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RESEARCH PAPER

Chemical Composition and Antifungal and Antibacterial Activity of *Mentha spicata* L. Volatile Oil

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Abstract

Y. Bayan, and M. Küsek. 2018. Chemical Composition and Antifungal and Antibacterial Activity of *Mentha spicata* L. Volatile Oil. Cien. Inv. Agr. 45(1): 64-69. In this study, we researched the chemical composition and the antifungal and antibacterial activity of volatile oil from *Mentha spicata*. The Gas chromatography/mass spectrometry (GC/MS) analysis of *M. spicata* showed that the main component was carvone (56.94%), followed by limonene (11.63%), sabinene hydrate (7.04%) and caryophyllene (4.06%). The antifungal activity of the volatile oil from *M. spicata* L. was determined with respect to plant pathogenic fungi, such as *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Sacc.) W.C. Synder & H.N. Hans (FORL), *Rhizoctonia solani* J.G. Kühn. (*R. solani*), *Alternaria solani* (*A. solani*), and *Verticillium dahliae* Kleb. (*V. dahliae*). The volatile oil was shown to have strong antifungal activity against plant pathogenic fungi. The result of the study was that at a dose of 12 μ L petri⁻¹, the volatile oil inhibited 100% of mycelium growth in *V. dahliae*, *A. solani* FORL and *R. solani*. Volatile oil exhibited remarkable activity against the selected bacterial strains of *Xanthomonas* spp. (ZI365, ZI366, ZI368, ZI370, ZI373, ZI375, ZI376, ZI378).

Keywords: Antibacterial, Antifungal, *Mentha spicata* L., Volatile oils.

Introduction

The Lamiaceae family contains more than 4000 species in 200 genera. In the Lamiaceae family, mint is one of the most cultivated and aromatic plants. Mint is grown in temperate regions in many parts of the world (South America, Antarctica, Europe and Asia) (Chambers, 1992; Kanatt *et al.*, 2007).

Mentha leaves have been traditionally used fresh and dried with various spices. *Mentha* species are comprised of biologically active components, which are used in traditional medicines. Additionally, mint species can be used in traditional medicine for common ailments, such as colds, coughs, sinusitis, fever, bronchitis, and nausea (Starburck, 2001; Dhifi *et al.*, 2011). In addition, the mint plant has been reported to have insecticidal, antimicrobial, antispasmodic, antioxidant and antifungal activities (Papachristos and Stamopoulos, 2001; Shetty 2001; Nosrati, *et al.*, 2011; Karagozlu *et al.*, 2011).

The main components of the volatile oil of *M. spicata* L. are carvone, limonene, cis-carveol, 1,8 cineol and cis-sabinene hydrate, of which carvone is the most important constituent (Baser, 1993; Wyk and Wink, 2008). The antifungal and antibacterial activities of the volatile oil components have been defined in the literature. Volatile oil molecules are less-produced by the plant, but they act as defense mechanisms against predator attacks, such as pathogens and insects (Bayan and Aksit, 2016; Silva and Câmara, 2013). The objectives of this study were to determine the chemical components of volatile oils from *M. spicata* L. and to investigate their antibacterial and antifungal activities.

Materials and methods

Preparation of plant materials and volatile oil

The *M. spicata* plant materials were collected from Kirsehir, Turkey in July 2016. The volatile oil was extracted from wet aerial parts by hydro-distillation in a Clevenger's apparatus for 2 h. The volatile oil was obtained from plant materials and was then kept in the dark at 4 °C until it was used.

Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analyses were done in an Agilent Technologies 7890A GC System, 5975C using a Triple-Axis Detector mass spectrometer with a built-in Autosampler, with the use of the HP-5MS capillary column (30 m × 0.25 mm × 0.25 mm). For GC/MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium was the transporter gas at a flow rate of 1 mL min⁻¹. The column temperature program was the same as defined upstairs. As in the gas chromatography, 1.0 L split/splitless (10:1) of the sample, diluted with hexane, was transferred to the clone. Identification of oil components was successful by comparison of their mass spectral fragmentation model by the available mass library (WILLEY and NIST).

In vitro antifungal effect of the volatile oils

The antifungal activities of volatile oil were determined using the agar well diffusion method (Tepe *et al.*, 2005). The Potato Dextrose Agar (PDA) was autoclaved and cooled to 40 °C. Then, it was transferred to 60-mm petri dishes (10 ml petri⁻¹). Next, 5-mm-diameter wells were opened on the PDA within the petri dishes. The plant volatile oils were applied at doses of 0.5, 1, 2, 4, 8 and 12 µl petri⁻¹ into the wells. Mycelium disks of 5 mm were then placed at equal distances from these wells. The fungi transferred to the petri dishes were incubated at 22±2 °C. The inhibition in development was compared to that in the control group, and percentile mycelial growth was calculated

$$I=100 \times (DC -DT)/DC$$

I: Inhibition percentage compared to the control (mycelium development)

DC: Mycelium development in the control

DT: Mycelium development in volatile oil applications.

Data were analyzed using the One-Way procedure of ANOVA (Windows version of SPSS, release 15.00). Differences among concentrations were compared using Duncan's Multiple Range Test with a level of $p < 0.05$.

In vitro antibacterial activity

In the plant bacterial sample, eight different *Xanthomonas* spp. (ZI365, ZI366, ZI368, ZI370, ZI373, ZI375, ZI376, ZI378) strains were isolated from pepper fields in Kahramanmaraş, Turkey. The bacteria cultures were grown in nutrient glucose agar solid medium, at 25 °C. After 24 h of growth, each bacteria strain, at a concentration of 0.1 OD density, was set with a spectrophotometer at 600 nm. Then, they were inoculated on the surface of nutrient glucose agar petri dishes with the aid of a drumstick. Later, filter paper discs (10 mm

in diameter) saturated with 10 μ l of volatile oil were placed on the surface of each inoculated petri dish. The petri dishes were incubated at 25 °C for 48 h. Bacteria were evaluated by measuring the undeveloped zone. The experiment was performed with four duplicates and was repeated twice.

Results and Discussion

Chemical Composition results

The compounds identified from *M. spicata* are shown in table 1. Thirty components, representing 100% of the total, were identified in *M. spicata* volatile oil. As the results of GC-MS analysis

showed, the main component of *M. spicata* L. volatile oil was Carvone (56.94%). The main component was followed by Limonene (11.63%), Sabinene hydrate (7.04%), Caryophyllene (4.06%), and Terpinen-4-ol (2.49%), as shown in table 1.

There is wide diversity in the chemical composition of *M. spicata* plants around the world. In previous studies, different chemotypes from the volatile oil of *M. spicata* have been described with other main components, with a prevalence of pulegone, carvone, limonene, 1,8-cineole and piperitone (Telci *et al.*, 2004; Morteza-Semnania *et al.*, 2006; Kokkini *et al.*, 1995). The chemical compositions of some differences were observed among volatile oils from *M. spicata*; the differ-

Table 1. Chemical composition of *M. spicata* L. volatile oil.

Compound number	¹ RT (min)	² RI	Name	%	Methods of identification
1	13,098	952	β -Pinene	0,45	RI, ³ MS
2	13,283	957	β -Pinene	0,45	RI, MS
3	13,472	963	β -Pinene	1,07	RI, MS
4	14,465	993	Terpinolene	0,35	RI, MS
5	14,889	1005	Limonene	11,63	RI, MS
6	15,018	1010	Eucalyptol	3,28	RI, MS
7	15,835	1035	γ -Terpinene	0,79	RI, MS
8	16,203	1046	Sabinene hydrate	7,04	RI, MS
9	16,876	1065	Terpinolene	0,24	RI, MS
10	17,25	1075	4-Thujanol	0,54	RI, MS
11	18,089	1098	Terpinolene	0,31	RI, MS
12	19,648	1145	α -Terpineol	0,37	RI, MS
13	20,047	1156	Terpinen-4-ol	2,49	RI, MS
14	20,712	1175	Dihydrocarvone	3,40	RI, MS
15	21,005	1183	Dihydrocarvone	0,18	RI, MS
16	21,517	1196	Carveol	0,35	RI, MS
17	22,604	1229	Carvone	56,94	RI, MS
18	23,431	1254	Carvone oxide	0,14	RI, MS
19	24,934	1297	Neodihydrocarveol	0,79	RI, MS
20	25,623	1318	Verbenone	0,53	RI, MS
21	26,049	1332	Carveol acetate	0,14	RI, MS
22	26,742	1353	alfa-Copaene	0,16	RI, MS
23	27,103	1364	β -Bourbonene	1,93	RI, MS
24	28,256	1398	Caryophyllene	4,06	RI, MS
25	28,485	1406	β -Cubebene	0,29	RI, MS
26	28,904	1420	β -Farnesene	0,53	RI, MS
27	29,284	1433	Caryophyllene	0,22	RI, MS
28	30,107	1459	β -Cubebene	0,96	RI, MS
29	31,214	1494	β -Cadinene	0,14	RI, MS
30	33,236	1563	Caryophyllene oxide	0,25	RI, MS
Total			100		

¹RT, retention time; ²RI, retention indices; ³MS, Mass spectra

ences may be due to genotype, environmental conditions or agronomic management, such as harvesting and date (Kokkini *et al.*, 1995; Dhifi *et al.*, 2011; Khan and Ahmad, 2011).

In vitro antifungal results

The volatile oil of *M. spicata* was used in the assay. The antifungal inhibition percentages of volatile oil from *M. spicata* L. on the mycelia growth of *V. dahliae*, *A. solani*, FORL and *R. solani* are shown in table 2.

The rate of mycelium growth inhibition was shown to vary with different doses. Compared with the control, volatile oil at a dose of 8 μL petri⁻¹ reduced the mycelium growth of *A. solani* and *R. solani* by 100%. *V. dahliae* and FORL mycelium growth was reduced by 57.61% and 64.09%, respectively. This result showed that, for *V. dahliae* and FORL, volatile oil had antifungal potential, and volatile oil showed a better effect on *A. solani* and *R. solani* at a dose of 8 μL petri⁻¹. At a dose of 10 μL petri⁻¹, the volatile oil blocked the plant pathogen mycelium growth by 100%. The biological activities of volatile oils from the mentha species were shown to have strong antimicrobial and antifungal effects. Additionally, volatile oil from *M. spicata* showed a fungitoxicity effect (Ramesh *et al.*, 2006; Hajlaoui *et al.*, 2009).

Gulluce *et al.* (2007), in their work on the antimicrobial properties of the volatile oils from *Mentha longifolia* L. ssp. *Longifolia*, showed the strong antimicrobial activity of volatile oil against all 30 microorganisms. The antimicrobial effects of volatile oils depend on the species of the compound and its chemical components. Previous studies support our results.

In vitro antibacterial results

The antimicrobial activity of the volatile oil from *M. spicata* was studied with a dose of 10 μL petri⁻¹ against eight plant pathogenic bacterial strains. The results of the antimicrobial potential of the volatile oil are presented in Table 3. The diameter of the growth inhibition zones ranged from 12 to 16 mm for all the *Xanthomonas* spp. strains.

M. spicata volatile oil showed remarkable activity against the tested *Xanthomonas* spp. strains. The highest effect of volatile oil was seen on the ZI365 strain. In previous studies, most researchers reported the antimicrobial, antifungal, and antioxidant properties of volatile oils (Pattnaik *et al.*, 1996; Sacchetti *et al.*, 2005; Gulluce *et al.*, 2007). Gulluce *et al.* (2007) reported that the volatile oils of *M. longifolia* ssp. *longifolia* showed significant antimicrobial activities against all 15 bacteria, 14 fungi and a yeast species tested. When

Table 2. Antifungal activity values (Inhibition (%) and Inhibition zone) for *M. spicata* volatile oil.

Doses (μL petri ⁻¹)	†Plant Pathogens							
	‡R.s		A.s		FORL		V.d	
	‡I(%)	§Iz (mm)	I(%)	Iz(mm)	I(%)	Iz(mm)	I(%)	Iz(mm)
(0) N.c	0.00 ^a ±0.0	60	0.00 ^d ±0.0	60	0.00 ^e ±0.0	60	0.00 ^f ±0.0	60
0.5	0.00 ^a ±0.0	60	0.00 ^d ±0.0	60	15.08 ^f ±0.79	50.95	28.42 ^g ±2.49	42.94
1	21.11 ^a ±1.46	47.33	0.00 ^d ±0.0	60	37.73 ^g ±5.02	37.35	36.56 ^g ±1.48	38.06
2	34.05 ^a ±2.38	39.57	31.39 ^a ±0.96	41.16	45.92 ^a ±1.17	32.44	43.63 ^d ±0.46	33.82
4	58.75 ^b ±1.22	24.75	46.61 ^b ±1.33	31.82	52.95 ^a ±0.95	28.23	50.88 ^c ±1.02	29.46
8	100 ^a ±0.0	0	100 ^a ±0.0	0	64.09 ^b ±1.52	21.54	57.61 ^c ±1.99	25.43
12	100 ^a ±0.0	0	100 ^a ±0.0	0	100 ^a ±0.0	0	100 ^a ±0.0	0

According to Duncan's test, the averages with different letters in the same column are different at the significance level of $p < 0.05$

†Plant pathogens: *R. solani* (R.s), *A. Solani* (A.s), *F. oxysporum* f. sp *radicis-lycopersici* (FORL), *V. dahliae* (V.d), Negative control (N.c). ‡I(%): Mycelium inhibition percentage. §Iz (mm): Mycelium development

Table 3. Antibacterial activity of volatile oil; pathogen inhibition zone (mm), according to the disk diffusion method.

<i>Xanthomonas</i> spp. strains	Control	ZI378	ZI376	ZI375	ZI373	ZI370	ZI368	ZI366	ZI365
inhibition zone (mm)		14	14	13	13	13	13	12	16

compared with earlier studies, similar results were obtained in our study. Previous studies have supported our findings.

The main conclusions are as follows. In this study, we determined the antibacterial and antifungal activities and chemical composition of volatile oil from *M. spicata* L. The results of this work have

shown that volatile oil from *M. spicata* has strong antifungal activity against plant pathogenic fungi. Additionally, it showed remarkable antibacterial activity. The chemical composition of volatile oil from *M. spicata* was determined, and its main component was carvone. These studies can be used in the management of plant pathogenic fungi and bacterial disease control.

Resumen

Y. Bayan, y M. Küsek. 2018. Composición química y actividad antifúngica y antibacteriana del aceite volátil *Mentha spicata* L. Cien. Inv. Agr. 45(1): 64-69. El objetivo del trabajo fue la investigación de la composición química, de la actividad antifúngica y antibacteriana del aceite volátil *Mentha spicata*. Desde el análisis Cromatografía de gases / espectrometría de masas (GC-MS), el componente principal *M. spicata*, resulta constituido por carvona (56.94%), limoneno (11.63%), hidrato de sabineno (7.04%) y cariofileno (4.06%). Se ha además detectado la actividad antifúngica del aceite volátil de *M. spicata* L. Contra hongos patógenos de plantas; *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Sacc.) W.C. Synder & H.N. Hans (FORL), *Rhizoctonia solani* J.G. Kühn. (*R. solani*), *Alternaria solani* (*A. solani*) y *Verticillium dahliae* Kleb (*V. dahliae*). Se ha demostrado una fuerte actividad antifúngica del aceite volátil contra los hongos patógenos de plantas. El resultado del estudio, a una dosis de 12 µL petri-1, inhibió el crecimiento del 100% del micelio de *V. dahliae*, *A. solani*, FORL y *R. solani*. El aceite volátil exhibió una actividad notable contra las *Xanthomonas* spp seleccionadas (ZI365, ZI366, ZI368, ZI370, ZI373, ZI375, ZI376, ZI378) aislados bacterianos.

Palabras clave: Aceites volátiles, antibacterial, antifúngico, *Mentha spicata* L.

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