*, *Mentha piperita*, *Clinopodium vulgare*, *Satureja hortensis*, gas chromatography/mass spectrometry (GC/MS).

INTRODUCTION

Herbal medicine has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources as a good choice, because these natural resources have ordinarily fewer side effects (Zargari, 1996). The medicinal plants have been proved effective in the treatment of infectious diseases and simultaneously decrease many of the side effects (Iwu et al., 1999). Also, they are costless and effective against a broad spectrum of antibiotic resistant microorganisms and they have very potent natural biologically active agents (Nychas et al., 2003). In many parts of the world, the extracts and essential oil of medicinal plants with active biological compounds are used for their antimicrobial and antiviral properties (Hassawi and Kharma, 2006) that have been used in folk medicine. The increasing occurrence of

*Corresponding author. E-mail: hossen253@yahoo.co.uk. Tel: +98 -231–3353170–3. Fax: +98-231-3354161.

antimicrobial resistance represents a worldwide major concern for both human and veterinary medicine (Lorian, 1996). For this reason, there is a growing interest in the antimicrobial screening of extracts and essential oils from plants in order to discover new antimicrobial agents. Nowadays, about 25% of the drugs prescribed worldwide come from plants and 252 of them are considered as basic and essential by the World Health Organization (WHO). The WHO considers phytotherapy in its health programs and suggests basic procedures for the validation of drugs in developing countries. Infectious diseases are the second leading cause of death worldwide (Fazly-Bazzaz et al., 2005). From the time of the ancient Iranian, the plants were considered to protect against diseases. Iran has a very honorable past in traditional medicine, which goes back to the time of Babylonian, Assyrian civilization. One of the most significant ancient heritages is sophisticated experience of people who have tried over millennia to find useful plants for health improvement, with each generation adding its own experience to this tradition (Naghibi et al., 2005). Based on literature search, 18% of the plant species are used for medicinal purposes in Iran. Treatment of infections continues to be a problem in modern time, because of side effects of some drugs and growing resistance to antimicrobial agents. To investigate for novel, safer and more potent antimicrobials is a pressing need. Herbal medicines have received much attention as a source of new antibacterial with low side effect and significant activity (Fazly-Bazzaz et al., 2005). In the present study, the biological activities of four plants: *Stachys pubescens, Mentha piperita, Satureja hortensis* and *Clinopodium vulgare* were evaluated. Nowadays, there is a considerable research interest towards the compositional analysis of essential oil and extract. It has been reported that essential oil yield and their components in plants is related to genetic (Mohammed and Al-Bayati, 2009), climate, elevation, topography (Pourohit and Vyas, 2004; Rahimmalek et al., 2009a) and genotype (G), growing conditions (E) and their interaction (G × E) (Basu et al., 2009; Shafie et al., 2009). Previous studies have shown that these selected plant species have potential medicinal activity (Iscan et al., 2002; Mimica-Dukić et al., 2003; Saeed and Tariq, 2005; Andoğan et al., 2002; Mathur et al., 2011; Hammer et al., 1999; Adam et al., 1998; Sahin et al., 2003; Gulluce et al., 2003; Razzaghi-Abyaneh et al., 2008; Adinguzel et al., 2007; Boyraz and Özcan, 2006; Dikbas et al., 2009; Azaz et al., 2005; Chorianopoulos et al., 2004; Mihajilov-Krstev et al., 2010; Mihajilov-Krstev et al., 2009; Karami-Osboo et al., 2010). The antibacterial activity of essential oil of *S. pubescens* and *C. vulgare* has never been evaluated and was carried out for the first time in this study. The widespread use of antibiotics both inside and outside medicine is playing a significant role in the emergence of bacterial resistant (Goossens et al., 2005). Although, there were low levels of preexisting antibiotic resistant bacteria before the widespread use of antibiotics; evolutionary pressure from their use has played a role in the development of multidrug resistance varieties and the spread of resistance between bacterial species (Hawkey and Jones, 2009). Biological cost or metabolic price is a measure of the increased energy metabolism required to achieve a function. Drug resistance has a high metabolic price (Steven and Timothy, 2010) in pathogens for which this concept is relevant (Wichelhaus et al., 2002). Although several strategies have been proposed to overcome and control this situation. However, a clear solution has not yet been elucidating due to the antibiotic resistance, consequences, and side effects of antimicrobial drugs. Many plants are used in Iran in the form of oils and crude extracts, infusion or plaster to treat common infections without any scientific evidence of efficacy. Pharmacological studies carried out on essential oils of some aromatic plants' species that were obtained in central regions of Iran, have shown antimicribial activity which is coherent with the use of these plants in folk medicine. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. In the present study, three medicinal plants were selected which are widely used in the folk medicine in our region. All the plants have been used in the treatment of infectious diseases with different geographical area (Rechinger, 1982a, b; Chevallier, 1996). The aim of this study was to evaluate the antibacterial potential of the essential oils derived from *S. pubescens*, *M. piperita, S. hortensis* and *C. vulgare* that grows in the wild in the central part of Iran against standard strains. The selected strains: *Staphylococcus aureus* (PTCC:1431), *Listeria monocytogenes* (PTCC:1163), *Streptococcus pneumoniae* (PTCC:1240), *Pseudomonas aeruginosa* (PTCC:1430), *Klebsiella pneumoniae* (PTCC:1053*), Escherichia coli* (PTCC:1329) and *Salmonella typhi* (PTCC:1609) were purchased from Iranian Research Organization for Science and Technology (IROST). The antimicrobial potential was performed by disc diffusion (DD) and broth microdilution method (BMD) to determine the minimum inhibitory concentration (MICs) and maximum bactericidal concentration (MBCs).

MATERIALS AND METHODS

Collection of plant and essential oil extraction

The plants were collected from their wild habitat in Semnan city in the central part of Iran between April and June, 2011 which are shown as geographical and environmental conditions in Table 1. Plants were identified by experts of the University of Applied Science and Technology (UAST) Education Center in Semnan branch. The leaves of *S. pubescens, M. piperita*, *S. hortensis* and *C. vulgare* in full flowering stage were collected to determine antibacterial activity. A voucher specimen for each plant has been deposited in the herbarium of Medicinal Plants Research, UAST.

Table 1. Geographical and environmental conditions.

S/N	Plant	Region	Altitude (m asl[1])	Latitude	Longitude
1	Mentha *piperita*	Garmsar,North Eyvanakey	2100	35.432670	53.256050
2	*Satureja hortensis*	Shahrood Mayamey Bekran	2150	36.43520	54.376050
3	*Clinopodium vulgare*	Semnan Fullad mahaleh	2650	35.78527	53.32405
4	*Stachys pubescens*	Shahrood, Semnan	2315	36.32415	54.35316

Air-drying of plant material was performed in a shady place at room temperature for 4 days. Ground and dried leaves of plants (100 g) were subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus. The distillated oils were dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4°C until analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis

The essential oils were analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. A fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) was used for the separation. Helium was used as a carrier gas at a flow rate of 1 ml/min. The oven temperature was programmed at 60°C (4 min), and then rising to 300°C at 4°C min^{-1}. The injector and detector temperature were kept at 250 and 300°C, respectively. The mass spectrometer was operated in electron-impact ionization (EI) mode with 70 eV energy with MS transfer line at temperature of 300°C was used. Ion source and interface temperatures were 200 and 250°C, respectively. The split ratio was 1:50. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250°C. The column was programmed as follows: 60°C for 2 min and then increased by 3°C min^{-1} up to 300°C. Volume of injected samples was 0.5 µl. Identification of components was based on the comparison of retention times (RT) and the computer mass spectra libraries using Wiley 275 GC/MS Library (Wiley, New York), those found in the literature (Adams, 2001; McLafferty, 1993) and the mass spectrometry data bank (NIST). The percentage composition of the essential oil was computed by the normalization method from the GC peak areas measurements (Table 4).

Microorganisms, inoculums and antibacterial assay

Bacterial strain

In the present study, a total of 7 standard isolates were obtained from IROST in 2011. Bacterial strains used in this study were four Gram-negative bacteria: *P. aeruginosa* (PTCC: 1430), *K. pneumoniae* (PTCC: 1053), *E. coli* (PTCC: 1329), *S. typhi* (PTCC: 1609) and three Gram-positive bacteria: *S. aureus* (PTCC: 1431), *L. monocytogenes* (PTCC: 1163), *S. pneumonia*e (PTCC: 1240), that were grown in Müeller–Hinton (MH) agar (Oxoid) and incubated for 24 h at 37°C. Cultures were used for making bacterial suspensions, and turbidity was adjusted to 0.5 McFarland and confirmed using a spectrophotometer (UV-VIS 1650, Shimatzu, Japan).

Preparation of inoculums

Bacterial strains were prepared by suspending one isolated colony from MH agar plates in 5 ml of MH broth and overnight broth cultures. The suspensions were adjusted in 0.5 McFarland standard turbidity to obtain final inoculums of 5×10^5 to 5×10^6 CFU/ml after 24 h of growth at 37°C and confirmed using a spectrophotometer. The essential oils were dissolved in dimethyl sulfoxide (DMSO, 25 mg/ml) and diluted to MH broth for antibacterial tested. All strains were tested by BMD and disk diffusion (DD) techniques according to the National Committee for Clinical Laboratory Standards (NCCLS, 2003a, b).

Serial dilution method

MICs and MBCs of essential oils were determined by using BMD method as described by NCCLS (2003a) in flat-bottomed 96-well clear plastic tissue-cultured plates (Greiner, 650161). The MIC was assayed using two-fold BMD method in MH Broth in 96-well plates. Plates contained two-fold dilutions of antibacterial agents at the concentration ranges: 0.5 to 64 µg/ml (25%, v/v). These dilutions were used to dispense 100 µl into each of the sterile 96-wells and an equal volume of bacterial inoculums was added to each well on the microtiter plate. After incubation for 24 h at 37 °C, the micro-dilution trays were checked with unaided eyes to detect the growth inhibition of the bacteria, and then the MICs were determined with spectrophotometer. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The final concentration of DMSO in the assays did not interfere with the bacterial proliferation which is used as a control. Negative controls were prepared with non-inoculated medium with oils, and one non-inoculated well, without antimicrobial agents, was also included to ensure medium sterility. The commercial antimicrobials Ciprofloxacin (Sigma) and Gentamicin (Merk) were included as positive controls. One inoculated well was included to allow control of the broth suitability for organism growth. To determine the MBCs, the suspensions (20 µl) were taken from each well without visible growth and inoculated in MH agar for 24 h at 37°C. The MBC was defined as the lowest concentration of the essential oil at which incubated micro-organisms are completely killed. Tests were performed in triplicate for each test concentration ($P > 0.05$).

Disc diffusion method

Agar diffusion method was carried out for the assessment of the essences antibacterial activity as recommended by NCCLS (2003b). The potential activity of oils were confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition zone (IZ) compared to known standard antibiotics. Essential oils were diluted in DMSO to different concentrations (0.5, 1, 2, 4, 8, 16, 32 and 64 µg/ml). 50 µl of standardized inoculums according to 0.5 McFarland turbidity standard solutions (10^5 to 10^6 CFU/ml) of the selected strains were spread onto the surface of Mueller Hinton (MH) agar and kept for 2 h at 4°C for absorption. Sterilized paper discs (Whatman, 6 mm diameter) containing approximately 20 µl of the essential oils were impregnated with different amount of essential oils. The prepared discs of the oils and standard antibiotics were placed on the surface of MH agar media. The inoculated plates were incubated at 37°C for 24 h and the resulting

Table 2. MIC and MBC (µg/ml) values for different essential oils of plants.

Bacteria	Gentamicin		Ciprofloxacin		*M. piperita*		*C. vulgare*		*S. hortensis*		*S. pubescens*	
	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]	MIC[a]	MBC[a]
S.p	1	2	0.5	0.5	0.5	0.5	1	1	1	2	1	1
S.a	0.5	1	1	2	0.5	1	0.5	1	4	4	0.5	1
P.a	1	1	0.125	0.5	1	2	2	4	1	2	2	2
E.c	2	4	0.5	1	2	2	8	8	8	8	1	2
S.t	1	2	0.5	0.5	4	8	4	4	16	32	4	8
L.m	2	2	1	1	8	16	16	32	32	32	8	8
K.p	2	4	1	2	8	16	16	64	-	-	8	16

MIC=Minimum inhibitory concentration; MBC= minimum bactericidal concentration; "-" No growth inhibition. E.c=*Escherichia coli*, P.a= *Pseudomonas aeruginosa*, S.a=*Staphylococcus aureus*, S.t=*Salmonella typhi*, S.p=*Streptococcus pneumonia*, K.p=*Klebsiella pneumoniae*, L.m=*Listeria monocytogenes*.

Table 3. Antibacterial activity screening of antibacterial agents by zone of inhibition (mm diameter) in disc diffusion method.

Bacterial strains	D.D of NC	D.D of PC		*M. piperita*	*C. vulgare*	*S. hortensis*	*S. pubescens*
		G	C	$D.D_T$	$D.D_T$	$D.D_T$	$D.D_T$
**S. pneumonia*	-	18	25	27	25	21	26
***S. aureus*	-	24	28	25	27	18	25
P. aeruginosa	-	19	22	23	15	20	21
E. coli	-	14	16	17	10	13	20
****S. typhi*	-	17	21	16	19	11	13
L. monocytogenes	-	16	15	10	8	5	11
K. pneumoniae	-	15	18	8	7	-	8

D.D= Diameter of inhibition zone (mm) including of disc diameter of 6mm. T=tested at a concentration of 20 µg /disc. NC=Negative Control. PC=Positive Control (G=Gentamicin, C=Ciprofloxacin,). "-" No growth inhibition. *S=Streptococcus, **S=Staphylococcus, P=Pseudomonas, E=Escherichia, ***S=Salmonella, L=Listeria, K=Klebsiella.

zone of inhibition (diameter) was measured in millimeters by comparing the different concentrations of oils and the standard antibiotics. The MIC was defined as the lowest concentration, resulting in a clear zone of growth inhibition around the disc after incubation period. Gentamicine (Merk) and Ciprofloxacin (Sigma) discs were applied over the test plates as a positive control. Negative controls were prepared using the solvent to dissolve the essential oil solution. All experiments were performed in triplicate.

Statistical analysis

Comparison of data was performed using the one way analysis of variance (ANOVA) or the unpaired Student's t-test and is presented as mean ± standard deviation. Comparison of MIC and MBC values, tests were made in triplicate for quantification. Values of $p < 0.05$ were considered significant.

RESULTS

All essential oils showed effective antibacterial activities on the selected pathogenic bacteria. Antibacterial activities of essential oils were investigated by broth microdilution and the disc diffusion method. The MICs and MBCs and diameter of inhibition zone (DD) of the selected oils on the bacteria are shown in Tables 2 and 3.

The results showed that essential oil of the plants were active against all the pathogenic bacteria species with different degree in the following range of concentrations: essential oil of *S. pubescens* and *M. piperita* had the best antibacterial effect and its MIC value was between 0.5 and 8 µg/ml. *C. vulgare* is the second degree with MIC values between 0.5 and 16 µg/ml. Otherwise, *S. hortensis* had a lowest antibacterial effect comparison to the earlier essences and its MIC value was 1 to 32 µg/ml. Ciprofloxacin and Gentamicin used as positive control as well as DMSO as a negative control which did not show any inhibition against the pathogens bacteria. MIC range of standard antibiotics "Ciprofloxacin and Gentamycin" were 0.5 to 1 µg/ml and 0.5 to 2 µg/ml, respectively. Even at low concentrations, the plant's species showed antibacterial activity more or nearly equal to the commercial bactericidal agents. All of the oils had the best inhibitory activities against *S. Pneumonia*, *S. aureus* and *P. aeruginosa*. The weakest activity was observed against *L. monocytogenes* and *K. pneumoniae* with the highest MIC and MBC, and *K. pneumoniae* was resistance against *S. hortensis*. The results of the chemical analyses using GC/MS of the essential oils were listed in Table 4. Number of indentified constituents

Table 4. Chemical analyses of essential oils.

S/N	*M. piperita*			*C. vulgare*			*S. hortensis*			*S. pubescens*		
	Component	P.A	RT	Component	P.A (%)	RT	Component	P.A (%)	RT	Component	P.A (%)	RT
1	α-Pinene	0.83	5.473	Terpinen-4-ol	4.84	5.479	α- Terpinene	0.58	6.760	β-Pinene	1.8	4.840
2	Sabinene	0.57	6.085	β-pinene	16.04	5.627	o-Cymene	16.91	6.880	1,4-Cyclohexadiene	0.4	5.412
3	β-Pinene	1.04	6.154	Sabinene	10.19	6.159	1,8-Cineole	0.93	7.018	Myrcene	0.9	6.124
4	dl-Limonene	3.09	6.944	Cymen-8-ol	2.24	6.972	γ-Terpinene	3.56	7.413	α- Terpinene	2.7	6.529
5	1,8-Cineole	5.12	7.001	Limonene	5.84	7.012	Terpineol	1.06	7.567	Benzene	0.9	6.790
6	Trans-abinene Hydrate	0.61	7.561	Terpinolene	1.52	7.212	2-Butoxyethy lacetate	1.69	7.853	Limonene	6.3	6.893
7	l-Menthone	18.68	8.923	1.8-cineole	6.76	7.319	Linalool	2.73	8.013	(*E*)-β-Ocimene	2.8	7.115
8	Menthofuran	25.70	9.066	β-(Z)-ocimene	3.61	7.364	Borneol	2.42	9.112	γ-terpinene	1.2	7.430
9	Menthol	23.17	9.198	γ- Terpinene	1.95	7.480	3-Cyclohexen -1-ol	0.64	9.267	3-Cyclohexen-1-ol	1.5	9.280
10	Cyclohexen	0.74	9.272	α-Copaene	3.27	8.114	α- Terpineol	1.06	9.455	Linalool	9.7	9.987
11	Cyclohexanol	0.43	9.358	linalool	2.26	8.049	Benzene, 1-methoxy-4-(2-propenyl)	0.64	9.541	2,6-Octadien	11.5	10.420
12	3-Cyclohexene-1-methanol	0.42	9.450	Camphor	1.72	9.112	1-Isopropyl	2.05	10.039	Octen-1-ol acetate	1.6	10.560
13	Pulegone	6.72	10.182	Pulegone	2.45	10.375	2,6-Octadien-1-ol	1.27	10.302	2,6-Octadienal	2.1	10.602
14	2-Cyclohexen-1-one	0.26	10.394	Thymol	5.32	10.743	Phenol	3.06	10.634	Linalyl acetate	1.2	10.742
15	3-Memthene	1.11	10.634	Camphene	0.48	11.802	Thymol	48.58	10.817	δ-Elemene	5.4	11.124
16	Camphane	7.40	10.897	Carvacrol	6.04	11.894	Carvacrol	5.19	11.526	β-Bourbonene	0.2	12.247
17	Cyclohexene	0.30	11.126	Isosativene	0.27	12.014	Nerol acetate	0.45	12.047	δ-Cadinene	19.7	13.905
18	trans-Caryophyllene	1.47	12.706	Caryophyllene	1.76	12.695	trans-Caryo phyllene	3.72	12.705	Naphthalene	1.2	13.926
19	Germacrene	1.16	13.478	Calarene	0.63	13.207	Naphthalene	1.05	13.867	β-Gurjunene	0.3	13.945
20	Mint furanone	0.47	13.690	γ-Cadinene	3.52	13.659	delta-Cadinene	0.69	13.947	Bicyclogermacrene	1.8	14.364
21	Hexahydrochrysene	0.67	14.308	Germacrene D	18.12	14.826	Nerolidol	0.90	14.342	Caryophyllene oxide	1.3	14.821
22	-	-	-	-	-	-	Caryophyllene oxide1	0.79	4.760	Spathulenol	0.8	14.834
23	-	-	-	-	-	-		-	-	Germacrene	22.4	15.248
Total	-	99.96	-	-	98.83	-	-	99.97	-	-	97.7	-

TR = Retention Time; PA = Peak Area

in *S. pubescens, S. hortensis*, *M. piperita* and *C. vulgare* were 23, 22, 21 and 21, respectively. Also, analysis of data with creditable library shows that, the main components of *S. pubescens* were: Germacrene (22.4%), δ-Cadinene (19.7%), 2,6-Octadien(11.5%), Linalool (9.7%); *M. piperita* were: Menthofuran (25.70%), Menthol (23.17%), Menthone (18.68%) and Camphane (7.40%), *C. vulgare* were: Germacrene D (18.12), β-pinene (16.04), Sabinene (10.19) and 1.8-cineole (6.76); and *S. hortensis* were Thymol (48.58%), o-Cymene (16.91%), Carvacrol(5.19), trans-Caryophyllene (3.72%) and γ-Terpinene (3.56%).

DISCUSSION

This study was attempted to purify the selected plant's oils that were native in our region in order to identify their essential oils as antibacterial properties. In addition, components of plants were determined and the result was compared with other studies. This is due to several reasons, namely, conventional medicine can have side effects, high coast, abusive or incorrect usage of synthetic drugs result in complications, and the large percentage of the world's population do not have access to conventional pharmacological treatment. The best antibacterial activities were seen in *M. piperita, S. pubescens* and *C. vulgare*, while *S. hortensis* displayed a moderate response against bacterial species. In comparison to the standard drugs, these data showed *M. piperita* and *S. pubescens* had the highest activity; *C. vulgare* had the lower activity but with the lowest different, while the different properties of *S. hortensis* was more. The results confirmed the antibacterial potency of these plants. In other studies concerning the antimicrobial activity of these plants, inhibition effects of *M. piperita* on some microorganisms such as *S. paratyphi*, *Proteus mirabilis, Proteus vulgaris, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes*, *Lactobacillus acidophilus, Bacillus subtilis, Enterobacter aerogenes, Shigella dysenteriae* and *Yersinia enterocolitica* growth was studied and this plant showed the highest antimicrobial activity (Iscan et al., 2002; Mimica-Dukić et al., 2003; Saeed and Tariq, 2005; Mathur et al., 2011; Andogan et al., 2002). In previous studies, *S. hortensis* showed antimicrobial activity against some of the standard and clinical microorganisms (Iscan et al., 2002; Sahin et al., 2003; Gulluce et al., 2003; Adinguzel et al., 2007; Boyraz and Özcan, 2006; Azaz et al., 2005; Chorianopoulos et al., 2004; Mihajilov-Krstev et al., 2010; Mihajilov-Krstev et al., 2009; Karami-Osboo et al., 2010; Baser et al., 2004). Other study concerning the extract *of C. vulgare*, inhibited the growth of some bacterial species with different degrees (Opalchenova and Obreshkova, 1999). Our result with other studies confirmed that a variety of bacterial species are affected by essential oil of selected plants, especially about the essential oils of *S. pubescens* and *C. vulgare* evaluated for the first time. The aforementioned finding supports their traditional usage of these oils as antibiotic and antiseptic (Riley, 2005). Briefly, the results of this study showed that the essential oils of these plants have a very broad spectrum of antibacterial activities with notable MICs and MBCs, which are near or lower than dose synthetic drugs. These plants could safely be used as organic preservatives to replace synthetic antibiotics in the prevention and cure of some human and animal infectious disease as well as food industrial preservatives. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. In comparison to the other studies, the composition of the plants showed little difference. Similar to our result, previous studies on *M. piperita* showed that the main components of the oils and extracts were menthol, menthofuran, menthone and menthyl acetate (Iscan et al., 2002; Maffei et al., 1999; Soković et al., 2009; Rohloff, 1999). There are three numbers of these components in our main components, but in the other study in Iran, the main components were very different: α-terpinene, isomenthone, *trans*-carveol, pipertitinone oxide (Rasooli et al., 2006). Menthone and menthol has been reported to be responsible for the antimicrobial activity of *M. piperita* (Gupta and Saxena, 2010; Bassolé et al., 2010; Kizil et al., 2010). But, the antibacterial activity of menthofuran has not been determined. Since *M. piperita* showed the highest antibacterial activities, then it was suggested the menthol, menthofuran or menthone alone or mix together (synergic effect) play a major antibacterial role. Since, each of menthol, menthofuran or menthone alone do not have considerable amount in the total extract; therefore, its antibacterial activity also belongs to the mixture of the whole components. With attention to other, its components were less than 8%. But, a point of consideration is the study that showed the good antimicrobial activity of *M. piperita*, while there are any these three components in their chemical composition (Rasooli et al., 2006). Concerning the *S. pubescens*, there are a few studies which determined the chemical composition. Many studies have not been conducted so far; Iranian researcher reported (*Z*)-β-Ocimene, Germacrene D and Bicyclogermacrene as the main components (Baher and Mirza, 2006). Previous studies related to the chemical composition of *S. hortensis,* thymol, Carvacrol, γ-terpinene and p-cymene reported as main components which are similar to our result (Gulluce et al., 2003; Adinguzel et al., 2007; Azaz et al., 2005; Sefidkon et al., 2006). Since, in these studies, *S. hortensis* display the high antimicrobial activity, therefore, it is concluded that the main components like thymol and carvacrol have an antimicrobial activity. But, the important point in these studies confirmed the antimicrobial activity of *S. hortensis* (Gulluce et al., 2003; Adinguzel et al., 2007; Mihajilov-Krstev et al., 2009) which is the lack of thymol or carvacrol as part of the main compounds, and the amount of these components was inconsiderable against whole extract. Therefore, it can be said that the total extract of *S. hortensis* has better antimicrobial activity than pure components, because in this status, there is synergistic effect between components as was observed in this study. Concerning the *C. vulgare*, in the previous study, the constituents of sabinene, germacrene D, E-caryophyllene, (Z)-β-ocimene and γ-terpinene was reported as the main components (Nurzyn´ska-Wierdak, 2009). The main components of their plants were similar to our results, but in the other study the main components were different to our result, in that the study of germacrene-D, b-caryophyllene and b-caryophyllene oxide was reported as the main components (Kökdil, 1998). In the present study, the similarity of composition to other studies is low. Nonetheless, all of them have a good antimicrobial activity and this subject describes that the special compound cannot have the major antimicrobial potency and the antibacterial of this plant yielded the mixture of their components. Although, these plants are in the same family, but their main component is different, and this variety in biological activity is related to their composition. The two Gram positive: *S. aureus, S. pneumonia* and two Gram negative: *P. aeruginosa, E. coli* bacteria showed high sensitivity against the essential oils with the lowest MICs and MBCs. Some studies reported that these plants inhibited both Gram positive and Gram negative bacteria (Dikbas et al., 2006; Hoferl et al., 2009). It was suggested that these differences in components could be due to the variety of the ecotype system reported by other scientists and references (Asbaghian et al., 2011). Since the essential oils are complex mixtures of several compounds, it is difficult to attribute their biological activity to a particular constituent. Usually, major compounds are the ones responsible for the antimicrobial activity of the essential oils. However, some studies showed that minor components may have a crucial role in the biological activity of the oils (Koroch et al., 2007). Further studies are needed to determine the antibacterial activities of the bioactive compounds responsible for the observed potential value. Natural plant-derived bactericidal may be a source of a new alternative active compounds. In attention to, in the present study, most isolates showed a less difference concentrations of essential oils between bacteriostatic and bactericidal values. Suggesting that the essential oils of the selected plants could be a possible source to obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms and also in the search for novel antibacterial agents with the potential application of some major or minor constituents alone, mixture of the extract or in combination with antibiotics for the treatment and prevention of pathologies associated with multi resistant bacteria. However, the mechanism of inhibitory effects of these plant's oils against infectious bacteria is still unclear. Further investigations regarding the *in vitro* and *in vivo* should be conducted in order to clear mechanisms pathway and develop such products.

REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography /quadrupole mass spectroscopy, *third ed.* Allured Publishing Corporation: Carol Stream, Illinois, USA.

Adinguzel A, Ozer H, Kilic H, Ctin B (2007). Screening of antimicrobial activity of essential oil and methanol exteract of *Satureja hortensis* on foodborne bacteria and fungi. Czeck J Food Sci. 25: 81-89.

Andoğan BC, Baydar H, Selçuk K, Demirci M, Özbaşar D, Mumcu E (2002). Antimicrobial activity and chemical composition of some essential oils. Pharmacol. Res. 25:860-864.

Asbaghian S, Shafaghat A, Zarea K, Kasimov F, Salimi F (2011). Comparison of volatile constituents, and antioxidant and antibacterial activities of the essential oils of *Thymus caucasicus*, *Mentha piperita* and *Satureja hortensis*. Nat Prod. Commun. 6:137-140.

Azaz AD, Kürkcüoglu M, Satil F, Can BKH, Tümen G (2005). *In vitro* antimicrobial activity and chemical composition of some *Satureja* essential oils. Flavour Fragr. J. 20:587–591.

Baher NZ, Mirza M (2006). Chemical composition of the essential oils of *Stachys pubescens* Ten. Flavour Fragr. J. 21:757–759.

Baser KHC, Özek T, Kirimer N, Tümen G (2004). A Comparative Study of the Essential Oils of Wild and Cultivated *Satureja hortensis* L. J. Essent. Oil Res. 16:422-424.

Bassolé IHN, Lamien-Meda A, Bayala B, Fran STC, Novak J, Nebié RC, Dicko MH (2010). Composition and Antimicrobial Activities of *Lippia multiflora* Moldenke, *Mentha* x *piperita* L. and *Ocimum basilicum* L. Essential Oils and Their Major Monoterpene Alcohols Alone and in Combination. Molecules 15:7825-7839.

Basu S, Chaplin WJ, Elsworth Y, New R, Serenelli AM (2009). Fresh insights on the structure of the solar core. Astrophys. J. 699:1403-1417.

Boyraz N, Özcan M (2006). Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey. Int. J. Food Microbiol. 107:238–242.

Chorianopoulos N, Kalpoutzakis E, Aligiannis N, Mitaku S, Nychas GJ, Haroutounian SA (2004). Essential Oils of *Satureja*, *Origanum*, and *Thymus* Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens. J. Agric. Food Chem. 52:8261–8267.

Dikbas N, Kotan R, Dadasoglu F, Karagöz K, Çakmakc R (2009). Correlation between Major Constituents and Antibacterial Activities of Some Plant Essential Oils against Some Pathogenic Bacteria. Turk. J. Sci. Technol. 4:57-64.

Fazly-Bazzaz BS, Khajehkaramadin M, Shokooheizadeh HR (2005). *In vitro* antibacterial activity of *Rheum ribes* extract obtained from various plant parts against clinical isolates of Gram-negative pathogens. Iranian J. Pharmacol. Res. 2:87-91.

Goossens H, Ferech M, Vander Stichele R, Elseviers M (2005). Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet Group Esac Project 365:579-587.

Gulluce M, Sökmen M, Daferera D, Ağar G, Özkan H, Kartal N, Polissiou M, Sökmen A, Şahin F (2003). *In Vitro* Antibacterial, Antifungal, and Antioxidant Activities of the Essential Oil and Methanol Extracts of Herbal Parts and Callus Cultures of *Satureja hortensis* L. J Agric. Food Chem. 51:3958–3965.

Gupta N, Saxena G (2010). Antimicrobial activity of constituents identified in essential oils from Mentha and Cinnamomum through GC/MS. Int. J. Pharm. Biol. Sci. 1:715-720.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86:985–990.

Hassawi D, Kharma A (2006). Antimicrobial activity of some medicinal plants against *Candida albicans*. J. Biol. Sci. 6:109-114.

Hawkey PM, Jones AM (2009). The changing epidemiology of resistance. J. Antimicrob. Chemother. 64:3–10.

Hoferl M, Buchbauer G, Jirovetz L, Schmidt E, Stoyanova A, Denkova Z, Slavchev A, Geissler M (2009). Correlation of Antimicrobial Activities of Various Essential Oils and Their Main Aromatic Volatile Constituents. J. Essent. Oil Res. 21:459-463.

Iscan G , Kirimer N , Kürkcüoğlu M , Can BKH , Demirci F (2002). Antimicrobial Screening of *Mentha piperita* Essential Oils. J. Agric. Food Chem. 50:3943–3946.

Iwu MW, Duncan AR, Okunji CO (1999). New antimicrobials of plant origin. In: J Janick (ed.), Perspectives on new crops and new uses. ASHS Press, Alexandria, VA. pp 457–462.

Karami-Osboo R, Khodaverdi M, Ali-Akbari F (2010). Antibacterial Effect of Effective Compounds of *Satureja hortensis* and *Thymus vulgaris* Essential Oils against *Erwinia amylovora*. J. Agric. Sci. Technol. 12:35-45.

Kizil S, Tolan V, Kilinc E, Yuksel U (2010). Mineral cntent, essential oil components and biological activity of two Mentha species (*M. piperita* L., *M. spicata* L.). Turk. J. Field Crops 15:148-153.

Kökdil G (1998). Composition of the essential oil of Clinopodium vulgare L. ssp. arundanum (Boiss.) Nyman collected from two different localities in Turkey. Flavour Fragr. J. 13:170-172.

Koroch AR, Juliani HR, Zygadlo JA (2007). Bioactivity of essential oils and their components, R.G. Berger, Editor, Flavour. Fragr. Springer-Verlag, Berlin, Heidelberg. pp 87–115.

Lorian V (1996). Antibiotics in Laboratory Medicine, fourth ed. Williams and Wilkins, Baltimore.

Maffei M, Canova D, Bertea CM, Scannerini S (1999). UV-A effects on photomorphogenesis and essential-oil composition in *Mentha piperita.* J. Photochem. Photobiol. 52:105–110.

Mathur A, Prasad, GBKS, Rao N, Babu P, Dua VK (2011). Isolation and identification of antimicribial compound from *Mentha pipirita*. Rasayan J. 4:36-42.

McLafferty FW (1993). Registry of Mass Spectral Data. Wiley, New York.

Mihajilov-Krstev T, Radnović D, Kitić D, Zlatković B, Ristić M, Branković S (2009). Chemical composition and antimicrobial activity of *Satureja hortensis* L. essential oil. Cent. Eur. J. Biol. 4:411-416.

Mihajilov-Krstev T, Radnovic D, Dusanka K, Stojanovic-Radic Z, Zlatkovic B (2010). Antimicrobial activity of *Satureja hortensis* L. essential oil against patho*Genic*. Arch. microbial Biol. Sci. 62:159-166.

Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M (2003). Antimicrobial and Antioxidant Activities of Three *Mentha* Species Essential Oils. Planta Med. 69:413-419.

Mohammed MJ, Al-Bayati FA (2009). Isolation and identification of antibacterial compounds from *Mentha piperita* aerial parts and *Dianthus caryophyllus* flower buds. Phytomed. 16:632-637.

Naghibi F, Mosaddegh M, Motamed MS, Ghorbani A (2005). Labiatae family in folk medicine in Iran from Ethnobotany to Pharmacology. Iran. J. Pharm. Res. 2:63-79.

National Committee for Clinical Laboratory Standards (NCCLS) (2003a). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. Wayne PA, USA.

National Committee for Clinical Laboratory StandardS(NCCLS) (2003b). Performance Standards for Antimicrobial Disc Susceptibility Tests. 8th ed. Approved Standard M2-A8. Wayne PA, USA.

Nurzyn´ska-Wierdak R (2009). Herb yield and chemical composition of common oregano (Origanum vulgare L.) essential oil according to the plant's developmental stage. Herba Polonica 55:55-62.

Nychas GJE, Tassou CC, Skandamis P (2003). Antimicrobials from herbs and spices (eds Roller, S.) in "Natural antimicrobials for the minimal processing of foods" CRC Press Woodhead Publishers, New York pp. 176–200.

Pourohit SS, Vyas SP (2004). Medicinal plants cultivation. Agrobios Press, India.

Rahimmalek M, Bahreininejad B, Khorami M, Sayed TBE (2009a). Genetic diversity and geographical differentiation of *Thymus daenensis*, an endangerd medicinal plant, as revealed by Inter Simple Sequence Repeat (ISSR) markers. Biochem. Genet. 47:831-842.

Rasooli I, Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Alipoor AS (2006). Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochem. 67:1249–1255.

Razzaghi-Abyaneh M, Shams-Ghahfarokhi M, Yoshinari T, Rezaee MB, Jaimand K, Nagasawa H, Sakuda S (2008). Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. Int J. Food Microbiol. 123:228–233.

Rechinger KH (1982a). *Labiatae* In: Flora Iranica. No. 150, Akademische Druch-u. Verlagsanstat, Austria.

Rechinger KH (1982b). Flora Iranica. No. 139. Graz: Akademische Druck. Verlagsanstalt.

Riley AP (2005). Food Policy, Control and research. Nova Science Publisher, Inc.

Rohloff J (1999). Monoterpene Composition of Essential Oil from Peppermint (*Mentha* × *piperita* L.) with Regard to Leaf Position Using Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry Analysis. J. Agric. Food Chem. 47:3782–3786.

Saeed S, Tariq P (2005). Antibacterial activities of *Mentha piperita*, Pisum sativum and Momordica charantia. Pak J Bot. 37:997-1001.

Sahin F, Karaman I, Güllüce E, Öğütçü H, Şengül M, Adıgüzel A, Öztürk S, Kotan R (2003). Evaluation of antimicrobial activities of *Satureja hortensis* L. J. Ethnopharmacol. 87:61–65.

Sefidkon F, Abbasi KH, Bakhshi KGH (2006). Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. Food Chem. 99:19–23.

Shafie MSB, Zain HSM, Shah MS (2009). Study of genetic variability of Wormwood capillary (Artemisia capillaris) using inter simple sequence repeat (ISSR) in Pahang region, Malaysia. Plant Omics J. 2: 127-134.

Soković MD, Vukojević J, Marin PD, Brkić DD, Vajs V, Van Griensven LJLD (2009). Chemical Composition of Essential Oils of *Thymus* and *Mentha* Species and Their Antifungal Activities. Molecules 14:238-249.

Steven HG, Timothy DM (2010). Antibiotic Resistance Protocols 2nd Edition. Springer-Verlag LLC, New York.

Wichelhaus TA, Böddinghaus B, Besier S, Schäfer V, Brade V, Ludwig A (2002). "Biological Cost of Rifampin Resistance from the Perspective of Staphylococcus aureus". Antimicrob. Agents Chemother. 46:3381–3385.

Zargari A (1996). Medicinal Plants, Tehran University Publications, Tehran, Iran, fourth edition.