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CHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITIES OF THE ESSENTIAL OILS OF *SATUREJA THYMBRA* L. AND *THYMBRA SPICATA* L. AND THEIR MAIN COMPONENTS

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Abstract - This work covers the chemical composition and antimicrobial activities of the essential oils isolated from *Satureja thymbra* and *Thymbra spicata* analyzed using GC/MS. The main components of *S. thymbra* oil were thymol (33.8 %), γ-terpinene (30.8 %) and p-cymene (11.8 %). The main components in *T. spicata* oil were carvacrol (74.5 %) and γ-terpinene (11.2 %). The oils and their main components, thymol and carvacrol, were assayed by applying the microdilution method for antibacterial and antifungal activity against food poisoning, plant, animal and human pathogenic microorganisms. The oil of *T. spicata* and carvacrol showed the highest antimicrobial activity.

Key words: *Satureja thymbra*, *Thymbra spicata,* essential oil, thymol, carvacrol, antimicrobial activity

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INTRODUCTION

From ancient times, in addition to spices and their derivatives being used for flavoring foods and beverages and for medication, essential oils have also been highly valued for their use as antimicrobials (Akin and Oguz, 2010).

We focused our investigation on the antimicrobial activities of the essential oils of *Satureja thymbra* and *Thymbra spicata* from the Lamiaceae family, which has a wide distribution in Greece and is of economic importance, and also of their components carvacrol, thymol, camphor, and 1,8-cineole, against soil-borne pathogens, food storage fungi, mycotoxic species, phytopathogens and human pathogens. This is the first report on the essential oil composition and antimicrobial activity of *Thymbra spicata* growing in Greece.

Thymbra spicata L. is used in the traditional medicinal system of Turks, Greeks, Egyptians and Romans to treat asthma and bronchitis as well as being used in the food industry for flavor, aroma and preservation (Daneshvar-Royandezagh et al., 2009). The leaves have recently gained much popularity as a remedy to combat hypercholesterolemia (Akkol et al., 2009). The essential oils of this plant have wide industrial applications, from the flavoring of foods, liqueur production, perfumery and antiseptic used as antimicrobial agents (Sarac et al., 2009). Besides, the dried plant, softened in boiled water used to be applied to wounds as a drug (Akin and Oguz, 2010).

Satureja thymbra L. has a concurrent warming, circulatory-stimulating action that makes it an excellent addition (at low levels) to massage blends for arthritis and rheumatism. It can also soothe painful joints and muscles (Simon et al., 1984). The acaricidal activity of the essential oil of *S. thymbra* and its major constituents, carvacrol and *γ*-terpinene, was also observed (Cetin et al., 2009).

MATERIALS AND METHODS

Plant material

The plant material (aerial parts of *S. thymbra* and *T. spicata*) was collected at full blooming stage from experimental plots at A.R.C.N.G –NAGREF (latitude 40°, 31' N, longitude 22°, 58' E). The cultivated plants were primarily originated from seeds native to Greece's *S. thymbra* and *T. spicata* populations.

Essential oil isolation procedure

The percentage oil content (as ml/100g of plant material) was determined using the European Pharmacopoeia apparatus (Clevenger-type). Dried inflorescences and the upper leaves (50 g) were chopped and distilled for 2 h. The obtained essential oils were dried over anhydrous sodium sulphate and stored in the refrigerator (-18 °C) until used.

Essential oil analyses procedure

The essential oil samples were analyzed by Gas Chromatograph Hewlett Packard 5890 Series II, connected to a chromatographic integrator (Hewlett Packard 3396 Series II Dual Channel). Three fused silica columns of different polarity were used: Durabond-DB 1, DB-Wax and CP-Sil 19 CB. Temperature program: 45 to 220° C at 3.5° C/min, carrier gas nitrogen: 140 Kpa, injection temperature: 220° C, detector temperature 300° C. Sample injection: 0.2–0.3 μl of a 10 % essential oil solution in pentane; split 1:20. The percentage compositions were computed after 3 GC runs of each sample from the peak areas without correction factors. The GC/MS analysis was performed on a fused silica column DB-5, using a Gas Chromatograph 17A Ver. 3 interfaced with a Mass Spectrometer Shimadzu QP-5050A supported by the Class 5000 software. The injection temperature was 260 °C, interface heating 300 °C, ion source heating 200 °C, EI mode 70 eV, scan range: 41 – 450 amu, and scan time 0.50 s. The oven temperature programs were: a) 55° – 120°C (3°/min), 120-200°C 4°/min, 200-220° (6°/min) and 220°C for 5 min and b) 60-240 C° at 3° C/min, carrier gas He, 54.8 kPa, split ratio 1:30.

Identification and quantification of the oil components

The identification of the constituents was based on comparison of their Kovats indices (RI) relative to *n*-alkanes with corresponding literature data (Adams, 1995), as far as matching a) their spectra with those from MS libraries (NIST 98, Adams 1995), and b) the RT of co-eluting reference compounds – peak enrichment technique (authentic samples by Roth and Sigma Aldrich).

Antibacterial activity

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Listeria monocytogenes* (NCTC 7973), *Enterococcus faecalis* (human isolate) and the following Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538).

The bioassay was carried out by the microdilution method (Hanel and Raether, 1988; Espinel-Ingroff, 1989; Daouk et al., 1995). The bacterial cell suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 μl per well. The microplates were incubated for 24 h and 48 h at 37° C. The lowest concentrations without visible growth (by the binocular microscope) were defined as MICs. The minimum bactericidal (MBCs) were determined by the serial subcultivation of 2 μl into microtiter plates containing 100 μl of broth per well and further incubation for 24 h and 48 h at 37° C.

The lowest concentration with no visible growth was defined as MFC respectively indicating = 99.5% killing of the original inoculum. Each experiment was repeated in triplicate. Streptomycin was used as positive controls (0.6-25 μg/ml).

Antifungal activity

For the antifungal bioassays, the following fungi were used: *Aspergillus fumigatus* (plant isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061).

The bioassay was carried out by the microdilution method (Hanel and Raether, 1988; Espinel-Ingroff, 1989; Daouk et al., 1995). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 μl per well. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (by the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 μl into microtiter plates containing 100 μl of broth per well and further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. Each experiment was repeated in triplicate. The commercial fungicide, ketoconazole, was used as positive control (0.6 - 25 μg/ml).

RESULTS

Essential oil analyses

The results of the chemical analysis of the oils from *S. thymbra* and *T. spicata* are presented in Table 1. We identified 24 compounds in *S. thymbra* oil, representing 98.70% of the total amounts, and 20 compounds in *T. spicata* oil (99.00%). It can be seen that thymol (33.8%), γ-terpinene (30.8%) and p-cymene (11.8%) were the main components of *S. thymbra* oil. Carvacrol (74.5%) was the main component of the *T. spicata* oil, while the biosynthetically related monoterpene hydrocarbons γ-terpinene and p-cymene amounted to the 8.12 % and 5.62% of the oil, respectively.

Previous studies on the essential oil of *Satureja thymbra* have shown that it contains carvacrol and thymol as major components. From the analysis of *S. thymbra* oil, carvacrol was found to be the most abundant component (48.5%); these results are similar to those reported by Lagouri et al. (1993) for the same Greek plant species. The biosynthetically related γ-terpinene and p-cymene were the other major components in the oils, representing 23.2% and 9.6%, respectively (Soković et al., 2002). The chemical composition of *S. thymbra* oil from Turkey showed that carvacrol (55.8%), γ-terpinene (21.7%) and p-cymene (8.7%) were the dominant components (Ugur et al., 2009) The chemical composition of the essential oils from *S. thymbra* species restricted to Greece and the eastern Mediterranean region, showed that the main components were thymol (41%), γ-terpinene (22.2%) and p-cymene (11.8%) (Chorianopoulos et al., 2008). Kokkini and Vokou (1998) showed that mostly thymol containing chemotypes of *S. thymbra* can be found in Greece but also in Sardinia.

It can be noticed that the oil examined in this paper is a thymol-type oil from the *Satureja* species.

The composition of the essential oil from the aerial parts of *Thymbra spicata* from Turkey resulted in the identification of 23 constituents, representing 97.04% of the oil. The major compounds detected in the essential oil were carvacrol (60.39%), γ-terpinene (12.95%), and p-cymene (9.61%) (Kiliç, 2006). Other authors, reporting also on the essential oil composition of *T. spicata* from Turkey, found that its main constituents were carvacrol (75.74%), γ-terpinene (9.28%), p-cymene (7.17%),

Table 1. Chemical composition of *S. thymbra* and *T. spicata* essential oils.

		S. thymbra	*T. spicata*	Identification method**
RI*	**Constituents**	Percentage yield		
928	α– thujene	1.92	0.84	a, b, c
935	*α*- pinene	1.32	0.55	a, b, c
951	camphene	0.41	0.09	a, b, c
976	sabinene	0.14	-	a, b, c
980	β- pinene	0.57	0.14	a, b, c
982	1-octen-3ol	0.23	0.30	a, b, c
995	myrcene	2.38	1.76	a, b, c
1000	octanal	0.18	0.38	a, b, c
1007	*α*- phellandrene	0.22	0.22	a, b, c
1019	α- terpinene	2.72	1.58	a, b, c
1028	*p*-cymene	11.8	5.62	a, b, c
1032	limonene	0.90	0.44	a, b, c
1033	1,8-cineole	-	0.14	a, b, c
1041	*cis* -b- ocimene	0.39	-	a, b, c
1051	*tr* - *β*- ocimene	0.55	-	a, b, c
1062	*γ* – terpinene	30.8	8.12	a, b, c
1069	*cis* - sabinene hydrate	0.34	-	a, b
1086	terpinolene	-	0.14	a, b, c
1096	linalool	0.70	-	a, b, c
1165	borneol	0.34	0.18	a, b, c
1176	terpinen-4- ol	0.50	0.92	a, b, c
1238	thymol methyl ether	1.57	-	a, b
1295	thymol	33.80	-	a, b, c
1304	carvacrol	3.00	74.5	a, b, c
1414	*β*- caryophyllene	2.36	2.50	a, b, c
1575	spathulenol	-	0.14	a, b, c
1579	caryophyllene oxide	1.65	0.46	a, b, c
	total identified compounds	98.70	99.00	

*RI, calculated retention indices on the DB 5 column
**Identification method: a) RI b) Mass spectra c) authentic samples (co-elution, RT)

myrcene (1.39%), β-caryophyllene (1.13%) and thymol (0.15%), respectively (Sarac *et al.*, 2009). The literature indicates that wild populations of *T. spicata* var. *spicata* are almost exclusively represented by the carvacrol containing chemotype (Dogan et al., 1987; Akgul et al., 1999; Fleisher and Fleisher, 2005). The oxygen-containing phenolic monoterpene carvacrol was found to be the major constituent in the essential oil of *T. spicata* plants originating from Greece, followed by moderate amounts of the monoterpenic hydrocarbons γ-terpinene and p-cymene, which are biosynthetically related (Poulose and Croteau, 1978).

The results of the antibacterial and antifungal activity of the essential oils tested are given in Table 2 and Table 3, respectively. Fungi proved to be more sensitive than bacteria to the effect of both essential oils. The oil of *S. thymbra* had minimal inhibitory concentration (MIC) in the range of 0.6-5.0 μg/ml, and minimal bactericidal concentration (MBC) in the range of 2.5-10.0 μg/ml. The most sensitive bac-

Table 2. Antibacterial activity of *S. thymbra* and *T. spicata* essential oils and streptomycin (MICs and MBCs in μg/ml).

Bacteria		S. thymbra	T. spicata	Thymol	Carvacrol	Streptomycin
S.aureus	MIC	2.50	0.60	0.25	0.25	3.10
	MBC	5.00	1.25	0.5	0.5	6.25
B.cereus	MIC	0.60	0.60	0.25	0.125	1.25
	MBC	2.50	1.25	0.5	0.25	2.50
M.flavus	MIC	2.50	0.60	0.25	0.02	0.60
	MBC	5.00	1.25	0.5	0.05	1.25
L.monoytogenes	MIC	5.00	1.25	1	0.5	12.50
	MBC	10.00	2.50	1	0.5	25.00
Ps.aeruginosa	MIC	1.25	0.15	1	0.5	1.60
	MBC	5.00	0.60	1.5	1	3.10
En.faecalis	MIC	5.00	0.60	1	0.5	0.60
	MBC	10.00	1.25	1.5	0.5	1.25
S. typhimurium	MIC	0.60	0.30	0.5	0.5	1.25
	MBC	5.00	0.60	1	0.5	2.50
E.coli	MIC	5.00	0.30	1	0.5	0.60
	MBC	10.00	0.60	1.5	0.5	1.25

Table 3. Antifungal activity of *S. thymbra* and *T. spicata* essential oils and ketoconazol (MICs and MFCs in μg/ml).

Fungi		S. thymbra	T. spicata	Thymol	Carvacrol	Ketoconazole
A. versicolor	MIC	1.25	0.30	0.125	0.05	0.60
	MFC	5.00	0.60	0.25	0.05	5.00
A. ochraceus	MIC	2.50	0.60	0.125	0.05	5.00
	MFC	5.00	1.25	0.25	0.05	25.00
A. niger	MIC	2.50	0.60	0.125	0.05	25.00
	MFC	5.00	0.60	0.25	0.05	25.00
A. fumigatus	MIC	1.25	0.30	0.125	0.05	2.50
	MFC	2.50	0.60	0.25	0.05	5.00
P. ochrochloron	MIC	1.25	0.30	0.25	0.125	5.00
	MFC	5.00	2.50	0.5	0.25	10.00
P. funiculosum	MIC	2.50	0.30	0.25	0.25	0.60
	MFC	5.00	1.25	0.5	0.25	1.25
T. viride	MIC	1.25	0.30	0.25	0.125	25.00
	MFC	2.50	0.60	0.5	0.25	50.00

teria was *B. cereus* with MIC of 0.6 μg/ml and MBC of 2.5 μg/ml, while the most resistant bacterial species were *L. monocytogenes, E. faecalis* and *E. coli* with MIC of 5 μg/ml and MBC of 10 μg/ml. In the cases of *B. cereus* and *L. monocytogenes*, the essential oil of *S. thymbra* was more effective than the commercial drug streptomycin (Table 2). The essential oil of *T. spicata* showed very strong antibacterial activity with MIC of 0.1-1.25 μg/ml and MBC of 0.6-2.5 μg/ml. This oil showed the best antibacterial activity against the Gram-negative bacteria, *P. aeruginosa,* with very low MIC and MBC of 0.1 μg/ml and 0.6 μg/ml, respectively. The most resistant bacteria proved to be *L. monocytogenes* with MIC of 1.25 μg/ml and MBC of 2.5 μg/ml. This oil showed a higher antibacterial potential than streptomycin in all the cases, except for *M. flavus* and *E. faecalis* where the activity was the same (Table 2). It is obvious that *T. spicata* oil showed a higher antibacterial activity than the *S. thymbra* oil.

Thymol showed very strong antibacterial activity with MIC at 0.25-1.0 μg/ml and 0.5-3.0 μg/ml, respectively, while a bactericidal effect was achieved at 0.5-1.5 μg/ml. Carvacrol showed a stronger antibacterial activity with MIC at 0.02-0.5 μg/ml and MBC at 0.05-1.0 μg/ml. Thymol and carvacrol showed higher antibacterial activity than streptomycin (MIC 0.6-12.5 μg/ml and MBC 1.2-25.0 μg/ml) (Table 2). The essential oils of both tested species showed considerable antifungal activity against all the fungi tested. The oil of *S. thymbra* showed an inhibitory effect on the tested fungi in the range of 1.25-2.5 μg/ml, and fungicidal activity in the range of 2.5-5.0 μg/ml. *A. fumigatus* and *T. viride* were the most sensitive fungi to this oil, while *A. ochraceus, A. niger* and *P. funiculosum* showed themselves to be more resistant to this oil. Ketoconazole showed a lower antifungal effect than *S. thymbra* oil in cases of almost all the fungi except for *A. versicolor*, where the activity was the same (Table 3).

The oil of *T. spicata* possessed a higher antifungal potential than *S. thymbra* oil. The MIC was 0.3-0.6 μg/ml and MFC in the range of 0.6-2.5 μg/ml. *A. versicolor* and *A. fumigatus* were the most sensitive, while *P. ochrochloron* was the most resistant to this oil. This oil showed much greater antifungal activity than the commercial antifungal agent ketoconazole (Table 3).

Thymol showed a great antifungal potential with an MIC of 0.125–0.2 μg/ml and MBC at 0.25-0.5 μg/ml. The *Penicillium* species and *Trichoderma viride* were more resistant than the *Aspergillus* species to thymol. Thymol showed a high antifungal potential with very low MIC values. Carvacrol exhibited very high antifungal activity, with MIC/MFC at 0.05-0.25 μg/ml. Both phenolic compounds tested showed much greater antifungal potential than commercial agents, even 10-500 times higher (Table 3).

Thus, it seems likely that the inhibitory effects of the examined essential oils are due to their major components. These inhibitory effects are interesting in connection with the presentation of mycotoxin contamination in many foods.

As a consequence, the chemical composition of the essential oils and their biological activities have been extensively studied, indicating that the essential oils possesses significant antimicrobial activities and may be used as effective agents that does not exhibit any genotoxic activities. Moreover, many reports concern the biological activities (antioxidant, antifungal, anti-inflammatory etc.) of the essential oils of *Satureja* and *Thymus* species (Chorianopoulos et al., 2004). The *in vitro* efficacy of *T. spicata* essential oil against 21 bacteria and seven *Candida* species was examined using disc diffusion and minimum inhibitory concentration (MIC) methods. The essential oil demonstrated strong anti-microbial activity in a wide spectrum against most microorganisms, particularly the yeasts tested. That is the first report on the anticandidal properties of the essential oil of *T. spicata* (Sarac et al., 2009). In previous studies, Yegen et al., (1992) and Müller-Riebau et al., (1995) have reported that the essential oils of *T. spicata* and *S. thymbra*, and their main phenolic constituents, carvacrol and thymol, show a remarkable antifungal activity by inhibition of the mycelial growth of the soil-borne phytopathogenic fungi *Fusarium moniliforme*, *Rhizocto-*

nia solani, *Sclerotinia sclerotiorum*, and *Phytophthora capsici*, one of the most important plant pathogens of pepper growing in the East Mediterranean region of Turkey.

These essential oils were effective against Gram-positive and Gram-negative bacteria, which included multiple antibiotic resistant strains. However, *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas fluorescens* MU 87 were resistant to these oils. The essential oils were very effective against *Candida albicans*. The antimicrobial activity of the essential oils showed some variations depending on the localities from which they were collected. The essential oil of the *S. thymbra* was especially very effective against the resistant strains such as *Stenotrophomonas maltophilia* MU 64, *S. maltophilia* MU 99 and *Chryseomonas luteola* MU 65 (Sarac and Ugur, 2008). The essential oil of *S. thymbra* (1% v/v) growing in Greece, and the decoction and hydrosol fraction of the essential oil were tested against biofilms formed by five bacterial species, either as monospecies, or as a mixed-culture of all species. The tested bacterial species were *Staphylococcus simulans* and *Lactobacillus fermentum* (useful technological bacteria), *Pseudomonas putida* (spoilage bacterium), *Salmonella enterica* and *Listeria monocytogenes* (pathogenic bacteria). Both methods revealed that the essential oil and the hydrosol of *S. thymbra* exhibited a strong antimicrobial action against both monospecies and mixed-culture biofilms (Chorianopoulos et al., 2008). The essential oil of *S. thymbra* was found to be active against the bacteria *E. coli, P. aeruginosa, S. typhimurium, S. sonnei* and *S. aureus* and the yeast *C. albicans* (Gören et al., 2004). *S. thymbra* essential oil originating from Greece possessed very good antifungal properties with low MIC (0.1-1.0 µl/ml) and MFC (0.2-2.0 µl/ml) values (Soković et al., 2002). The results of the antifungal activity of *S. thymbra* oil against *M. perniciosa,* a contaminator of *Agaricus bisporus,* obtained by the micro atmosphere method, showed an MIQ of 0.001-0.05 µl/ml and MFQ of 0.1-0.25 µl/ml (Glamočlija et al., 2006). Previous investigation confirms the high antifungal activity of thymol against food storage, phytopathogenic fungi (Müller-Riebau et al., 1995) and dermatomycetes (Adam et al., 1998).

DISCUSSION

After comparison of the presented data with the chemical composition of the oil, it is evident that there is a relationship between the high activity of *Satureja* and *Thymus* oils and their high phenolic content. Although this study has mainly identified compounds, thymol and carvacrol, which appear to contribute significantly to the antifungal activity of oils, the possibility remains that other minor components contribute to the antimicrobial activity.

The results showed that *S. thymbra* and *T. spicata* essential oils and their components (thymol and carvacrol) are potent bacterial and mould inhibitors, confirming the possibility of using them in food preservation. The result of this study supports the traditional use of these plants.

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REFERENCES

Adam, K., Sivropoulu, A., Kokkini, S., Lanaras, T., and *M. Arsenakis* (1998). Antifungal activities of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia* and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem*. **46,** 1739-1745.

Adams, R (1995). Identification of essential oil components by Gas Chromatography/Mass spectroscopy, Allured Publish. Co, Carol Stream, Ilinois.

Akkgul, A., Ozcan, M., Chialva, F., and *F. Monguzzi* (1999). Essential oils of four Turkish wild-growing Labiatae herbs: *Salvia cryptantha* Montbr. Et Auch., *Satureja cuneifolia* Ten., *Thymbra spicata* L. and *Thymus cilicicus* Boiss. Et Bal. *J. Essent. Oil Res.* **11**, 209-214.

Akin, M., Oguz, D., and *H. T. Saracoglu* (2010). Antibacterial Activity of Essential oil from *Thymbra spicata* var. *spicata* L. and *Teucrium polium* (Stapf Brig.). *Internat. J. Pharm. App. Sci.* **1** (1), 55.

Akkol, E. K., Avci, G., Küçükkurt, I., Keleş, H., Tamer, U., Ince, S., and E. *Yesilada* (2009). Cholesterol-reducer, antioxidant and liver protective effects of *Thymbra spicata* L. var. *spicata*. *J Ethnopharmacol.* **126** (2), 314-319.

Cetin, H., Cilek, J. E., Oz, E., Aydin, L., Deveci, O., and *A. Yanikoglu* (2009). Comparative efficacy of spinosad with conventional acaricides against hard and soft tick populations from Antalya, Turkey, *Vet. Parasitol.* **163,** 101–104.

Chorianopoulos, G. N., Kalpoutzakis, E., Aligiannis, N., Mitaku, S., Nychas, J. G., and *S. A. Haroutounian* (2004). Essential oils of *Satureja, Origanum*, and *Thymus* Species: Chemical composition and antibacterial activities against foodborne pathogens *J. Agric. Food Chem.* **52**, 8261-8267.

Chorianopoulos, G. N., Giaouris, E. D., Skandamis, P. N., Haroutounian, S. A., and *G. J. Nychas* (2008). Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid–base sanitizers *J Appl Microbiol.* **104** 1586-1596.

Daneshvar-Royandezagh, S., Khawar, K. M., and *S. Ozcan* (2009). *In vitro* micropropagation of garden thyme (*Thymbra spicata* L. var. *spicata* L.) collected from southeastern Turkey using cotyledon node. *Biotechnol. & Biotechnol. Eq.* **3** 1319-1321.

Daouk, K. D., Dagher, M. S., and *J. E. Sattout* (1995). Antifungal activity of the essential oil of *Oryganum syriacum* L. *Journal of Food Protection*, **58**, 1147-1149.

Dogan, A., Bayrak, A., and *A. Akgul* (1987). Thymol/Carvacrol containing Labiates species and volatile composition of essential oil of *Thymbra spicata*. *Gıda* **6**, 359-362.

Espinel-Ingfroff, A. and *S. Shadomy* (1989). *In vitro* and *in vivo* evaluation of antifungal agents. *Eur. J. Clinic Microb. Infec. Dis*., 352-361.

Fleisher, A. and *Z. Fleisher* (2005). Extract analyses of *Satureja thymbra* l. and *Thymbra spicata* L. aromatic plants of the Holy Land and the Sinai. Part XVII, *J. Essent. Oil Res.* **17,** 32–35.

Glamočlija, J., Soković, M., Vukojević, J., Milenković, I., and *L. J. L. D. Van Griensven* (2006). Chemical composition and antifungal activities of essential oils of *Satureja thymbra* L and *Salvia pomifera*, subsp. *calycina* (Sm.) Hayek. *J. Essent. Oil Res.* **18**, 115-117.

Goren, A. C., Topçu, G., Bilsel, G., Bilsel, M., Wilkinson, J. M, and *H. M. Cavanagh* (2004). Analysis of essential oil of *Satureja thymbra* by hydrodistillation, thermal desorber and headspace GC/MS techniques and its antimicrobial activity. *Nat. Prod. Res.* **18**, 189-195.

Hanel, H., and *W. Raether* (1988). A more sophisticated method for determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example. *Mycoses* **31,** 148-150.

Kiliç, T. Z (2006). Analysis of essential oil composition of *Thymbra spicata* var. *spicata*: antifungal, antibacterial and antimycobacterial activities. *Naturforsch C.* **61** (5-6), 324-328.

Kokkini, S. and *D. Vokou* (1998). Carvacrol–rich plants in Greece, *Flav. Fragr. J.* **4,** 1–7.

Lagouri, V., Blekas, G., Tsimidou, M., Kokkini, S., and *D. Boskou* (1993). Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece, *Z. Lebensm. Unters. Forsh.* **197,** 20–23.

Muller-Riebau, F., Berger, B., and *O. Yegen* (1995). Chemical composition and fungitoxic properties to phytopathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *J. Agric. Food Chem.* **43**, 2262-2266.

Poulose, A. J., and *R. Croteau* (1978). Biosynthesis of aromatic monoterpenes. Conversion of γ-terpinene to p-cymene and thymol in *Thymus vulgaris* L. *Arch. Biochem. Biophys*. **187,** 307–315.

Sarac, N., and A. *Ugur* (2008). Antimicrobial Activities of the Essential Oils of *Origanum onites* L., *Origanum vulgare* L. subspecies *hirtum* (Link) Ietswaart, *Satureja thymbra* L., and *Thymus cilicicus* Boiss. & Bal. Growing Wild in Turkey. *J Med Food.* **11**, 568-573.

Sarac, N., Ugur, A., and *M. E. Duru* (2009). Antimicrobial activity and chemical composition of the essential oils of *Thymbra spicata* var. *intricata*. *Int J Green Pharm*. **3**, 24-28.

Simon, J. E., Chadwick, A. F., and *L. E. Craker* (1984). Herbs: an indexed bibliography, 1971-1980. The scientific literature on selected herbs and medicinal plants of the temperate zone. Hamden, Connecticut, Archon Books. 770 pp.

Soković, M., Tzakou, O., Pitarokili, D., and *M. Couladis* (2002). Antifungal activities of selected aromatic plants growing wild in Greece. *Nahrung*, **46,** 317–320.

Ugur, A., Sarac, N., and *S. E. Duru* (2009). Antimicrobial activitz and chemical composition of essential oil of the essential oils of *Satureja thymbra* L. from Mugla (Turkey) *ISHS Act. Hortic.* **826**, 111.

Yegen, O., Berger, B., and *R. Heitefuss* (1992). Untersuchungen zur fungitoxischen Wirkung der Extrakte sechs ausgewählter Pflanzen aus der Türkei auf phytopathogene Pilze (Investigations on the Fungitoxicity of Extracts of Six Selected Plants from Turkey against Phytopathogenic Fungi). *Z. Pflanzenkrankh. Pflanzenschutz,* **99,** 349–359.