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Chemical Composition of *Mentha spicata* L. subsp. *tomentosa* and *M. pulegium* L., and their Antimicrobial Activity on Strong Pathogen Microorganisms

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Abstract

Mentha L., recognized as a medical and aromatic plant, is a general name affiliated to mint species and belongs to Labiatae family. Some species are used as fresh vegetables in the Turkish kitchen and they can also be used in salads. In addition, some species have been used as a spice in food. In this study, chemical composition and antimicrobial activity towards some pathogenics (gram + and gram -) microorganisms of the essential oils *Mentha spicata* L. subsp. *tomentosa* (Briq.) Harley, *Mentha pulegium* L. grown under West Anatolian ecological conditions were investigated. Extractions were carried out with Clevenger apparatus and essential oil composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS). Microorganisms used for the antimicrobial studies were Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa, Enterococcus faecium* DSM 13590, *Escherichia coli* Q157:H7 and *Bacillus cereus* CCM99. As a result, *M. pulegium* and *M. spicata* subsp. *tomentosa* were found to be rich in piperitenone oxide: 72.77% and 25.84%, respectively. Each of the oils was found to possess antimicrobial properties against test microorganisms. Essential oils obtained from *Mentha* species give positive effect on all microorganisms.

Keywords: *Mentha, essential oils, GC-MS, antimicrobial, Turkey*

Introduction

Since ancient times, the crude herbal extracts of aromatic plants have been in use for different purposes such as food, perfumery, and drugs. The medicinal properties of these plants have been investigated in the recent scientific developments (Ekren *et al*., 2013). Recently, there has been considerable interest in essential oils and extracts of medicinal and edible plants, herbs, and spices for the development of alternative food additives, in order to prevent the growth of foodborne pathogens or to delay the onset of food spoilage (Çetin *et al*., 2011). Labiatae family, spreading on a large area in the world, consists of 236 genus and 7133 species. Labiatae exists especially in tropical and mild regions like Mediterranean region, on tropical high plains having seasonal climate (Yılar *et al*., 2015). *Mentha* L., recognized as a medical and aromatic plant, is a general name affiliated to mint species and belongs to Labiatae family. This plant is represented by 62 taxon in the world and 15 taxon in Turkey (Başer *et al*., 2012). Some members of this genus are also used as herbal teas and condiments both in fresh and dried form due to their distinct aroma (Başer *et al*., 1999). *Mentha* is a plant of economic importance, distributed over a wide area in the world. Some species are used as vegetables in the Turkish kitchen and in salads. In addition, some species have been used as a spice in food (Deniz *et al*., 2010).

The aim of the present study was to determine the essential oil contents of *Mentha spicata* subsp. *tomentosa,* and *Mentha pulegium* growing under West Anatolian ecological conditions and to investigate their antimicrobial effect on various bacteria.

Materials and Methods

Plant material

M. spicata subsp. *tomentosa* and *M. pulegium* aerial parts of the plants were collected in July 2015, which is their blooming period in Aydın and its surroundings (Fig. 1). The collected samples were placed in fabric bags and kept in a room with no sunlight.

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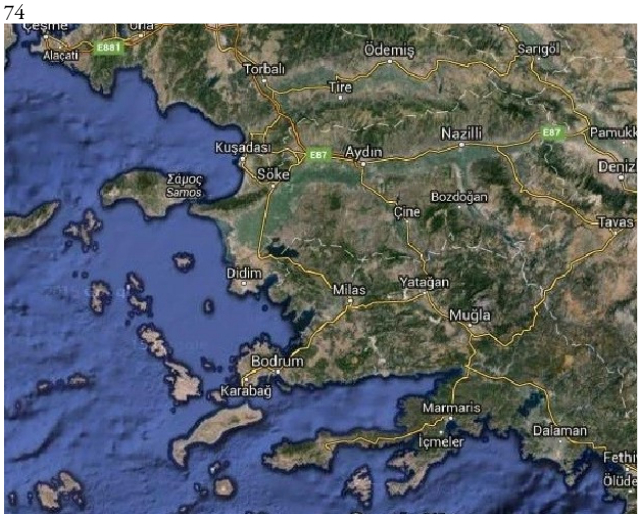

Fig. 1. Location of the Aydın region. Map data: US Dept of State Geographer 2015 Google 2015 Basarsoft Image Landsat

Isolation of essential oils

Approximately 150 g of plant samples were used for the essential oil extraction process. Extraction was performed with Clevenger apparatus (Basaran glass, Turkey and Misung Scientific Co., Korea) using water distillation.

GC-MS analysis

Qualitative and quantitative essential oil analysis were conducted at Eskisehir Anadolu University Medicinal Plants, Drugs and Scientific Research Center (AUBİBAM) by Hewlett Packard 5973 Mass Selective Detector System and GC-MS 6890 instrument equippped withan Agilent HP-Innowax colon (60m X 0.25 mm film, 0.25 μm thickness). Hellium was used as a carrier gas. Conditions were as follows; from 50 °C to 240 °C by an increase of 4 °C / minutes. At 240 °C, 40 minutes of waiting time were implemented. Injection port and detector temperature were 240 °C and 280 °C respectively. Chracterization of essential oil components was based on the library (Wiley and NIST) comparison with the mass spectra of the injected essential oil samples.

Preparation of microorganism cultures

Antimicrobial activity of the plants extracts was detected by four different gram positive (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* ATCC 6538, *Enterecoccus faecium* DSM 13590, *Bacillus cereus* CCM99) and two different gram negative (*Pseudomonas aeruginosa, Escherichia coli* Q157:H7) microorganisms using disc diffusion method. Stock bacterial cultures in Brain Heart Infusion Broth incubated at 37 °C for 24 hours. Microorganisms were adjusted to Mc Farland 0.5 density with sterile physiological water. Each microorganism was planted into petri dishes containing Muller Hinton Agar with expansion method. Then, the petri dishes were allowed to dry at room temperature for 20-25 minutes. Afterwards, for the disc diffusion method, each disk was impregnated with 10 μL of oil and allowed to incubate at 37 °C for 24 hours. At the end of the incubation period, inhibition zones were measured from the lower surface of the petri dish. Tobramycin antibiotic was used as a control in all experiments. All trials were performed with two repetitions.

Results

Chemical composition of the essential oils

Essential oils are liquid, volatile, and rich in phenolic compounds (Santos *et al*., 2016). They are produced by the plants' secondary metabolism and are complex mixtures of low molecular weight terpenes. Their environmental function is related to defense against phytophagous insects and/or attraction of pollinators, as well as to intra- or inter-specific allelopathic phenomena (Tommasi *et al*., 2009). In this study, the chemical composition of *M. spicata* subsp. *tomentosa* and *M. pulegium* were given in Table 1.In total, 90 component ewere detected as *M. spicata* subsp. *tomentosa* aerial parts essential oil composition. 90.35% of the total essential oils in 20 components (components which are ≥0.4% in total ratio). The essential oils obtained from the *M. spicata* subsp. *tomentosa* plant were detected to contain piperitenone oxide (25.84%), pulegone (24.72%), cis piperitenone oxide (12.55%) at most. In a recent revision, this taxon was given as a synonym of *Mentha spicata* subsp. *condensata* (Briq.) Greuter & Burdet (Tucker and Naczi, 2007).

Mentha pulegium spreads on almost every place in the world, except for tropical regions. Topsoil parts of this plant are used for a traditional food or medical purposes as a guaiacol and against cholera, tuberculosis and poisoning (Tanker and Sezik, 1965; Yasa *et al*., 2012). In our study, a total of 76 components were detected as *M. pulegium* aerial parts essential oil composition. 93.22% of the total essential oils in 18 components (components which are ≥ 0.4% in total ratio) were given in Table 1. The essential oils obtained from the *M. pulegium* plant were detected to contain piperitenone oxide (72.77%), carvacrol (4.03%), germacrene-D (3.79%) at most. In past studies obtained from findings, our results have been determined to be proportionally different (Stoyanova *et al*., 2005; Mkaddem *et al*., 2007; Chalchat *et al*., 2000; Morteza-Semnani *et al*., 2011; Yasa *et al*., 2012).

In general, these findings confirmed that the essential oil composition of the plants can have different quality and quantity in different geographical and environmental conditions, and during different periods of the plant growth (Mazandarani *et al*., 2013; Dastjerdi and Mazoji, 2015; Sevindik *et al*., 2016).

Antimicrobial activity of the essential oils

The most commonly studies of essential oils is related to antimicrobial activities. These oils have different antimicrobial effects on a variety of microorganisms including gram (-) and gram (+) bacteria. As the essential oils are complex mixtures comprising different components, action levels thereof vary depending on diversity and amount of active substances. Despite having limited information relating to their mechanisms of action, this seems to be associated with lipophilic features and chemical structures of the oils (Bayaz, 2014). In this study, the disc diffusion method was used for determination of the *in vitro* antimicrobial activity of essential oils. The trials were performed with two repetitions and the average values are given in Table 2.

The essential oil obtained from *M. pulegium* plant was effective against MRSA, *E. faecium* DSM 13590, *S. aureus* ATCC 6538, *P. aeruginosa*, *E. coli* Q157:H7 and *B. cereus*

Table 1. Essential oil composition of *M. spicata* subsp. *tomentosa* and *M. pulegium*

M. spicata subsp. *tomentosa*			*M. pulegium*		
RT	Component	Percent (%)	RT	Component	Percent (%)
11.55	α-pinene	0.79	11.55	α- pinene	0.56
15.95	β-pinene	1.03	15.96	β-pinene	0.74
16.71	sabinene	0.51	16.72	sabinene	0.40
19.04	β-myrcene	0.59	19.06	β-myrcene	0.63
20.77	limonene	1.59	20.79	limonene	1.82
21.14	1,8-cineole	1.72	37.32	terpinen-4-ol	0.40
29.34	3-octanol	0.59	39.07	pulegone	0.79
33.25	menthofuran	1.78	39.83	epibicyclosesquiphellandrene	0.65
36.58	isopulegone	0.51	41.09	germacrene D	3.79
37.42	caryophyllene	1.38	45.05	p-cymen-8-ol	0.63
39.34	pulegone	24.72	45.21	3,5-dimethylcyclohexen-1-one	0.71
41.34	cis piperitone oxide	12.55	45.77	(1'-butenyl)thiophene	0.47
41.61	2-cyclohexen-1-ol	0.88	48.02	piperitenone oxide	72.77
41.83	piperitone	6.08	49.25	benzene	1.69
41.61	4-(acetylamino) crotonic acid lactam	0.88	49.54	3,6,6-trimethyl -8-oxabicyclo[5.1.0]oct-2-en-4-one	0.52
47.71	piperitenone	5.29	49.95	4,6-dimethyl-2-methyl pyrimidine	2.18
48.35	piperitenone oxide	25.84	51.18	aromadendren	0.44
49.23	benzene	0.52	54.94	carvacrol	4.03
49.51	caryophyllene oxide	1.80	**Total**		**93.22**
54.94	carvacrol	1.30			
Total		**90.35**			

Table 2. Antimicrobial activity (inhibition zones) of the essential oils from *M. pulegium* and *M. spicata* L. subsp. *tomentosa*

Microorganisms	Inhibition zone (mm) (Disc diffusion method)		
	M. pulegium	*M. spicata* subsp. *tomentosa*	Antibiotic Tobramycin
Methicillin-resistant *Staphylococcus aureus* (MRSA)	12±1.4	17.5±0.7	0±0
Staphylococcus aureus ATCC 6538	16.5±0.7	11±1.4	22±2.8
Pseudomonas aeruginosa	12.5±0.7	21±8.4	12±0
Escherichia coli Q157:H7	9±1.4	20.5±2.1	20.5±4.9
Bacillus cereus CCM99	23±1.4	22.5±0.7	19±1.4
Enterococcus faecium DSM 13590	9±4.2	13±4.2	12±2.8

Note: Results are expressed as mean; ± standard deviation (SD)

CCM 99 in varying degrees. The biggest inhibition zone (23 mm) generated from the essential oil was against *B. cereus* and the smallest inhibition zone (9 mm) was generated against *E. faecium* and *E. coli* Q157:H7. Morteza-Semnanı *et al*. (2011) studied that impact of different concentration of the essential oil from *M. pulegium* was gotten from Mazandaran/Iran. The essential oil generated average 7.7 mm resistance zone against the *E. coli* PTCC 1330 and 9 mm resistance zone against the *S. aureus* end of the study. In this study, it was observed that, for *Mentha spicata* subsp. *tomentosa*, essential oil constituted a resistance zone of 22.5 mm against *B. cereus* at the most while it constituted a resistence zone of 11 mm *S. aureus* at the least (Table 2).

Essential oil components and antimicrobial effect of *M. spicata* L. subsp. *tomentosa* and *M. pulegium* grown under West Anatolian ecological conditions were determined in this study.The difference between our work and the previous studies may be explained by the usage of different methods and different plant collecting periods from different geographical regions and by the different type of bacterial strains used. The observation of an antimicrobial effect against microorganisms shows us that plants containing etheric oils may be used for treatment purposes and may be an alternative to synthetic antibiotics.

Conclusions

According to the results obtained in this study, it is possible to conclude that antimicrobial activity of the essential oils was slightly lower in comparison to antibiotic (tobramycin) effect on tested six different bacteria genus (MRSA, *S. aureus*, *P. aeruginosa, E. faecium*, *E. coli* and *B. cereus*). These findings may be a valuable resource for further food microbiology, biotechnological, and medicinal plants studies. It will also help to understand the importance of the biological diversity and conservation biology efforts.

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