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Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart from the Marmara region, Turkey

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ABSTRACT: The family Lamiaceae is represented by 45 genera, 546 species and 730 taxa in Turkey. The genus *Origanum* is represented in Turkey by 22 species; the ratio of endemism in the genus is 63%. *Origanum vulgare* subsp. *hirtum* collected from different localities in Marmara region and their cultivated forms provided by the Atatürk Central Horticultural Research Institute Yalova/Turkey were subjected to hydrodistillation to yield essential oils which were subsequently analysed by GC and GC–MS. The main constituents of the oils were identified and antimicrobial bioassays were applied. The analyses showed that wild and cultivated *Origanum vulgare* subsp. *hirtum* oils contained carvacrol (82.9–7.5% and 85.4–5.3%, respectively) and thymol (60.1–0.3% and 68.0–0.3%, respectively) as the main components. The essential oils showed strong antimicrobial activity against all the microorganisms tested. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: *Origanum vulgare* subsp. *hirtum*; Lamiaceae; essential oil; GC–MS analysis; antibacterial activity; antifungal activity; carvacrol; thymol

Introduction

Turkey is regarded as an important gene-centre for the family Lamiaceae. The family is represented by 45 genera, 546 species and 730 taxa in Turkey. The rate of endemism in the family is 42.2%.[1] The *Flora of Turkey* has 22 species (32 taxa) of *Origanum*, 21 being endemic to Turkey, and the ratio of endemism in the genus is 63%. Of the 52 known taxa of *Origanum*, 32 are distributed in Anatolia, meaning that 60% of all *Origanum* taxa are recorded to grow in Turkey. One of them is *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart. This plant is known in Turkey as 'Istanbul kekigi' and is widely used as kekik in Marmara and Thrace regions.[2]

Essential oils have many applications in folk medicine and for food flavouring and preservation, as well as in the fragrance and pharmaceutical industries. The antimicrobial and antioxidant properties of essential oils have been known for a long time, and a number of investigations have been conducted on their antimicrobial activities, using bacteria, viruses and fungi.[3–5]

The essential oil of *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart has been analysed by several authors[6–10] and its thymol and carvacrol chemotypes were identified in *O. vulgare* subsp. *hirtum*.[11] Baser *et al.*[2,10] reported that there was a correlation between the oil yield and the carvacrol content, best oil yields (2.9–6.5%) and highest carvacrol contents (up to 78.73%) were obtained.

The other subspecies of *O. vulgare* growing in Turkey are poor in oil yield.[10] The main constituents of *O. vulgare* L. subsp. *viride* growing in Iran were reported as linalyl acetate, β-caryophyllene and sabinene.[12] Carvacrol, γ-terpinene, *p*-cymene were characteristic for plants cultivated in the Kishenev Botanical Garden, Moldova.[13] The *O. vulgare* subsp. *virens* plants produce linalool, β-caryophyllene, linalool-α-terpineol, linalool-terpinen-4-ol, terpineol (-linalool) and terpineol (-carvacrol)[14] chemotypes of essential oils.

The composition of the essential oil depends on plant type, geographical location and collection season.[15] The chemical composition and antioxidant effect of the essential oil from species have been reviewed recently by Milos *et al.*[16] They reported the main components as thymol (40.4%), carvacrol (24.8%) and *p*-cymene (16.8%). The antimicrobial activity of major oil compounds, according to Faid *et al.*,[17] has the following order: phenols > alcohols > aldehydes > ketones > ethers > hydrocarbons. This action of essential oils may be due to the impairment of a variety of enzyme systems, including those involved in energy production and structural component synthesis, and distort the lipid–protein interaction in the cytoplasmic membrane.[18,19]

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The aim of this study was to determine the composition and the antimicrobial activities of the essential oils of *O. vulgare* subsp. *hirtum* growing in different localities in the Marmara Region in Turkey, and their cultivated forms.

Experimental

Plant Material

Information on the plant material used in this study is given in Tables 1 and 2. The voucher specimens have been deposited at the Department of Biology, Balikesir University, Balikesir, Turkey, and in the Herbarium of the Faculty of the Pharmacy, Anadolu University (ESSE), Eskisehir, Turkey.

Extraction of the Essential Oil

Air-dried aerial parts of plants were hydrodistilled for 3 h using a Clevenger-type apparatus. The percentage yields (%) of the oils calculated on moisture-free basis are given in Table 2.

Gas Chromatography (GC)

GC analysis was carried out using a Shimadzu GC-9A with CR4-A integrator. A polar Thermon 600T FSC column (50 m × 0.25 mm i.d., film thickness 0.2 μm) was used with nitrogen as the carrier gas. Oven temperature was kept at 70 °C for 10 min and programmed to 180 °C at a rate of 2 °C/min, then kept constant at 180 °C for 30 min. The split ratio was adjusted to 60:1. The injector and FID detector temperature was 250 °C.

Table 1. Information on collection of wild and cultivated *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart

Plant code	Localities
101	Yalova
202A	Çan/Çanakkale
202B	Çan/Çanakkale
203B	Bayramiç/Çanakkale
203C	Bayramiç/Çanakkale
205C	Bayramiç/Çanakkale
206	Bayramiç/Çanakkale
208A	Gökçeada/Çanakkale
211	Gökçeada/Çanakkale
212	Yenice/Çanakkale
306	Sındırgı/Balıkesir
309	İvrindi/Balıkesir
311	Edremit/Balıkesir
313	Gönen/Balıkesir
315	Erdek/Balıkesir
316	Bandırma/Balıkesir
407	Mudanya/Bursa
407A	Mudanya/Bursa
419	İznik/Bursa
213	Lapseki/Çanakkale

* Cultivated samples.

GC–MS Analysis Conditions

The following GC–MS systems were both used during the analysis for determination of the chemical compounds:

Table 2. Essential oil yields and main compounds of wild and cultivated *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart

Plant code	Essential oil yield (%)		Main compounds of the essential oils									
			Carvacrol		Thymol		Linalool		γ-Terpinene		*p*-Cymene	
	W	C	W	C	W	C	W	C	W	C	W	C
101	3.9	4.3	70.9	78.7	3.4	0.3	0.1	0.2	0.9	5.5	13.0	5.0
202A	4.5	5.7	80.9	81.0	1.4	0.6	0.1	0.2	0.8	3.3	9.2	6.7
202B	4.5	3.0	80.9	72.3	1.8	0.6	0.1	0.3	0.7	6.4	7.8	10.7
203B	4.3	4.0	74.4	77.3	2.0	6.8	0.1	0.1	1.3	4.9	13.2	4.4
203C	5.1	5.1	82.9	85.2	1.2	0.5	0.1	—	0.1	3.6	8.8	4.7
205C	4.2	4.5	74.2	53.2	1.2	2.7	0.03	0.1	4.9	9.6	10.4	22.1
206	4.4	5.5	45.5	39.1	4.6	8.0	0.1	0.1	7.2	19.5	31.1	17.5
208A	4.4	5.4	31.9	15.9	48.1	42.3	0.03	tr.	3.6	12.0	8.0	16.7
211	3.8	5.4	75.7	88.6	1.8	0.7	0.1	0.1	0.9	1.7	13.4	2.8
212	3.0	4.9	73.2	69.6	5.0	8.3	0.1	0.1	tr.	6.8	12.1	6.3
306	4.3	4.6	7.5	8.7	60.1	68.0	0.1	0.1	2.9	6.2	17.8	6.0
309	4.1	4.2	8.7	5.3	57.8	67.7	0.1	0.1	7.8	8.0	15.3	8.8
311	4.1	4.9	60.2	60.3	5.5	18.4	0.1	0.1	3.5	6.6	21.1	6.6
313	4.0	4.6	68.8	84.0	1.9	1.3	0.1	0.1	0.2	3.5	20.4	4.6
315	4.0	4.0	68.9	85.4	1.4	1.1	0.1	0.1	—	3.0	20.5	3.6
316	4.7	3.7	73.4	76.0	2.0	5.4	0.1	0.1	0.3	3.6	14.6	5.6
407	3.1	4.2	70.4	70.0	6.1	1.5	0.1	tr.	0.1	12.9	13.1	5.5
407A	3.1	3.2	53.9	66.0	0.7	0.6	0.04	0.1	2.8	3.9	28.1	16.5
419	3.7	4.4	78.3	79.2	0.3	0.4	0.2	0.1	0.5	5.3	10.0	5.2
213	6.1	5.0	29.1	14.2	52.3	36.6	tr.	0.1	4.7	4.8	6.4	31.6

W: Wild. C: Cultivated. tr: less than 0.1%.

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1. A Hewlett-Packard GCD system, with an Innowax FSC column (60 m × 0.25 mm i.d.), was used, with helium as the carrier gas. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and then kept constant at 220 °C for 10 min. Split flow was adjusted to 50 ml/min. The injector temperature was 250 °C. MS were taken at 70 eV. Mass range was *m*/*z* 35–425.
2. A Shimadzu GCMS-QP5050A system, with CP-Sil 5CB column (25 m × 0.25 mm i.d.) was used, with helium as the carrier gas. GC oven temperature was kept at 60 °C and programmed to 260 °C at a rate of 5 °C/min, and then kept constant at 260 °C for 40 min. Split flow was adjusted to 50 ml/min. The injector temperature was at 250 °C. MS were taken at 70 eV. Mass range was *m*/*z* 30–425.

A library search was carried out using the in-house Baser Library of Essential Oil Constituents. The MSs were also compared with those of reference compounds and confirmed with the aid of retention indices from published sources. The percentage compositions were obtained from electronic integration measurements using flame ionization detection (FID; 250 °C). The components identified in the oils tested are listed in Table 2.

Antimicrobial Screening

The agar disc diffusion method was employed for the determination of antimicrobial screening of the essential oils.[20] Suspension of the tested microorganisms (10^8 CFU/µl) was spread on the solid media plates. Each test solutions are prepared in DMSO. Then filter paper discs (6 mm in diameter) were soaked with 20 µl of the stock solutions and placed on the inoculated plates. After keeping at 2 °C for 2 h, they were incubated at 37 °C for 24 h for bacteria and *Candida albicans*. The diameter of the inhibition zones were measured in millimetres (Table 3).

Determination of Minimum Inhibitory Concentration (MIC)

Microdilution broth susceptibility assay was used (21). Stock solutions of essential oils were prepared in dimethylsulphoxide (DMSO). Serial dilutions of essential oils were prepared in sterile distilled water in 96-well microtitre plates. Freshly grown bacterial suspension in double-strength Mueller–Hinton broth and yeast suspension of *Candida albicans* in Saboraud dextrose broth were standardized at 10^8 CFU/ml (McFarland No. 0.5). The microtitre plates were incubated at 37 °C for 3 days. Each test was performed in duplicate. Chloramphenicol and ketoconazole served as positive controls (Table 4).

Fungal Spore Inhibition Assay

In order to obtain conidia, the fungi were cultured on Czapex Dox agar and malt extract agar medium in 9 cm Petri dishes at 25 °C for 10 days. Harvesting was carried out by suspending the conidia in a 1% w/v sodium chloride solution containing 5% w/v DMSO. The spore suspension was then filtered and transferred into tubes and stored at –20 °C, according to the method of Hadacek and Greger.[22]

Screening for antifungal activities of the stock solution of the essential oils was performed qualitatively using the disc

Table 3. Antibacterial screening of wild and cultivated *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart essential oils, according to the agar disc diffusion method (mm)

Plant code	Stock solution of the essential oils											
	E. coli		*S. aureus*		*P. aeruginosa*		*E. aerogenes*		*P. vulgaris*		*C. albicans*	
		*		*		*		*		*		*
101	8	10	8	10	9	11	9	11	9	10	6	8
202A	8	10	7	9	8	10	8	11	8	10	9	8
202B	7	8	8	7	7	8	8	8	8	10	9	7
203B	10	11	10	10	9	8	10	9	8	9	9	8
203C	9	8	9	8	8	9	10	9	9	9	10	9
205C	10	8	11	9	11	9	9	9	9	8	10	9
206	8	10	8	10	9	10	8	10	9	10	9	10
208A	11	8	10	9	10	8	10	10	10	9	8	7
211	9	10	10	10	9	10	9	9	9	9	9	7
212	10	8	9	8	10	7	9	8	8	8	9	9
306	10	8	10	7	11	7	11	7	10	7	11	8
309	11	10	11	10	9	9	8	10	9	10	12	9
311	9	9	13	10	10	9	8	12	8	10	7	9
313	8	9	9	9	9	8	8	9	9	9	8	7
315	10	10	10	9	9	11	10	11	9	10	10	7
316	8	8	7	8	8	8	8	7	7	7	9	7
407	9	10	8	9	8	10	9	8	8	9	9	7
407A	9	8	9	8	10	7	10	7	9	8	9	7
419	10	9	12	8	11	8	10	8	10	7	11	7
213	8	9	8	7	9	8	9	7	8	7	8	7
Control	29[c]		30[c]		33[c]		28[c]		26[c]		34[k]	

* Cultivated samples. [c]Chloramphenicol. [k]Ketokonazol.

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Table 4. Antibacterial activities of wild and cultivated *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart (MIC)

Plant code	*E. coli*		*S. aureus*		*P. aeruginosa*		*E. aerogenes*		*P. vulgaris*		*C. albicans*	
		*		*		*		*		*		*
101	250	125	125	125	250	125	125	250	62.5	125	62.5	250
202A	500	250	500	125	500	125	250	125	250	125	125	500
202B	250	250	250	250	250	500	125	250	250	250	500	500
203B	125	250	250	250	250	250	250	500	250	125	125	250
203C	500	250	500	500	250	125	250	250	500	500	125	250
205C	250	250	125	125	125	125	125	125	125	125	125	250
206	250	500	125	500	125	250	125	250	250	500	125	250
208A	250	250	250	250	250	250	250	250	250	250	125	500
211	500	250	250	250	500	250	250	250	500	250	125	500
212	250	250	500	250	250	250	500	250	250	500	250	250
306	62.5	500	62.5	500	125	500	125	500	125	500	125	500
309	125	125	250	250	250	250	125	250	125	250	62.5	250
311	250	250	125	250	250	500	250	125	250	250	125	500
313	250	125	250	125	62.5	250	125	250	125	250	125	500
315	250	125	250	250	500	250	250	250	500	125	500	500
316	500	500	500	250	500	500	500	500	125	500	125	500
407	125	250	250	250	125	250	125	250	250	250	500	500
407A	500	250	250	250	250	250	125	250	125	250	125	500
419	125	250	125	250	125	250	125	125	125	125	62.5	500
213	125	250	125	250	500	250	500	500	125	250	500	500

* Cultivated samples.

diffusion method (Table 5) against the saprophytic fungi *Aspergillus flavus*, *A. niger*, *Penicillium expansum* and *Alternaria brassicola*, cultured on malt extract and Czapex Dox agar medium. One loopful of the spore suspension was applied to the centre of the Petri dishes and 20 µl of each essential oil was applied onto sterile paper discs (6 mm in diameter) and placed in the Petri dishes, which were incubated at 25 °C for 3 days. The test solution was prepared in DMSO. The percentage inhibition of the fungal growths was determined on the growth in test plates compared to the respective control plates (23) according to the equation:

$$\text{Inhibition } \% = 100\ (C - T)/C$$

where C is the diameter of fungal growth on the control and T is the diameter of the fungal growth on the test plate. The activities of the essential oils were compared with the activity of standard fungicide ketoconazol.

Results and Discussion

Wild-growing *Origanum vulgare* subsp. *hirtum* samples from the Marmara region of Turkey, cultivated in Yalova (Table 1), were hydrodistilled and the oils were analysed by GC and GC–MS. The analyses showed that wild and cultivated *O. vulgare* subsp. *hirtum* oils obtained from wild plants and cultivars contained carvacrol (82.9–7.5% and 85.4–5.3%, respectively), and thymol (60.1–0.3% and 68.0–0.3%, respectively) as the main components. Other major components identified in oils from wild and from cultivated samples, respectively, were as follows: *p*-cymene, 31.1–6.4% and 31.6–2.8%; γ-terpinene, 7.8–0.1% and 19.5–3.0%; linalool, 0.4–0.1% and 0.3–0.1%. The basic composition of the essential oils from wild plants was the same as those from cultivated plants. However, the γ-terpinene content was lower in wild collections (trace–7.8%) than those cultivated in Yalova (3.0–19.5%) (Table 2). The main components and their retention indices are summarized in Table 2, while the results of the antibacterial and antifungal activities of the essential oils are presented in Tables 3–5.

In general, the essential oils possessing the strongest antibacterial properties against bacteria contain a high percentage of phenolic compounds, such as carvacrol and thymol. The possibility that the other minor components may possess some antimicrobial power or synergistic effect still remains unclear.[24,25] Also, another major component of the tested essential oils is *p*-cymene, which is not an effective antibacterial when used alone;[25,28] however, when combined with carvacrol, a synergistic effect against *B. cereus* has been reported.[26]

Ultee *et al*.[27] proposed a mechanism of action for carvacrol based on its acidity, according to which the activity of carvacrol is related to the loss of the proton gradient. Thus, the proton motive force disturbs the bacterial membrane. Briefly, carvacrol may diffuse back and forth through the bacterial membrane, while exchanging the acidic proton for another cation on the cytosolic side of the membrane and the opposite cation exchange at the exterior side.[28]

A recent study, using the agar disc diffusion method and microdilution broth susceptibility assay, indicated that all the tested bacteria were sensitive against the oils (Tables 3 and 4). Also, no obvious difference in susceptibility between Gram-negative and Gram-positive bacteria was measured after 24 h. Ratledge and Wilkinson reported that Gram-negative microorganisms are less

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Table 5. Antifungal activities of wild and cultivated *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart (inhibition %)

Plant code	Stock solution of the essential oils							
	Aspergillus flavus		*Aspergillus niger*		*Penicillum expansum*		*Alternaria brassicola*	
		*		*		*		*
101	100	5.45	100	16.98	100	13.63	100	—
202A	100	27.27	60	11.32	100	100	68.7	8.33
202B	5.4	100	—	15	22.7	100	—	—
203B	—	18.18	5.6	16.98	13.6	100	—	—
203C	25.4	16.36	—	15	22.7	100	58.3	—
205C	—	40	20.7	15	100	27.27	—	31.25
206	27.27	12.72	18.86	5.66	—	—	12.5	—
208A	100	45.45	45.28	24.52	100	9	—	50
211	—	23.63	—	24.52	27.27	18.18	—	37.50
212	54.5	9	43.39	24.52	100	9	100	—
306	—	3.63	—	1.88	100	18.18	43.75	33.33
309	10.9	41.81	—	20.75	9	9	29.16	58.33
311	65.45	16.36	56.6	13.20	100	100	100	37.50
313	78.18	—	15	15	100	9	100	—
315	100	9	26.41	15	100	9	100	45.83
316	—	—	—	18.86	100	31.81	—	12.50
407	—	23.63	1.92	24.52	50	18.18	14.58	37.50
407A	—	27.27	—	30.18	18.18	—	—	—
419	34.54	9	100	43.39	100	9	—	58.33
213	78.18	—	100	—	100	9	100	27
Ketokonazol	100		100		100		100	

* Cultivated samples.

susceptible to the action of antibacterials, since they possess an outer membrane surrounding the cell wall.[29] However, not all studies on essential oils have concluded that Gram-positives bacteria are more susceptible. Also, Dorman and Deans[30] reported that the volatile oils of *O. vulgare* subsp. *hirtum* appeared to be equally effective against both Gram-positive and Gram-negative microorganisms. Components with phenolic structures, such as carvacrol and thymol, were highly active against the test microorganisms.

Dortunc and Cevikbas showed that the essential oils of *O. onites* and *O. vulgare* subsp. *hirtum* were effective against *Bacillus subtilis*, *Staphylococcus aureus*, *Echerichia coli*, *Candida albicans*, *Aspergillus niger*, *Penicillium chrysogenum* and *Fusarium* sp.[31] The antibacterial and antifungal effects of the essential oils from several medicinal plants are apparently related to their phenolic contents.[32,33]

In view of the observed inhibitory features of these essential oils, it is suggested that they could be used as preventatives against microfungal and bacterial contamination in many foods, instead of the common synthetic antimicrobial products.

References

1. Baser KHC. *Acta Horticult*. 1993; **333**: 217–238.
2. Baser KHC. The Turkish *Origanum s*pecies. In *Oregano. The Genera Origanum and Lippia*, Kintzios SE (ed.). Taylor & Francis: London, 2002; 109.
3. Sarer E. *Origanum Species*, *Their Essential Oil Content and Main Components of Oils*, Baser KHC, Guler N (eds). AREP: Istanbul, 1993; 20.
4. Azaz AD, Irtem HA, Kurkcuoglu M, KHC Baser. *Z. Naturforsch*. 2004; **59**: 75–80.
5. Alma MH, Mavi A, Yildirim A, Digrak M, T Hirata. *Biol. Pharm. Bull*. 2003; **26**: 1725–1729.
6. Baser KHC. *Lamiales Newslett*. 1994; **3**: 217–238.
7. Fleisher A, Sneer N. *J. Sci. Food Agric*. 1982; **33**: 441–446.
8. Lawrence BM. *Perfum. Flavor*. 1989; **14**: 36–40.
9. Sezik E, Tumen G, Kirimer N, Özek T, Baser KHC. *J. Essent. Oil Res*. 1993; **5**(4): 425–431.
10. Baser KHC, Ozek T, Kurkcuoglu M, Tümen G. *J. Essent. Oil Res*. 1994; **6**: 31–36.
11. Skoula M, Gotsiou P, Naxakis G, Johnson BC. *Phytochemistry* 1999; **52**: 649–657.
12. Afsharypuor S, Sajjadi ES, Erfan-Manesh M. *Planta Med*. 1997; **63**: 179–180.
13. Bodrug MV, Dragalin IP, PF Vlad. Proceedings of the Simpheropol Conference, Ukrania, 1990; 165.
14. Azcan N, Kara M, Demirci B, Baser KHC. *Lipids* 2004; **39**(5): 487–489.
15. Milos M, Mastelic J, Jerkoviç I. *Food Chem*. 2000; **71**: 79–83.
16. Faid M, Charai M, Mosaddak M. *J. Essent. Oil Res*. 1996; **8**: 657–664.
17. Kokkini S, Karaousou R, Dardioti A, Krigas N, Lanaras T. *Phytochemistry* 1997; **44**: 883–886.
18. Juven BJ, Kanner J, Schved F, Weisslowicz H. *J. Appl. Bacteriol*. 1994; **76**: 626–631.
19. National Committee for Clinical Laboratory Standards (NCCLS). *Performance Standards for Antimicrobial Disc Susceptibility Test*, 6th edn, Approved Standard, M2-A6. NCCLS: Wayne, PA, 1997.
20. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Antimicrobial susceptibility testing. In *Color Atlas and*

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Textbook of Diagnostic Microbiology. Lippincott-Raven: Philadelphia, PA, 1997; 785.
21. Hadecek F, Greger H. *Phytochem. Anal*. 2000; **11**: 137–147.
22. Dharmaraj N. *Transit. Metal Chem.* 2001; **26**: 105–109.
23. Juliano C, Mattana A, Usai M. *J. Essent. Oil Res*. 2000; **12**: 516–522.
24. Lambert RJW, Skandamis PN, Coote P, GJE Nychas. *J. Appl. Microbiol*. 2001; **91**: 453–462.
25. Ultee A, Slump RA, Steging G, Smid EJ. *J. Food Protect*. 2000; **63**(5): 620–624.
26. Ultee A, Bennik MH, Moezelaar R. *Appl. Environ. Microbiol*. 2002; **68**(4): 1561–1568.
27. Veldhuızen EJA, Tjeerdsma-Van Bokhoven JLM, Zweıjtzer C, Burt S, Haagsman HP. *J. Agric. Food Chem.* 2006; **54**: 1874–1879.
28. Burt S. Essential oils: their antibacterial properties and potential applications in foods — a review of the literature. *Int. J. Food Microbiol*. 2004; **94**: 223–253.
29. Dorman HJD, Deans SG. *J. Appl. Microbiol*. 2000; **88**: 308–316.
30. Dortunc T, Cevikbas A. *J. Pharm. Univ. Marmara* 1992; **8**: 117–128.
31. Consentino S, Tuberosa CIG, Pisano B *et al*. *Lett. Appl. Microbiol*. 1999; **29**: 130–135.
32. Farag RS, Daw ZY, Hewedi FM, El-Baroly GSA. *J. Food Protect*. 1989, **52**: 665–667.

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