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Characterization of the Essential Oil Volatiles of *Satureja thymbra* and *Satureja parnassica*: Influence of Harvesting Time and Antimicrobial Activity

NIKOS CHORIANOPOULOS,[†] EPAMEINONTAS EVERGETIS,[†] ATHANASIOS MALLOUCHOS,[†] ELEFTHERIOS KALPOUTZAKIS,[§] GEORGE-JOHN NYCHAS,[†] AND SERKOS A. HAROUTOUNIAN*,[†]

Agricultural University of Athens, Iera odos 75, Athens 11855, Greece, and School of Pharmacy, University of Athens, Panepistimiopolis Zografou, Athens 15771, Greece

Seasonal variation in chemical composition of essential oils obtained from *Satureja parnassica* subsp. *parnassica* Heldr. & Sart ex Boiss. and *Satureja thymbra* L. was determined by GC and GC-MS analysis. The in vitro antibacterial activities of these essential oils were evaluated at two different initial levels against two common foodborne bacteria. Their minimum inhibitory concentration (MIC) and noninhibitory concentration (NIC) values were determined via an innovative automated technique, which was applied on a Malthus apparatus combining the conductance measurements with the common dilution method. The essential oil concentration fluctuated greatly during the period examined and established that the *Satureja* oils obtained during the flowering period were the most potent, exhibiting the lowest MIC values and retaining remarkable bactericidal properties. Thus, they represent an inexpensive source of natural antibacterial substances that exhibit potential for use in food systems to prevent the growth of foodborne bacteria and to extend the shelf life of the processed food.

KEYWORDS: Seasonal variation; *Satureja* sp.; essential oil; antibacterial activity; food preservation; MIC; NIC

INTRODUCTION

The increase of foodborne illness incidences that have resulted from the consumption of food contaminated with bacteria and/or their toxins has initiated considerable research activity toward the discovery of potent antimicrobial agents (*1*). For this purpose, a broad variety of food preservatives such as organic acids and synthetic compounds have been screened in regard to their ability to inhibit foodborne bacteria and extend the shelf life of the processed food (*2*−*4*). The recent negative consumer perception against artificial preservatives, however, has shifted the research effort toward the development of alternatives that consumers perceive as naturals. Previous studies have indicated that the essential oils and extracts of edible and medicinal plants, herbs, and spices constitute a class of very potent natural antibacterial agents (*5*). Their bactericidal activities have established that their use in food systems may be considered as an additional intrinsic determinant to increase the safety and shelf life of foods (*6*−*8*). Today, the practical application of essential oils in food preservation has been hampered by various drawbacks and limitations, such as their strong smell when applied at effective doses and/or a decrease of their effectiveness when involved in complicated food ecosystems (*9*). In addition, the determination of the capability to prevent microbial growth [minimum inhibitory concentration (MIC)] constitutes a very important aspect of a potential food preservative and biocide (*10*).

Recently, we have undertaken an ongoing effort toward the use of various plant species of Greek flora as sources of biologically interesting compounds (*11*, *12*). In this context, we were intrigued to study the essential oils derived from *Satureja* plants, well-known ornamentals, pharmaceutical, and bee plants of the Mediterranean region. The most common *Satureja* specimen is *S. thymbra* L., which is sampled in countries of the Mediterranean region for use as a local spice. *S. thymbra* is also known as an herbal home remedy, due to its antiseptic, gastrosedative, and diuretic properties (*13*). Previous investigations on the chemical composition of the essential oil of *S. thymbra* indicated that it contains carvacrol and thymol as major components (*14*−*16*). On the other hand, biological activity studies have established that the essential oil possesses significant antibacterial and antifungal activities (*17*, *18*) and may be applied as an effective insecticide that does not exhibit any genotoxic activity (*19*). Similar reports, concerning the chemical composition of other *Satureja* essential oils—in respect to intraspecies variations—such as *S. montana*, *S. kitaibelii*, and *S. cuneifolia* (*20*, *21*) or their dependence on seasonal variation

* Corresponding author (telephone +30 2105294247; e-mail sehar@aua.gr).
[†] Agricultural University of Athens.
[§] University of Athens.

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Table 1. Collection Data and Yields of the Essential Oils

species	sample	vegetative stage	date	location	altitude (m)	part distilled[a]	wt of aerial parts (g)	vol of oil (mL)
S. thymbra	1	just before flowering	June 7, 2004	Mt. Immitos[b]	350	stems and leaves	210	4.1
	2	full flowering	July 7, 2004	Mt. Immitos[b]	350	stems, leaves, and flowers	190	4.2
	3	after flowering	Aug 7, 2004	Mt. Immitos[b]	350	stems, leaves, and flowers	230	4.3
	4	fruiting	Sept 7, 2004	Mt. Immitos[b]	350	stems and leaves	180	3.8
	5		Nov 7, 2004	Mt. Immitos[b]	350	stems and leaves	200	3.4
	6		Feb 7, 2005	Mt. Immitos[b]	350	stems and leaves	220	3.2
	7	before flowering	May 7, 2005	Mt. Immitos[b]	350	stems and leaves	230	4.2
S. parnassica	8	before flowering	June 16, 2004	Mt. Parnon[c]	1800	stems and leaves	240	2.9
ssp. *parnassica*	9	just before flowering	July 16, 2004	Mt. Parnon[c]	1800	stems and leaves	220	3
	10	full flowering	Aug 16, 2004	Mt. Parnon[c]	1800	stems, leaves, and flowers	250	3.6
	11	after flowering	Sept 16, 2004	Mt. Parnon[c]	1800	stems, leaves, and flowers	230	3.5

[a] All fresh. [b] Continental Greece. [c] Peloponnese.

and/or plant developmental stages have also been published (*22*, *23*). Finally, many papers have appeared concerning the biological activities (antioxidant, antifungal, antiinflammatory, and antinociceptive) of the essential oil of *S. hortensis* (*24*, *25*) and the in vitro antibacterial activities of the essential oils of *S. parnassica* subsp. *parnassica* and *Satureja spinosa* L. (*17*, *26*, *27*). On the contrary, research investigations into their antibacterial properties against foodborne pathogens with the aim of using them in food ecosystems are limited.

The main subject of the present paper constitutes the study of the effect of seasonal variation on the chemical composition of essential oils obtained from plants endemic to central and southern Greece, *S. parnassica* subsp. *parnassica* Heldr. & Sart ex Boiss. and from a plant widely distributed in the Mediterranean region, *S. thymbra* L. Subsequently, we have carried out a comparative determination of their minimum inhibitory concentrations (MICs) and noninhibitory concentrations (NICs) against representative Gram-negative (*Salmonella enterica* serovar Enteritidis PT4) and Gram-positive (*Listeria monocytogenes* Scott A) foodborne bacteria at two different initial levels. Thus, it will be possible to determine the optimum harvesting period to obtain the most potent oil that is capable of preventing the growth of foodborne bacteria and extending the shelf life of the processed food.

MATERIALS AND METHODS

Plant Material. Fresh plant materials were obtained in 2004 and 2005. Full collection details are provided in **Table 1**. A voucher specimen of each plant has been deposited in the herbarium of the Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece.

Standards. The identification of the chemical constituents of the essential oils was performed using as reference compounds the following chemicals (purchased from the respective commercial sources): borneol, β-caryophyllene, geraniol, 1,8-cineole, γ-terpinene, limonene, thymol (Sigma); α-bisabolol, carvacrol, caryophyllene oxide, cymene, α-pinene, β-pinene, α-terpinene, myrcene, linalool (Aldrich); terpin-4-ol and nerol (Fluka).

Isolation of the Essential Oils. Samples of each species collected (**Table 1**) were chopped and subjected to hydrodistillation in a Clevenger apparatus for 3 h with 3 L of H_2O. The resulting essential oils were dried over anhydrous sodium sulfate and stored at 4 °C. Their respective colors varied from light yellow for *S. thymbra* to light amber for *S. parnassica*. Yields of the essential oils are presented in **Table 1**.

Gas Chromatography (GC). All GC analyses were carried out on a Perkin-Elmer, Clarus 500 gas chromatograph, fitted with a HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column and FID. The column temperature was programmed from 60 to 280 °C at a initial rate of 3 °C/min. The injector and detector temperatures were programmed at 230 and 300 °C, respectively. Helium was used as the carrier gas at a flow rate 1 mL/min.

Gas Chromatography−Mass Spectrometry (GC-MS). The GC-MS analyses were performed using a Hewlett-Packard 5973-6890 GC-MS system operating in EI mode (equipped with a HP 5MS 30 m × 0.25 mm × 0.25 μm film thickness capillary column), using He (1 mL/min) as the carrier gas. The initial temperature of the column was 60 °C. The column was heated gradually to 280 °C with a 3 °C/min rate. The identification of the compounds was based on comparison of their retention indices (RI) (*28*), obtained using various *n*-alkanes (C_9−C_{24}). Also, their EI-mass spectra were compared with the NIST/NBS and Wiley library spectra and the literature (*29*, *30*). Additionally, the identity of the indicated phytochemicals was confirmed by comparison with available authentic samples.

Bacterial Cultures. *L. monocytogenes* Scott A (kindly provided by Dr. Eddy Smid, ATO-DLO) and *S. enterica* serovar Enteritidis PT4 (kindly provided by the University of Surrey) were grown overnight in flasks containing brain heart (BH) broth (Merck catalog no. 1.100493), with shaking, at the appropriate temperature for each bacteria. The cells were harvested by centrifugation at 3000*g* for 15 min at 4 °C (ALC 4239R). Subsequently, they were washed twice and resuspended in Ringer solution (LabM) to provide bacterial concentration between 10^6−10^7 colony-forming units (CFU)/mL.

Antibacterial Assays. Bacterial growth was monitored by conductance measurements using the Malthus system (Radiometer International, Copenhagen, Denmark). A typical Malthus conductivity cell contains platinum electrodes that allow the detection of the conductance changes as a response to the bacterial metabolism in the growth medium. Thus, it is feasible to monitor the conductance changes at defined intervals and record the corresponding data. The detected changes are expressed in microsiemens (μS), which are recorded graphically as conductance curves.

Three stock solutions of each essential oil directly mixed in BH broth (0.5, 0.4, and 0.3% v/v) were prepared. From these stock solutions, seven half-fold serial dilutions were made using the growth medium, covering the range from 0.5 to 0.00469% v/v essential oil. The serial dilutions were dispensed in 2.0 mL final volume into the sterile glass reaction cells of the Malthus. The solutions were well vortexed before each dilution to avoid bad commixtures caused at the lipophilic state of the essential oil. The reaction cells were incubated at 37 °C for 30 min and subsequently inoculated with 0.2 mL of an 18 h culture of each pathogen grown at optimum temperature in the same growth medium. To examine the possible effect of inoculum size on the MIC, two levels of inoculation (10^5 and 10^2 CFU/mL) were used. Each experiment was carried out in triplicate. The reaction cells were incubated for 48 h at 37 °C for *Salmonella* Enteritidis PT4 and at 30 °C for *L. monocytogenes*.

Analysis. The basis of the data analyses is the comparison of the area under the conductance/time curve. The effect on the growth is manifested by a reduction in the area under the conductance/time curve relative to a control well at any specified time (*27*). By calculating the area using the trapezoidal rule, the relative amount of growth can be

Table 2. Chemical Constituents of *S. thymbra* Essential Oils Tested

		GC area %							
compound	KI[a]	*S. thymbra* 1	*S. thymbra* 2	*S. thymbra* 3	*S. thymbra* 4	*S. thymbra* 5	*S. thymbra* 6	*S. thymbra* 7	identification[b]
α-thujene	923	0.40	0.48	0.27	0.36	0.33	0.45	0.38	a
α-pinene	932	0.58	0.44	0.32	0.66	0.77	0.63	0.72	a, b
camphene	947	1.14	1.03	1.18	1.09	1.06	1.64	1.38	a
sabinene	974		0.05	0.10	0.12	tr[c]			a
β-pinene	976	0.07	0.05	0.15	0.10	0.12	0.15	0.09	a, b
myrcene	990	2.21	2.12	1.70	3.59	2.66	2.82	2.44	a, b
α-phellandrene	1001	0.16	0.14	0.27	0.25	0.44	0.32	0.10	a
α-terpinene	1015	1.59	1.48	0.81	1.83	0.98	0.50	0.56	a, b
p-cymene	1024	13.60	10.89	8.83	15.58	18.20	14.65	12.18	a, b
limonene	1027	0.79	0.82	1.05	3.71	1.85	1.72	0.96	a, b
1,8-cineole	1030	0.28	0.38	0.42	0.51	1.23	0.88	0.74	a, b
cis-β-ocimene	1039	0.43	0.38	0.51	0.60	0.28	0.33	0.39	a
trans-β-ocimene	1048	0.09	tr	0.10	0.05	0.06		0.08	a
γ-terpinene	1058	17.02	12.45	10.61	14.91	18.25	25.12	19.19	a, b
cis-sabinene hydrate	1068	0.28	0.44	0.33	0.25	0.67	0.70	0.54	a
terpinolene	1089	0.37	0.29	0.42	0.61	0.26	0.36	0.22	a
linalool	1099	0.61	1.14	2.03	1.35	0.72	0.86	0.38	a, b
borneol	1166	1.51	1.94	3.30	3.98	3.44	2.09	2.30	a, b
terpin-4-ol	1177	1.20	1.50	0.74	1.18	0.89	1.86	1.41	a, b
α-terpineol	1187	0.15		0.16	tr	0.18	0.09	0.11	a
nerol	1229		0.31	tr		0.42	0.34	0.09	a, b
thymol, methyl ether	1235	2.11	2.47	2.23	3.02	3.28	2.44	2.39	a
carvone	1241		tr	0.05	0.09	tr			a
geraniol	1250	0.11	tr			0.08	0.09	tr	a, b
thymol	1286	27.88	17.22	12.59	20.88	20.73	20.21	26.49	a, b
carvacrol	1295	11.88	29.18	39.10	12.80	4.69	4.16	9.17	a, b
thymol acetate	1357	0.32	0.08	tr	tr	0.44	0.31	0.26	a
eugenol	1358	0.74	0.30	0.22	0.10	0.96	0.88	0.79	a
geranyl acetate	1379	0.07	0.24			0.42	0.37	0.28	a
β-caryophyllene	1418	8.05	6.88	4.29	3.92	5.24	6.78	7.94	a, b
aromadendrene	1444	0.15	tr	0.10	0.14	0.39	0.29	0.21	a
α-humulene	1450	0.24	0.38	0.34	0.35	0.32	0.38	0.29	a
germacrene D	1483	0.45	0.32	1.20	1.47	1.48	1.32	0.87	a
bicyclogermacrene	1494	0.56	0.18	0.24	0.21	1.09	0.83	0.47	a
β-bisabolene	1508	0.45	0.48	0.37	0.24	1.03	0.73	0.54	a
δ-cadinene	1524		0.05	tr	tr				a
spathulenol	1571	0.32	tr	0.09	0.11	0.28	0.34	0.26	a, b
caryophyllene oxide	1581	2.03	2.12	2.93	3.98	4.91	3.48	2.87	a, b
viridiflorol	1588		0.25	0.10	tr				a
α-bisabolol	1681	0.11	tr			0.24	0.16	0.07	a, b
total		97.95	96.48	97.15	98.04	98.39	98.28	97.16	

[a] KI, Kovats indices calculated against C_8–C_{24} *n*-alkanes on the HP 5MS column. [b] Identification method: a, comparison of mass spectra with MS libraries and retention times; b, comparison with authentic compounds. [c] tr, concentration of <0.05%.

obtained using the ratio of the test area to that of the control, termed the fractional area, f_a (*10*).

Measurement of MIC and NIC. The respective MIC and NIC values were determined using a modification of the Lambert−Pearson model (LPM), which was originally employed by Lambert and Pearson (*10*). In this context, the Gompertz function was used to fit their observations and produce a dose−response profile. This procedure has been updated by Lambert and Lambert (*31*) in the form of eq 1, where

$$fa = \exp\left[-\left(\frac{x}{P_1}\right)^{P_2}\right] \quad (1)$$

f_a represents the fractional area, x is the inhibitor concentration (mg/L), P_1 is the concentration at maximum slope (of a log x vs f_a plot), and P_2 is a slope parameter. In this context, the MIC of each substance may be defined as the intercept of the concentration axis to the tangent at the maximum gradient of the f_a/log concentration curve. Thus, from the LPM may be formulated the following equation:

$$\mathrm{MIC} = P_1 \exp\left(\frac{1}{P_2}\right) \quad (2)$$

On the other hand, the essential noninhibitory concentration below which normal visible growth was observed may be defined as the intercept of the tangent at the maximum gradient of the f_a/log concentration curve to the $f_a = 1$ contour. From the LPM this can be formulated as

$$\mathrm{NIC} = P_1 \exp\left(\frac{1-e}{P_2}\right) \quad (3)$$

where e is the value of the exponential of 1 (approximately 2.718).

Data were fitted to the LPM using nonlinear least-squares regression analysis assuming equal variance. A more rigorous approach has been developed by Lambert (*32*), but in this work the discrepancy between the assumptions made and the use of a weighting scheme to correct for variance had little impact on the parameters found.

RESULTS AND DISCUSSION

Phytochemical Analysis. More than 40 phytochemicals representing 95.86−98.39% of the respective samples have been identified as constituents of the essential oils, by combined GC and GC-MS analyses. The detailed qualitative and quantitative analytical data of the main constituents (and their respective retention indices) of steam volatiles for *S. thymbra* and *S. parnassica* essential oils have been summarized in **Tables 3** and **4**, respectively. It is evident that the phytochemical content of the essential oils for both *Satureja* species varied greatly,

Table 3. Chemical Constituents of *S. parnassica* Subsp. *parnassica* Essential Oils Tested

		GC area %				
compound	KI[a]	*S. parnassica* 8	*S. parnassica* 9	*S. parnassica* 10	*S. parnassica* 11	identification[b]
α-thujene	923	1.78	0.57	0.65	0.54	a
α-pinene	932	1.39	0.68	0.87	0.80	a, b
camphene	947	0.18	0.10	0.05	0.09	a
sabinene	974	0.24	0.19	0.06	tr[c]	a
β-pinene	976	0.62	0.42	0.48	0.49	a, b
1-octen-3-ol	979	0.32	0.28	0.10	tr	a
myrcene	990	2.40	1.29	1.26	1.32	a, b
α-phellandrene	1001	0.22	0.12	0.14	0.14	a
α-terpinene	1015	3.93	1.68	1.62	1.56	a, b
p-cymene	1024	12.65	6.34	6.87	10.32	a, b
limonene	1027	0.60	0.36	1.35	0.40	a, b
1,8-cineole	1030	tr	tr	0.05	tr	a, b
cis-β-ocimene	1039	0.14	0.38	0.50	0.51	a
trans-β-ocimene	1048	0.25	0.39	0.60	0.54	a
γ-terpinene	1058	34.28	16.51	16.68	15.47	a, b
cis-sabinene hydrate	1068	0.33	0.22	0.24	0.26	a
terpinolene	1089	0.08	0.07	0.09	0.10	a
linalool	1099	0.61	0.89	1.75	1.68	a, b
nonanal	1102	0.24	0.13	0.08	0.19	a
allo-ocimene	1130	0.09	0.05	tr		a
borneol	1166	0.79	1.09	1.11	1.10	a, b
terpin-4-ol	1177	0.72	0.50	0.22	0.08	a, b
α-terpineol	1187	0.15	0.17	tr	0.15	a
cis-dihydrocarvone	1192	0.18	0.15	0.07	0.19	a
nerol	1229	0.14	0.31	tr		a, b
thymol, methyl ether	1235	0.28	0.10	0.22	0.29	a
geraniol	1250	0.19	tr			a, b
thymol	1286	19.96	38.51	20.33	17.82	a, b
carvacrol	1295	3.55	16.42	34.61	33.72	a, b
thymol acetate	1357	0.32	0.36	0.38	0.44	a
α-copaene	1370	0.12	0.25	0.10	0.28	a
β-bourbonene	1380	0.34	0.27	0.10	0.21	a
β-caryophyllene	1418	5.44	4.35	4.82	4.85	a, b
β-gurjunene	1426		tr	0.07	tr	a
aromadendrene	1444	0.15	0.20	0.10	0.14	a
α-humulene	1450	0.20	0.38	0.33	0.35	a
allo-aromadendrene	1458	0.11	0.24			a
γ-muurolene	1475	0.12	0.19	0.10		a
germacrene D	1483		0.12	0.15	0.19	a
bicyclogermacrene	1494	0.56	0.35	0.24	0.21	a
β-bisabolene	1508	0.32	0.48	0.28	0.31	a
γ-cadinene	1513	tr	0.05	0.10		a
δ-cadinene	1524	0.08	0.05	0.32	0.15	a
spathulenol	1571	0.32	0.89	0.14	0.11	a, b
caryophyllene oxide	1581	0.81	0.74	0.70	1.09	a, b
viridiflorol	1588	0.34	0.25	0.10	0.18	a
epi-α-cadinol	1633	0.21	0.16	0.12	0.14	a
α-bisabolol	1681	0.11		tr		a, b
total		95.86	97.25	98.15	96.41	

[a] KI, Kovats indices calculated against C_8–C_{24} *n*-alkanes on the HP 5MS column. [b] Identification method: a, comparison of mass spectra with MS libraries and retention times; b, comparison with authentic compounds. [c] tr, concentration of <0.05%.

depending on the period examined, and showed large prevalence of phenolic content. In most cases, however, thymol constitutes the major component of the oil.

It must also be pointed out that regardless of the vegetative stage of the plant collected, the sum of the two isomeric phenol monoterpenes (carvacrol and thymol) and their biosynthetic monoterpene precursors *p*-cymene and γ-terpinene (*33*) represented always the bulk of each essential oil (∼76%). More specifically—for both species—during their premature vegetative stage, γ-terpinene constitutes the major component (samples 6 and 8, **Tables 2** and **3**) of their essential oils. The approach of the flowering period results in the simultaneous gradual diminishment of monoterpene precursors and the prevalence of their phenolic metabolites. Thus, essential oils obtained from plants collected during the "just before their flowering" stage contain thymol as their major component, which constitutes 27.88 and 38.51% of the total oil content for *S. thymbra* and *S. parnassica*, respectively. On the other hand, during their full flowering period carvacrol prevails as the major component, accounting for 39.10% for *S. thymbra* and for 34.61% for *S. parnassica*. The end of the flowering stage delineates a sharp decrease of carvacrol levels and the predominance of thymol as the major component of the essential oils. A few months later, as the premature vegetative stage approached, the level of γ-terpinene was restored (**Table 2**; **Figure 1**). The content of *p*-cymene—the other major monoterpene precursor—fluctuated seasonally in a manner similar to that shown by γ-terpinene (**Tables 3** and **4**; **Figure 1**). The aforementioned results are in agreement with previous observations and reports on the seasonal variation in chemical composition of the essential oils of other wild populations of plants and species of the same taxon (*21*, *22*, *34*, *35*). Other monoterpene hydrocarbons such as

Table 4. MIC and NIC Values and Their Standard Errors (Percent v/v Concentration of Essential Oil in BH Broth)

	Salmonella serovar Enteritidis PT4				*L. monocytogenes* Scott A			
	high[a]		low[b]		high[a]		low[b]	
essential oil	MIC	NIC	MIC	NIC	MIC	NIC	MIC	NIC
S. thymbra 1	0.074 ± 0.001	0.052 ± 0.003	0.065 ± 0.002	0.048 ± 0.004	0.061 ± 0.003	0.044 ± 0.005	0.053 ± 0.004	0.025 ± 0.006
S. thymbra 2	0.068 ± 0.003	0.045 ± 0.002	0.060 ± 0.004	0.042 ± 0.003	0.054 ± 0.005	0.038 ± 0.004	0.048 ± 0.006	0.024 ± 0.002
S. thymbra 3	0.071 ± 0.004	0.049 ± 0.001	0.063 ± 0.001	0.045 ± 0.004	0.058 ± 0.001	0.042 ± 0.001	0.054 ± 0.003	0.029 ± 0.001
S. thymbra 4	0.079 ± 0.002	0.055 ± 0.002	0.067 ± 0.001	0.052 ± 0.001	0.068 ± 0.003	0.048 ± 0.001	0.060 ± 0.001	0.034 ± 0.004
S. thymbra 5	0.089 ± 0.001	0.067 ± 0.007	0.078 ± 0.002	0.054 ± 0.001	0.077 ± 0.002	0.050 ± 0.006	0.068 ± 0.002	0.035 ± 0.001
S. thymbra 6	0.094 ± 0.003	0.069 ± 0.004	0.084 ± 0.001	0.055 ± 0.004	0.080 ± 0.002	0.052 ± 0.003	0.072 ± 0.001	0.039 ± 0.002
S. thymbra 7	0.080 ± 0.002	0.054 ± 0.003	0.069 ± 0.001	0.050 ± 0.002	0.064 ± 0.003	0.049 ± 0.001	0.054 ± 0.004	0.028 ± 0.001
S. parnassica 8	0.078 ± 0.000	0.061 ± 0.005	0.072 ± 0.002	0.054 ± 0.004	0.064 ± 0.001	0.044 ± 0.002	0.056 ± 0.002	0.035 ± 0.003
S. parnassica 9	0.069 ± 0.004	0.043 ± 0.001	0.061 ± 0.004	0.040 ± 0.003	0.052 ± 0.004	0.036 ± 0.001	0.044 ± 0.001	0.022 ± 0.002
S. parnassica 10	0.064 ± 0.003	0.039 ± 0.005	0.056 ± 0.003	0.032 ± 0.000	0.049 ± 0.001	0.024 ± 0.003	0.035 ± 0.000	0.016 ± 0.004
S. parnassica 11	0.068 ± 0.005	0.042 ± 0.003	0.060 ± 0.001	0.037 ± 0.001	0.050 ± 0.002	0.031 ± 0.002	0.039 ± 0.004	0.019 ± 0.002

[a] High level of inoculation (10^5 CFU/mL). [b] Low level of inoculation (10^2 CFU/mL).

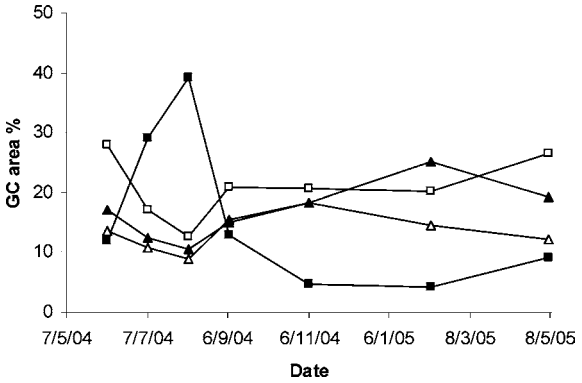

Figure 1. Seasonal variation of the major constituents (■, carvacrol; □, thymol; ▲, γ-terpinene; △, *p*-cymene) of *S. thymbra* plants. Values at (*X*) axis cover a period of 12 months (from June 7, 2004, to May 7, 2005; see **Table 1** for complete collection data).

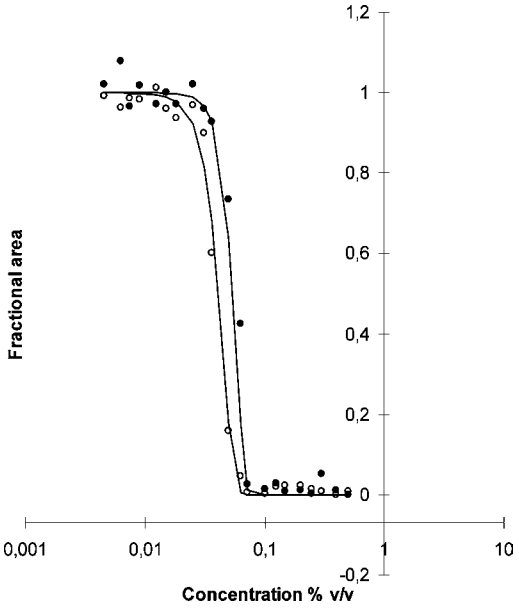

Figure 2. Inhibition profile of *S. parnassica* subsp. *parnassica* essential oil against *Salmonella* serovar enteritidis PT4 [●, high level of inoculation (10^5 CFU/mL); ○, low level of inoculation (10^2 CFU/mL); ●, observed fractional area; –, predicted fractional area].

myrcene and α-terpinene were also detected in smaller quantities, whereas various monoterpene alcohols such as linalool, borneol, and terpin-4-ol were found mainly in the oils obtained after the flowering stage (**Tables 3** and **4**). Finally, it is notable that the oils obtained during the just before the full flowering period contain β-caryophyllene as one of their major components.

Antimicrobial Assays. There is a broad variety of methods in use to assay the MIC of antimicrobials. Among them, the method developed by Lambert and Pearson (*10*) constitutes an innovative automated technique that combines the absorbance measurements with the common dilution method (*36*). This method has the advantage of allowing the quick and efficient MIC assay and may be applied either for single compounds (e.g., thymol) or for a mixture of preservatives (*31*, *36*). Significant improvement of this method constitutes the replacement of the absorbance with conductance measurements (*27*). The latter have the advantage of not depending on the active state of cells (including their shape and size) and not being affected by the possible oxidation of the essential oil (*5*). In this study, this method was applied on a Malthus apparatus, which allows the measurement of the cell metabolism rate, thereby showing the inhibition caused by the essential oil as a delay in bacteria metabolism compared to the control. Moreover, the accuracy of the MIC determinations was ensured via the mathematical process of data (*27*). More specifically, the areas under microsiemens/time curves were calculated using the trapezoidal rule. Thus, the relative amount of growth—termed the fractional area—was obtained using the ratio of the test area to that of the control (*27*). Plotting the inhibitor concentration on a logarithmic scale with the fractional area gave a characteristic sigmoid-shaped curve (**Figure 2**). Terms have been assigned to two specific concentrations: the noninhibitory concentration (NIC), that is, the concentration above which the inhibitor begins to have a negative effect on growth, and the minimum inhibitory concentration (MIC), which marks the concentration above which no growth is observed relative to the control (*27*).

The experimental data have been modeled using eq 1. The MIC and NIC values for each pathogen and the inoculum's level were calculated from eqs 2 and 3, respectively, and are presented in **Table 4**. It is notable, however, that the essential oils of both *Satureja* specimens, obtained during the full flowering period, showed the lowest MIC and NIC values against the pathogens tested (**Table 4**). This result is presumably connected to the high levels of their phenolic content (carvacrol and thymol) as compared with the oils obtained during the other vegetative stages (**Tables 2** and **4**). After the end of the flowering stage the assayed MIC and NIC values gradually increased, presumably reflecting the respective diminishment of the concentration of the aforementioned phenols. It must also be noted that the

oils tested possess higher activities (**Table 4**) against the Gram-positive bacterial strain of *L. monocytogenes* Scott A in comparison with the respective Gram-negative strain (*Salmonella* serovar Enteritidis PT4). These results were verified at two different initial levels of inoculation, that is, one high (10^5 CFU/mL) and one low (10^2 CFU/mL), indicating that lower essential oil concentrations are required to inhibit lower populations of pathogens (**Table 4**). This is in agreement with previous results (*27*, *37*) establishing that the amount of essential oil needed for MIC and NIC is dependent on the inoculum size.

These results may also be compared to those of other active essential oils against foodborne pathogens. Recently, a literature review (*38*) has summarized the MIC values of the most commonly used antibacterial essential oils, such as the oils of oregano, thyme, sage, clove, lemongrass, tea bush, rosemary, and turmeric. The reported MIC values of the aforementioned essential oils ranged from 0.05 to >2% v/v and from 0.02 to 0.05% v/v for *Salmonella* and *L. monocytogenes*, respectively, indicating that the assayed MIC values of *S. thymbra* and *S. parnassica* essential oils were of the same magnitude as the most active one (oregano). The aforementioned essential oils were obtained during the flowering stage and were found to contain the largest amounts of phenolic constituents (carvacrol and thymol). This is in agreement with a previous paper (*36*) which established that the phenolic content (sum of carvacrol and thymol) of oregano oil accounted for its antibacterial activity. Moreover, authors have shown that the combination of large proportions of thymol and carvacrol causes an inhibitory effect similar to that observed for the oregano essential oil. The strong inhibitory effect of these essential oils on bacterial membranes (*9*, *39*) is highly associated with the presence of carvacrol and thymol, presumably due to their ability to cause structural and functional damages to plasma membranes (*40*).

In conclusion, the essential oils tested represent an inexpensive source of natural antibacterial substances for use in food systems to prevent the growth of foodborne bacteria and extend the shelf life of the processed food. It is further notable that the essential oils of both *Satureja* specimens, obtained during the full flowering period, showed the lowest MIC and NIC values against the pathogens tested However, further research is needed to evaluate the effectiveness of *S. thymbra* and *S. parnassica* essential oils in food ecosystems to establish their utility as natural antimicrobial agents in food preservation and safety.

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