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Chemical composition and antibacterial activity of the essential oils of some medicinal plants

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Infectious diseases are the second leading cause of death worldwide. Treatment of infections continues to be a problem in modern time because of the side effects of some drugs and resistance to antimicrobial agents. Herbal medicines have received much attention as a source of safe and effective antibacterial drugs. In the present study, essential oils of some medicinal plants: *Stachys pubescens*, *Thymus kotschyanus, Thymus daenensis* and *Bupleurum falcatum* were investigated for antibacterial activity against *Staphylococcus aureus, Listeria monocytogenes, Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and Salmonella typhi*. This study determines the MIC and MBC of the essential oils and comparison with reference antibiotics "Ciprofloxacin and Gentamicin". The chemical composition of the essential oils was analyzed by Gas chromatography (GC) and Gas chromatography-mass spectrometry (GC-MS). The essential oils isolated from the leaves of *S. pubescens*, *T. kotschyanus and B. falcatum* showed a high level antibacterial activity. *T. daenensis* essential oil showed less activity comparing to other oils. The result showed the presence of 24, 23, 20 and 15 components in the essential oils of *T. kotschyanus*, *S. pubescens*, *T. daenensis,* and *B. falcatum* respectively. Resistance to first-line drugs in most of the pathogens causing these diseases ranges from zero to almost 100%. Thus, these essential oils exhibited interesting bacteriostatic and bactericidal activity.

Key words: Antibacterial, *Stachys pubescens*, *Thymus kotschyanus, Thymus daenensis. Bupleurum falcatum,* GC-MS.

INTRODUCTION

The use of medicinal plants in the world and especially in Asian countries, contributes significantly to primary health care. Researchers and pharmaceutical industries are considering medicinal plants as a good choice, because these natural resources have ordinarily fewer side effects (Zargari, 1996). Also they are costless and effective against a broad spectrum of antibiotic resistant microorganisms. In many parts of the world, the extracts and essential oil of medicinal plants are used in folk medicine for their antimicrobial and antiviral properties (Hassawi and Kharma, 2006), that have been used. The increasing occurrence of 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 came from plants and 252 of them are considered as basic and essential by the World Health Organization (WHO). The WHO

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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). Also, the food contamination is important to health that occurs to unwanted microorganisms. Most of the time, the contamination is natural, but sometimes it is artificial. The herbal medicine has been used for thousands of centuries by many cultures to enhance the flavor and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some herbs, and their components (Zaika, 1988).

Studies in the past decade confirm that, the growth of both gram-positive and gram-negative food borne bacteria, yeast and mold can be inhibited by medicinal plants. 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. The medicinal properties of the genus *thymus* have made it one of the most popular medicinal plants (Nickavar et al., 2005). 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 antibacterial 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). Among the *Lamiaceae* family, *Thymus* with about 215 species is a significant genus (Rechinger, 1982a; Stahl-Biskup and Saez, 2002). It is well documented that, some plants belonging to this family display antimicrobial properties (Vila, 2002) however, the reported aspects are not constant. 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., 2009) and genotype (G), growing conditions (E) and their interaction (G × E) (Basu et al., 2009; Shafie et al., 2009). Recent studies have shown that, herbal medicine especially these selected species have strong biological activity (Vardar-Unlü et al., 2003; Pina-Vaz et al., 2004; Karaman et al., 2001; Couladis et al., 2004; Essawi and Srour, 2000; Mojab et al., 2008; Schwartz et al., 1996; Rasooli and Mirmostafa, 2002; Mothana and Lindequist, 2005; Mohammed and Al-Bayati, 2009; Asbaghian et al., 2011; Ghasemi et al., 2010a, b, c; Cimanga et al., 2002) and food derived microbial stains (Cosentino et al., 1999).

The antibacterial activity of *S. pubescens* and *B. falcatum* was not determined until now, and it was carried out for the first time in this study. The widespread use of antibiotics both inside and outside of 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 in pathogens for which this concept is relevant (Steven and Timothy, 2010). 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 of them have been used in the treatment of infectious diseases with different geographical area (Rechinger, 1982a, b; Duke, 2002; Chevallier, 1996). The aim of this study was to evaluate the antibacterial potential of the essential oils derived from *Stachys pubescens*, *Thymus kotschyanus, Thymus daenensis,* and *Bupleurum falcatum* which brings about the growth of the wild in the central part of Iran against standard strains. The selected strains; *Staphylococcus aureus* (PTCC 1431), *Listeria monocytogenes* (PTCC 1163), *Streptococcus pneumoniae* (PTCC1240), *Pseudomonas aeruginosa* (PTCC: 1430), *K. pneumoniae* (PTCC 1053*), Escherichia coli* (PTCC 1329) and *Salmonella typhi* (PTCC 1609) purchased from Iranian Research Organization for Science and Technology (IROST). The antibacterial potential was performed by disc diffusion (DD) and broth microdilution (BMD) methods to determine the Minimum Inhibitory Concentrations (MICs) and Maximum Bactericidal Concentrations (MBCs).

MATERIALS AND METHODS

Collection of plant materials and essential oil extraction

The leaves of *S. pubescens*, *T. kotschyanus, T. daenensis* and *B. falcatum* were collected between April and June 2011 from their

Table 1. Geographical and environmental condition.

S/N	Plant	Region	Altitude (m asl[1])	Latitude	Longitude
1	*T. kotschyanus*	Shahmirzad, semnan	2100	35.432670	53.256050
2	*B. falcatum*	Semnan fullad mahaleh	2650	35.78527	53.28145
3	*T. daenensis*	Shahmirzad, semnan	2650	35.63567	53.32405
4	*S. pubescens*	Shahrood, semnan	2315	36.32415	54.35316

wild habitat in the central part of Iran (Table 1). Plants were identified by experienced botanists of the University of Applied Science and Technology (UAST) Education Center in Semnan branch. A voucher specimen "*S. pubescens* (L-1220-1215-1216), *T. kotschyanus* (L-1184-1008), *T. daenensis* (L-1184-1038), and *B. falcatum* (A-2849-848-262)" for each plant has been deposited in the herbarium of Medicinal Plants Research UAST. Air-drying of plant material was performed in a shady place at room temperature for 4 days. Grinding and dried leaves of plants (100 g) were subjected to hydro-distillation for 3 h, using a Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate, and stored in amber vials at +4°C until analysis.

Gas chromatography-mass spectrometry (GC-MS) analyses

The essential oils were analyzed on an Agilent Technologies 7890A Gas chromatography (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) were 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.

The injector and detector temperature were kept at 250°C 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 300ºC was used. Ion source and interface temperatures were 200°C and 250°C, respectively. The split ratio was 1:50. The percentage compositions were obtained from electronic integration measurements using FID, set at 250°C. The column was programmed as follows: 60°C for 2 min and then increased by 3°C/min 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. We can identify the 97 to 99% of all components in these plants by this method (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: *Pseudomonas 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), *Streptococcus pneumoniae* (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

The inocula of the bacterial strains were prepared by suspending one isolated colony from MH agar plates in 5 ml of MH broth (Oxoid) 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 broth microdilution (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 the NCCLS in flat-bottomed 96-well clear plastic tissue-cultured plates (NCCLS, 2003a). The MIC was assayed using two-fold BMD method in MH Broth in 96-well plates. Plates contained two fold dilutions of antimicrobial 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 microdilution 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 37ºC. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganisms 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 which as recommended by NCCLS (NCCLS, 2003b). The potential activities of oils were confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition

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

Bacteria	Gentamicin		Ciprofloxacin		*T. kutschyanus*		*B. falcatum*		*T. daenensis*		*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.a*	1	2	0.5	0.5	0.5	1	1	2	1	4	1	1
E.c	0.5	1	1	2	0.5	2	0.5	1	4	8	0.5	1
P.a	1	1	0.125	0.5	1	2	2	4	2	2	2	2
***S.t*	2	4	0.5	1	2	4	8	16	8	16	1	2
****S.p*	1	2	0.5	0.5	4	16	8	8	16	32	4	8
K.p	2	2	1	1	8	8	16	32	32	64	8	8
L.m	2	4	1	2	8	16	16	64	-	-	8	16

MIC = Minimum Inhibitory Concentration; MBC = Minimum bactericidal concentration, "-" No growth inhibition. *S.a= *S. aureus*, E.c = *E. coli*, P.a = *P. aeruginosa*, **S.t = *S. typhi*, ***S.p = *S. pneumonia*, K.p = *K. pneumoniae*, L.m = *L. monocytogenes.*

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

Bacterial strain	DD OF NC	DD OF PC		*T. kotschyanus*	*B. falcatum*	*T. daenensis*	*S. pubescens*
		D	C	$D.D^T$	DD^T	DD^T	DD^T
**S. aureus*	-	18	25	28	21	21	25
E. coli	-	24	28	25	18	18	28
P. aeruginosa	-	19	22	25	20	20	21
***S. typhi*	-	14	16	19	13	13	24
****S. pneumoniae*	-	17	21	18	16	16	18
K. pneumoniae	-	16	15	10	10	5	9
L. monocytogenes	-	15	18	7	5	-	10

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

zone compared to known standard antibiotics. Essential oils were diluted in DMSO to different test concentrations. 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 on to the surface of Mueller Hinton (MH) agar and kept for 2 h at 4ºC 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 (0.5, 1, 2, 4, 8, 16, 32 and 64 µg/ml). 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 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 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 MBCs and MICs and diameter of inhibition zone 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 *T. kotschyanus* and *S. pubescens* had a best antibacterial activity and its MIC values was between 0.5 to 8 µg/ml. *B. falcatum* is second degree with MIC values between 0.5 to 16 µg/ml. while, *T. daenensis* had a lowest antibacterial activity comparison to above essential oils and its MIC values was 1 to 32 µg/ml. Ciprofloxasin and Gentamicin used as positive control

Table 4. Chemical analyses constituents of essential oils.

S/N	*T. daenensis*			*T. kotschyanus*			*B. falcatum*			*S. pubescens*		
	Component	**PA(%)**	**RT**	**Component**	**PA(%)**	**RT**	**Component**	**PA(%)**	**RT**	**Component**	**PA(%)**	**RT**
1	β-Myrcene	1.25	6.308	α- Terpinene	0.58	6.755	α-Pinene	3.5	5.240	β-Pinene	1.8	4.840
2	Benzene	1.27	6.875	Benzene	6.88	6.871	Pinocarvone	4	5.285	1,4-Cyclohexadiene	0.4	5.412
3	Limonene	0.37	6.943	1,8-Cineole	0.92	7.012	α-Cubebene	8.1	5.432	Myrcene	0.9	6.124
4	1,8-Cineole	0.51	7.012	γ-Terpinene	3.58	7.413	Pinocamphone	1.7	6.641	α- Terpinene	2.7	6.529
5	γ-Terpinene	1.11	7.407	Terpineol	1.07	7.567	Heptanal	4.2	7.405	Benzene	0.9	6.790
6	Trans-Sabinene hydrate	0.56	7.561	2-Butoxyethyl acetate	1.70	7.848	cis-Verbenol	1.5	7.910	Limonene	6.3	6.893
7	Linalool	0.78	8.008	Linalool	2.88	8.013	Myrtenal	2.9	10.470	(*E*)-β-Ocimene	2.8	7.115
8	Borneol	2.17	9.106	Borneol	2.51	9.106	Thyopsene	3.1	10.958	γ-terpinene	1.2	7.430
9	3-Cyclohexen-1-ol	0.44	9.261	3-Cyclohexen-1-ol	0.65	9.437	Trans-Pinocarveol	4.1	11.690	3-Cyclohexen-1-ol	1.5	9.280
10	α- Terpineol	22.95	9.461	α- Terpineol	1.07	9.450	Trans-Verbenol	0.3	11.825	Linalool	9.7	9.987
11	dl-Limonene	1.76	9.884	Benzene (1-methoxy-4-(2-ropenyl-)	0.65	9.541	Cuparene	2.8	11.931	2,6-Octadien	11.5	10.420
12	Camphene	6.27	9.925	Isopropyl	2.07	10.033	Torilenol	39.1	11.965	Octen-1-ol acetate	1.6	10.560
13	Cyclohexanon	2.10	10.182	Carvacrol methyl ether	1.51	10.176	Spathulenol	19.6	12.560	2,6-Octadienal	2.1	10.602
14	2,6-Octadien	2.22	10.302	Octadien-1-ol	1.34	10.297	α-Calacorene	2.4	12.940	Linalyl acetate	1.2	10.742
15	2,6-Octadienal	0.33	10.565	Phenol	2.61	10.634	Pentacosane	1.5	13.850	δ-Elemene	5.4	11.124
16	Thymol	20.20	10.817	Thyme camphor	1.58	10.714				β-Bourbonene	0.2	12.247
17	Carvacrol	31.46	10.977	Thymol	46.72	10.817				δ-Cadinene	19.7	13.905
18	β-caryophyllene	1.59	12.705	m-Thymol	0.61	10.874				Naphthalene	1.2	13.926
19	β-Cubebene	0.86	13.478	Carvacrol	3.73	10.955				β-Gurjunene	0.3	13.945
20	Bicyclogermacrene	0.58	13.667	Nerol	0.48	12.042				Bicyclogermacrene	1.8	14.364
21	β-bisabolene	0.47	13.712	Trans-Caryophyllene	3.39	12.705				Caryophyllene oxide	1.3	14.821
22	CIS-α-Bisabolemne	0.74	14.113	Naphthalene	0.50	13.861				Spathulenol	0.8	14.834
23	-	-	-	Delta-Cadinene	0.69	13.947	-	-	-	Germacrene	22.4	15.248
24	-	-	-	Nerolidol	0.92	14.336	-	-	-	-	-	-
25	-	-	-	Caryophyllene oxide	0.78	14.760	-	-	-	-	-	-
26	-	-	-	Germacrene	0.60	15.360	-	-	-	-	-	-
Total		99.99			90.02			98.8			97.7	

as well as DMSO as a negative control which did nott show any inhibition against the pathogens bacteria. MIC range of standard antibiotics "Ciprofloxasine and Gentamycin" were 0.5-1 and 0.5–2 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. aureus*, *P. aeruginosa*, and *E. coli*. The weakest activity was observed against *K. pneumoniae* and *L. monocytogenes* with the highest MIC and MBC, and *L. monocytogenes* was resistance against *T. daenensis*. The results of the chemical analyses using GC-MS of the essential oils were listed in Table 4. The number of indentified constituents in *T. kotschyanus, S. pubescens*, *T. daenensis* and *B. falcatum* were

24, 23, 20 and 15, respectively. Also, analysis of data shows that, the main components of *S. pubescens* were: Germacrene(22.4%), δ-Cadinene (19.7%), 2,6-Octadien (11.5%), Linalool (9.7%); and in *B. falcatum* were: Torilenol (39.1%), Spathulenol(19.6) and α-Cubebene(8.1%), *in T. kotschyanus* were: Thymol (46.72%), Benzene (6.880%), Carvacrol (3.73%), γ-Terpinene (3.58%), trans-Caryophyllene (3.39%) and *T. daenensis* was include: Carvacrol (31.46), α- Terpineol (22.95), Thymol (20.20), Bicycloheptane (6.27), 2,6-Octadien (2.26).

DISCUSSION

This study attempted to purify the selected plant's oils that are native in our region in order to identify the antibacterial activity of their essential oils. The selected bacteria in this assay are important in food poisoning and human and animal infections. 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 world's population do not have access to conventional pharmacological treatment. The best antibacterial activities were seen in *T. kotschyanus* and *S. pubescens* essence, whilst *B. falcatum* displayed a moderate response against bacterial species and *T. daenensis* displayed less susceptibility against all bacteria. In comparison to the standard drugs, these data showed that *T. kotschyanus* and *S. pubescens* had the highest activity; *B. falcatum* had the lower activity but with the lowest different, while the different properties of *T. daenensis* was more. Antibacterial properties of selected plants were confirmed the potential role of these plants.

In other studies concerning the antimicrobial of this family, inhibition effects of *T. kotschyanus* on the some gram-positive and gram-negative bacteria such as *B. subtilis, Bacillus cereus*, *K. pneumoniae* and *Proteus mirabilis* growth was studied and this plant showed the highest bactericidal activity (Rasooli and Mirmostafa, 2003; Mohammed and Al-Bayati, 2009; Asbaghian et al., 2011). In the previous studies, *T. daenensis* showed the antibacterial activity against some of standard and clinical isolates of both gram-positive and gram-negative bacteria (Mothana and Lindequist, 2005; Essawi and Srour, 2000; Mohammed and Al-Bayati, 2009; Proestos et al., 2005). Since in the other study concerning the *T. daenensis*, it inhibited the growth gram-positive bacteria, such as *S. aureus*, *Micrococcus luteus*, *Entrococcus faecalis*, *Streptococcus pyogenes*, but it showed no activity against gram-negative bacteria (Mojab et al., 2008). It was concluded that, these oils exhibit significant antibacterial activity which support their traditional usage as well as established as an antiseptics (Riley, 2005).

These plants could safely be used as organic preservatives to replace synthetic antibiotics in the food preservation and cure of some human and animal infectious disease as well as food industrial preservatives. Concerning the *S. pubescens* and *B. falcatum,* we cannot find other antibacterial assay. The results of this study showed that, essential oils of plants have a very broad spectrum of antibacterial activities. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties. In this assay the composition of the plants were determined by GC-MS which are different from other regions in the world. Concerning the *S. pubescens* many studies have not been conducted so far; Iranian researcher reported; (*Z*)-β-Ocimene, Germacrene D and Bicyclogermacrene as main components (Baher Nik and Mirza, 2006). Previous studies related to the chemical composition of *B. falcatum,* α-pinene reported as main component which are not similar to our result (Saraçoğlu et al., 2012). Since, *B. falcatum* display the high antimicrobial activity, therefore, we concluded that, the most main components as Torilenol and Spathulenol have an antimicrobial activity alone or mix to other components. Similar to our result, in previous studies on *Thymus* species showed that, the main components of the oils and extracts were carvacrol and thymol (Rustaiyan et al., 2000; Sefidkon et al., 2002; Rasooli and Mirmostafsa, 2003; Asbaghian et al., 2011; Sajjadi and Khatamsaz, 2003; Nickavar et al., 2005; Mojab and Nickavar, 2006; Mohammed and Al-Bayati, 2009; Nejad et al., 2008; Safaei-ghomi et al., 2009) (Table 4).

The percentage of carvacrol in the *T. daenensis* was 31.46%. As a result it showed carvacrol is the first main component in *T. daenensis*. In the other reported carvacrol was as the first major constituent (Asbaghian et al., 2011) which agree to our data for *T. daenansis*. In *T. kotschyanus* and *T. daenensis*, the percentage of thymol was 46.72 and 20%, respectively while the thymol was only identified as the first main components in *T. kotschyanus*. In the other words, the thymol was not the first main components in *T. daenensis*. Since *T. kotschyanus* and *S. pubescens* showed the highest antibacterial activities, then it was suggested that, the thymol and Germacrene may be play a major antibacterial role, while the rest of its components were less than 7%.

Another study concerning the *T. kotschyanus* showed that, the major components were carvacrol, γ-terpinene, thymol, myrcene, p-cymene, β-caryophyllene and borneol, α-phellandrene, (Asbaghian et al., 2011; Jamshidi et al., 2006) as can be seen, the carvacrol reported as first main component, whereas, the percentage of thymol was lower than the carvacrol in them while at the present study, the thymol was first main component. In agreement to our data, the important point in the previous studies presented the carvacrol and

thymol which are the two medically important constituents (Guarda et al., 2011; Nostro et al., 2007), as main components with different percentages except for a few examples. It was suggested that, these differences in components could be due to the variety of the ecotype system that were reported by other scientists and references (Asbaghian et al., 2011).

Conclusion

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 biological 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 compounds for the observed potential value.

In the present study, results 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 and also in the search for novel antibacterial agents with the potential application of some major or minor constituents alone, mixed of presented essential oils 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, and further investigations regarding the *in vitro* and *in vivo* should be conducted in order to clear mechanisms pathway and develop such products. The whole extract of these plants may be a potential new drug for the treatment of bacterial infections that could become available for low-income populations. This study is part of a continued search for new drugs with high activity and few side effects that can be used to treat diseases associated with pathogen bacteria strains. More studies are needed to determine the substances are selective for certain bacterial species.

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