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Essential Oils of *Satureja*, *Origanum*, and *Thymus* Species: Chemical Composition and Antibacterial Activities Against Foodborne Pathogens

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The chemical composition of the essential oils obtained from the species restricted to Greece and the eastern Mediterranean region, *Satureja spinosa* L. and *Thymus longicaulis* L.; species endemic to central and south Greece, *Satureja parnassica* ssp. *parnassica* Heldr. and Sart ex Boiss.; species endemic to the island of Crete, *Origanum dictamnus* L.; and species widely distributed in the Mediterranean region, *Satureja thymbra* L. and *Origanum vulgare* L. subsp. *hirtum*, were determined by gas chromatography (GC) and GC/mass spectrometry (MS) analysis. The in vitro antibacterial activities of the essential oils were evaluated against a panel of five foodborne bacteria (*Escherichia coli* 0157:H7 NCTC 12900, *Salmonella enteritidis* PT4, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ScottA, and *Bacillus cereus* FSS 134). The analytical data indicated that various monoterpene hydrocarbons and phenolic monoterpenes constitute the major components of the oils, but their concentrations varied greatly among the oils examined. The antibacterial assay results showed that 5 μL doses of the essential oils extracted from the endemic *Satureja* species in Greece possess remarkable bactericidal properties, which are clearly superior as compared to those of *Origanum* and *Thymus* species essential oils. Therefore, they represent an inexpensive source of natural mixtures of antibacterial compounds that exhibit potentials for use in food systems to prevent the growth of foodborne bacteria and extend the shelf life of the processed food.

KEYWORDS: ***Satureja*** **sp.; essential oil; antibacterial activity; food preservation**

INTRODUCTION

Food preservation has become a complex problem. New food products are frequently being introduced onto the market. Generally, they require a longer shelf life and greater assurance of freedom from foodborne pathogenic organisms (*1*). The excessive use of chemical preservatives, many of which are suspect because of their potential carcinogenic and teratogenic attributes or residual toxicity, has resulted in an increasing pressure on food manufacturers either to completely remove chemical preservatives from their food products or to adopt alternatives that consumers conceive as "natural". Consequently, there is considerable research interest in the possible use of natural products, such as essential oils and extracts of edible and medicinal plants, herbs, and spices, for the development of alternative food additives in order to prevent the growth of foodborne pathogens or to delay the onset of food spoilage (*2*−*6*). In this context, essential oils derived from plants of *Origanum* and *Thymus* species (Lamiacae family) have been found to possess significant antifungal, insecticidal, and antimicrobial activities (*7*, *8*). These properties depend greatly on their chemical compositions and are mainly attributed to their contents in carvacrol and thymol (noncrystallizable and crystallizable phenols, respectively). The literature abounds with reports concerning the determination of chemical compositions and antimicrobial properties of the essential oils of various *Origanum* and *Thymus* species, as well as their applications in various commercial preparations, mainly as antimicrobial and antioxidant agents (*9*−*11*). It is noteworthy, however, that the antibacterial activities of the aforementioned essential oils against foodborne bacteria are limited, prohibiting their commercial application in food preservation or the extension of the processed food shelf life (*11*). Thus, there is vigorous research activity directed toward the discovery of more potent essential oils against foodorne bacteria.

Recently, we have undertaken an ongoing investigation concerning the determination of the phytochemical contents and

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Table 1. Collection Data

Latin name	abbreviation	vegetative stage	date	location	altitude (m)
Thymus longicaulis	TLK	in fruiting	03/06/02	Mt. Kitheron, continental Greece	600
Thymus longicaulis	TLP	full flowering	17/06/02	Mt. Parnon, Peloponnesus	1650
Satureja spinosa	SSPPS	just before flowering	08/06/02	Mt. Psiloritis, Crete	1700
Satureja thymbra	ST	full flowering	05/06/02	Mt. Immitos, continental Greece	350
Satureja spinosa	SSPDI	just before flowering	10/06/02	Mt. Dikti, Crete	1450
Satureja parnassica ssp. *parnassica*	SAPP	just before flowering	24/06/02	Mt. Parnon, Peloponnesus	1800
Origanum vulgare ssp. *hirtum*	ORH	full flowering	29/07/02	Kozani, continental Greece	650
Origanum dictamnus	OD	full flowering	06/08/02	Mt. Psiloritis, Crete	1750

biological activities of various wild-growing plant species of Greece (*12*). In this context, we have considered the study of *Satureja* L., a well-known ornamental plant of the Mediterranean region, as an intriguing case. We were particularly interested in *Satureja spinosa* L. (SSPPS and SSPDI), a specimen widely distributed almost exclusively on the islands of Crete and Samos (*13*). This plant is being used as an ornamental, pharmaceutical, and bee plant in the Cretan diet, which constitutes the best example of the Mediterranean diet with respect to cardiovascular disease prevention ability (*14*). Previous studies on the essential oil of *Satureja thymbra* L. (ST), the most common *Satureja* specimen, have shown that it contains carvacrol and thymol as major components. As a consequence, its chemical composition (*15*−*17*) and biological activities have been extensively studied, indicating that this essential oil possesses significant antifungal activities (*18*) and may be used as an effective insecticide that does not exhibit any genotoxic activities (*19*). Similar reports exist, concerning the chemical composition of the essential oil with regard to variations between the intraspecies such as *Satureja montana*, *Satureja kitaibelii* (*20*), and *Satureja cuneifolia* (*11*) or their dependence on seasonal variations, and plant developmental stages were also published (*21*, *22*). Moreover, many reports concern the biological activities (antioxidant, antifungal, antiinflammatory, and antinociceptive) of the essential oil of *Satureja hortensis* (*23*, *24*) or the anti-HP (*Helicobacter pylori*) activity of the essential oil of *Satureja parnassica* ssp. *parnassica* (SAPP) (*25*). On the contrary, the antibacterial properties of the essential oils of *Satureja* species against foodborne pathogens have not been thoroughly investigated.

The main subject of the current study is the determination of the chemical composition of the essential oil of the species restricted to Greece and eastern Mediterranean region, SSPPS, SSPDI, and *Thymus longicaulis* L. (TLK or TLP); the species endemic to central and south Greece, SAPP Heldr. and Sart ex Boiss.; the species endemic to the island of Crete, *Origanum dictamnus* L. (OD); and the species widely distributed in the Mediterranean region, ST and *Origanum vulgare* L. ssp. *hirtum* (ORH). Subsequently, we carry out a comparative evaluation (with respect to their chemical compositions) against the following five representative foodborne bacteria: *Escherichia coli* 0157:H7 NCTC 12900, *Salmonella enteritidis* PT4, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ScottA, and *Bacillus cereus* FSS 134.

MATERIALS AND METHODS

Plant Material. Fresh plant materials were obtained on 2002. Full collection details are provided in **Table 1**. A voucher specimen of each plant is deposited in the herbarium of the Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece.

Isolation of the Essential Oils. Samples of each species collected (**Table 2**) were chopped and subjected to steam distillation 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 ST to light amber for SSPPS and SSPDI. Yields of the essential oils are displayed in **Table 2**.

Table 2. Yields of the Essential Oils

name	part distilled	weight of aerial parts (g)	volume of oil (mL)
TLK	stems, leaves, calyx, and seeds (fresh)	250	2.1
TLP	stems, leaves, and flowers (fresh)	270	2.3
SSPPS	stems and leaves (fresh)	75	0.35
ST	stems, leaves, and flowers (fresh)	190	4.5
SSPDI	stems and leaves (fresh)	190	0.4
SAPP	stems and leaves (fresh)	80	1
ORH	stems, leaves, and flowers (air-dried)	30	1.0
OD	stems, leaves, and flowers (fresh)	80	1.0

Gas Chromatography−Mass Spectrometry (GC/MS). GC analysis was 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 flame ionization detector. The column temperature was programmed from 60 to 280 °C at a 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 of 1 mL/min. The GC/MS analyses were performed using a Hewlett-Packard 5973-6890 GC/MS system operating on electrospray ionization (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, and then, it was gradually heated to 280 °C with a 3 °C/min rate. The identification of the compounds was based on comparison of their retention indices (RI) (*26*) obtained using various *n*-alkanes (C_9−C_{24}) and by comparing their EI mass spectra with the NIST/NBS, Wiley libraries spectra and literature (*27*, *28*). Additionally, the identity of each compound was confirmed by comparison with available authentic samples.

Bacterial Cultures. *S. aureus* (ATCC 6538), *E. coli* O157:H7 (NCTC 12900), *L. monocytogenes* Scott A (kindly provided by Dr. Eddy Smid ATO-DLO), *S. enteritidis* PT4 (kindly provided from University of Surrey), and *B. cereus* FSS 134 were grown overnight in flasks containing BH broth (Merck catalog 1.100493), with shaking, at the appropriate temperature for each bacteria. The cells were harvested by centrifugation at 3000*g* for 15 min and 4 °C (ALC 4239R). Subsequently, they were washed twice and resuspended in Ringer solution (LabM) to provide a bacterial concentration between 10^6 and 10^7 cfu/mL.

Antibacterial Assays. The antimicrobial activities of the tested essential oils were monitored using the following two methods.

Disk Diffusion Method. The bacterial suspensions were diluted 10-fold in Ringer's solution (LabM). A 0.1 mL portion from the appropriate dilution was spread plated on BH Agar (Merck catalog 1.13825) in order to give a population of approximately 10^6 cfu/plate. Subsequently, sterile paper disks of 6.48 mm diameter (Whatman no. 2) were placed onto the inoculated agar surface and 5 μL of each essential oil was added to the paper disks. Each experiment was carried out in triplicate. Petries were incubated for 48 h, at 37 °C for *E. coli* 0157:H7 NCTC 12900 and *S. enteritidis* PT4, 35 °C for *B. cereus* FSS 134, and 30 °C

Table 3. Chemical Constituents of the Essential Oils Tested

	GC area %									
compound	SSPDI (from Dikti)	SSPPS (from Psiloritis)	SAPP	ST	TLK (from Parnon)	TLP (from Kitheron)	OVH	OD	KI[c]	identification
α-thujene	0.36	0.28	0.78	1.50	0.63	0.66	0.54	0.93	923	*a*
α-pinene	0.25	0.26	0.63	2.06	0.72	0.63	0.28	0.64	932	*a, b*
camphene	0.05	0.10	0.18	1.09	1.06	0.64		0.69	947	*a*
sabinene		0.05	0.10	0.10	tr			tr	974	*a*
β-pinene	0.06	0.05	0.15	1.30					976	*a, b*
1-octen-3-ol	0.34	0.10	0.28		0.69	0.90		tr	979	*a*
myrcene	0.59	0.66	1.20	1.45	0.59	0.56	0.93	0.86	990	*a, b*
3-octanol	0.27	0.27							991	*a*
α-phellandrene	0.16	0.14	0.27	0.40					1001	*a*
α-terpinene	0.88	1.07	1.68	1.98	0.89	1.39	0.56	1.37	1015	*a, b*
p-cymene	6.45	9.35	8.24	11.80	2.77	6.58	3.43	13.34	1024	*a, b*
limonene	0.53	0.61	1.81						1027	*a, b*
1,8-cineole	0.28		1.24		1.07	0.82			1030	*a, b*
cis-β-ocimene		0.23	1.51	0.23					1039	*a*
benzene acetaldehyde	0.54								1041	*a*
trans-β-ocimene	0.05	0.28	1.10	tr					1048	*a*
γ-terpinene	5.56	10.90	9.35	22.2	4.64	4.93	3.18	6.98	1058	*a, b*
cis-sabinene hydrate	0.28	0.44	0.33	0.25	tr	0.70		tr	1068	*a*
terpinolene	0.13	0.08	0.15	0.15	tr	0.36		tr	1089	*a*
p-cymenene		0.05							1093	*a*
linalool	0.07	0.81	0.76	0.45	4,29	4.18		4.39	1099	*a, b*
nonanal	0.11	0.13	0.24						1102	*a*
allo-ocimene			tr						1130	*a*
1-terpineol	0.15								1131	*a*
camphor		tr							1144	*a, b*
borneol	0.28	0.72	0.23	0.09	3.61	2.09	0.30	2.03	1166	*a, b*
terpin-4-ol	1.20	0.50	0.74	0.08	0.71	2.86	0.23	0.52	1177	*a, b*
p-cymen-8-ol	0.07	0.10							1180	*a*
α-terpineol	0.15		0.16						1187	*a*
cis-dihydrocarvone	0.18	0.05	0.07						1192	*a*
trans-dihydrocarvone	0.22	0.12							1199	*a*
nerol		0.31							1229	*a, b*
thymol, methyl ether	5.11	0.10	0.22	3.02					1235	*a*
neral		0.36							1236	*a*
carvone		tr							1241	*a*
geraniol		3.68			27.35				1250	*a, b*
thymol	15.49	24.17	43.48	41.0	8.65	3.53		0.35	1286	*a, b*
carvacrol	44.23	27.24	4.76	1.39	19.77	60.82	88.71	55.10	1295	*a, b*
thymol acetate	0.32		0.38	tr					1357	*a*
eugenol				0.10					1358	*a*
α-copaene			0.10						1370	*a*
geranyl acetate		0.24			2.98				1379	*a*
β-bourbonene			0.13						1380	*a*
cis-carvacryl acetate	1.90	0.08			tr				1381	*a*
β-elemene		0.06							1390	*a*
isocaryophyllene		0.07							1408	*a*
β-caryophyllene	4.76	9.37	5.79	5.92	6,20	0.96	1.08	1.76	1418	*a, b*
β-gurjunene			0.07						1426	*a*
aromadendrene	0.15	tr	0.10						1444	*a*
α-humulene	0.20	0.38	0.33	0.35	0.32			tr	1450	*a*
trans-β-farnesene	0.09								1456	*a*
allo-aromadendrene			0.11						1458	*a*
γ-muurolene			0.10						1475	*a*
germacrene D		0.12	1.15	0.47	1.48	0.89		0.36	1483	*a*
β-selinene		0.05							1489	*a*
bicyclogermacrene	0.56	0.35	1.27	0.21	1.29	tr			1494	*a*
α-muurolene			tr						1496	*a*
β-bisabolene	3.25	0.48	2.0		2.03	2.73	0.54	0.30	1508	*a*
γ-cadinene			0.10						1513	*a*
δ-cadinene		0.05	0.32	tr					1524	*a*
β-sesquiphellandrene	0.05								1525	*a*
spathulenol	0.32		0.89	0.11					1571	*a, b*
caryophyllene oxide	0.99	2.54	1.18	0.47	0.72	tr		0.57	1581	*a, b*
viridiflorol		0.25	0.10						1588	*a*
epi-α-cadinol			0.12						1633	*a*
α-bisabolol	0.11								1681	*a, b*
phytol	0.21								1939	*a*
total	96.95	97.25	93.90	98.17	92.46	96.23	99.78	90.19		

[a] Comparison of mass spectra with MS libraries and retention times. [b] Comparison with authentic compounds; tr = concentration less than 0.05%. [c] KI, Kovats indices calculated against C_8 to C_{24} *n*-alkanes on the HP 5MS column.

for *L. monocytogenes* Scott A and *S. aureus* ATCC 6538. After incubation, the inhibition zones were estimated by taking photos of Petries with a SONY camera (x-wave HAD SSC-DC50AP) and processed using the Impuls Vision XL 2.5 software. Each inhibition zone diameter was measured three times, and the average is presented.

Conductance Method. 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. In the present study, the growth medium (BH broth) was dispensed to 2.0 mL final volume into the previously described sterile closed glass reaction cells (121 °C/15 min). The essential oils were tested directly (solution 1% v/v) or dripped (5 μL) on a sterile Whatman disk (diameter 6 mm). The latter was introduced within the testing tube's wall (not in touch with the growth medium) in order to evaluate the effect of volatile compounds. The reaction tubes were incubated at 37 °C for 30 min and subsequently inoculated with 0.2 mL of an 18 h culture for each pathogen grown at optimum temperature in the same growth media. Two levels of inoculation (10^{5-6} and 10^{2-3} cfu/mL) were used to monitor this effect. The final population of pathogens in the vessels was determined by removing 1 mL aseptically after 48 h of incubation.

RESULTS AND DISCUSSION

Phytochemical Analysis. More than 70 phytochemicals representing 90.19−99.78% 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 RI) of steam volatiles have been summarized in **Table 3**. The phytochemical contents among the respective essential oils of *Satureja*, *Origanum*, and *Thymus* species varied greatly. Moreover, different *S. spinosa* and *T. longicaulis* specimens, obtained from two ecologically varied stations, showed large prevalent phenolic contents. In most cases, however, carvacrol constituted the major component (27.24−88.71%) of the oil, while the sum of the two isomeric phenolic monoterpenes (carvacrol and thymol) and their biosynthetic precursors *p*-cymene and γ-terpinene (*29*) was the bulk of each essential oil (ca 75%). Only the sample of TLK was exceptionally poor in phenolic monoterpenes (35.83%) and the essential oil of OVH was perticularly rich in carvacrol (88.71%). Consequently, the concentration of monoterpene hydrocarbons *p*-cymene and γ-terpinene was high only in poor carvacrol and thymol respective oils, such as the essential oil of ST. These results are in accordance with previous observations concerning several other Greek wild populations of the same taxon (*30*). On the contrary, previous work on the phytochemical content of the essential oil of *S. spinosa* (*31*) (sample obtained from different country, same maturation stage) has identified linalool as the major component (61.5%). Furthermore, they reported that only a small amount of monoterpene phenols (thymol, 1.2%, and carvacrol, 0.1%) is present, presumably because their samples have been derived from different chemotypes of *Satureja* species. Other monoterpene hydrocarbons, such as myrcene and α-terpinene, were also present in small quantities, while monoterpene alcohols such as linalool, borneol, and terpin-4-ol were detected mainly in the oils of *Origanum* and *Thymus* species, while a large amount of geraniol was detected only in the oil of TLP. Finally, it is interesting to point out that β-caryophyllene was detected as the major component mainly in the oils of *Satureja* species and the essential oils of SSPDI and ST were found to contain large amounts of thymol and methyl ether (5.11 and 3.02%, respectively).

Table 4. Antibacterial Activity of the Eight Essential Oils against Five Common Foodborne Pathogens[a]

	bacteria				
essential oil	*E. coli*	*S. enteritidis*	*S. aureus*	*L. monocytogenes*	*B. cereus*
SSPS	33.2	27.62	56.29	38.82	27.12
SSPDI	30.88	35.36	52.32	48.61	33.08
ST	24.05	23.07	31.84	28.11	27.07
SAPP	26.2	24.95	51.65	35.61	23.19
ORH	27.63	33.24	33.62	34.53	33.05
OD	25.35	24.53	26.41	22.71	22.43
TLK	14.72	10.82	11.4	12.03	11.43
TLP	23.21	24.31	31.38	22.72	21.65
			control		
gentamycin[b]	32.21	19.92	21.54	28.32	32.31
tetracycline[b]	16.32	12.55	12.60	17.32	16.98
carvacrol[c]	7.12	7.08	8.01	7.44	7.76
thymol[c]	9.86	9.34	11.75	10.48	11.32

[a] The diameter (mm) of the inhibition zone is the mean of six observations taken from two different experiments (coefficient of variation of mean of treatments taken from different experiments <5%). Each treatment was analyzed in triplicate (coefficient of variation of observations—zone of inhibition—from the same experiment <0.65%); sample amount 5 μL ($n = 3$). [b] Twelve μg/mL of DMSO. [c] Twenty mg/mL of ethanol. Solvents show no antibacterial activity.

Antimicrobial Assays. The assessment of inhibitory activity of various antimicrobials (natural or not) can be performed by using the following general methodologies: (i) assessment of the inhibition of the bacterial growth on an agar medium in comparison with the behavior of an antimicrobial compound diffused in the agar, (ii) the determination of the minimum inhibitory concentration of the antimicrobial compound in liquid media, (iii) the measurement of the radius or diameter of the inhibition zone of bacterial growth around paper disks impregnated with an antimicrobial compound, and (iv) the measurement of indirect indicators that evaluate their growth (e.g., optical density or impedance) in a liquid growth medium containing the antibacterial compound. In the present study, the last two methods were used to evaluate the antimicrobial activities of the essential oils derived from *Satureja*, *Origanum*, and *Thymus* species. The corresponding results are depicted in **Tables 4**−**9**. More specifically, we initially evaluated the antibacterial activities of the essential oils derived from four *Satureja* and four characteristic *Origanum* and *Thymus* specimens by the disk diffusion assay against a panel of five of the most common foodborne bacterial strains. Furthermore, we have determined the corresponding activities of the major monoterpenic phenols (carvacrol and thymol) and two common antibiotics (gentamycin and tetracycline). The results, presented in **Table 4**, indicated that all oils possess noteworthy antibacterial activities against the different strains tested. It must be noted, however, that the antibacterial activity of *S. spinosa* against the pathogenic bacteria was higher (or equal) as compared to the well-documented inhibitory activities of the respective *Origanum* or *Thymus* essential oils. Presumably this activity is not derived only from the presence of monoterpene phenols, but part of the activity resulted from the secondary and synergistic presence of minor active constituents, such as γ-terpinene and *p*-cymene. This is in accordance with a previous report (*11*) indicating that an essential oil containing 53% carvacrol and thymol was found to possess superior antibacterial activity as compared to respective oils that were rich in monoterpene phenols (87% content).

Table 5. Growth of *E. coli* 0157:H7 NCTC 12900 in BHI Broth for 48 h at 37 °C; Effect of Essential Oil on Detection Time, Maximum Conductance, and Final Viable Count

inoculum (cfu/mL)	10^5			10^2		
essential oil	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)
control	4	410	9.15	7.2	420	9.32
SSPPS 1%[a]			<1			<1
SSPDI 1%[a]			<1			<1
SAPP 1%[a]			<1			<1
ST 1%[a]			<1			<1
SSPPS 5 μL[b]	5.4	310	7.48	11.4	140	8.08
SSPDI 5 μL[b]	5	300	7.30	14.8	120	7.18
SAPP 5 μL[b]	4.8	70	7.62	8.6	310	7.51
ST 5 μL[b]	8.2	260	6.85	11.2	300	7.60

[a] Essential oil mixed in 1% concentration with BHI broth. [b] Sterile Whatman disks dipped with 5 μL of the essential oil (effect of volatiles).

Table 6. Growth of *S. enteritidis* PT4 in BH Broth and the Effect of Essential Oil on Detection Time, Maximum Conductance, and Final Viable Count

inoculum (cfu/mL)	10^5			10^2		
essential oil	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)
control	4.4	700	9.56	8	720	9.51
SSPPS 1%[a]			<1			<1
SSPDI 1%[a]			<1			<1
SAPP 1%[a]			<1			<1
ST 1%[a]			<1			<1
SSPPS 5 μL[b]	5.4	240	8.08	14.6	180	7.18
SSPDI 5 μL[b]	6	65	8.48	18.8	40	6.90
SAPP 5 μL[b]	6.4	200	7.48	9.8	310	7.30
ST 5 μL[b]	9	360	7.48	34.8	20	7.60

[a] Essential oil mixed in 1% concentration with BHI broth. [b] Sterile Whatman disks dipped with 5 μL of the essential oil (effect of volatiles).

Table 7. Growth of *S. aureus* ATCC 6538 in BH Broth and the Effect of Essential Oil on Detection Time, Maximum Conductance, and Final Viable Count

inoculum (cfu/mL)	10^5			10^2		
essential oil	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)
control	9.2	310	8.98	18	40	8.70
SSPPS 1%[a]			<1			<1
SSPDI 1%[a]			<1			<1
SAPP 1%[a]			<1			<1
ST 1%[a]			<1			<1
SSPPS 5 μL[b]			<1			<1
SSPDI 5 μL[b]			<1			<1
SAPP 5 μL[b]			<1			<1
ST 5 μL[b]			<1			<1

[a] Essential oil mixed in 1% concentration with BHI broth. [b] Sterile Whatman disks dipped with 5 μL of the essential oil (effect of volatiles).

Finally, the afforementioned results are in agreement with previous literature reports indicating that the Gram-positive bacteria (*S. aureus*, *L. monocytogenes*, and *B. cereus*) are more susceptible to essential oils than the Gram-negative bacteria (*E. coli* and *S. enteritidis*) (*32*, *33*).

When the inhibition zone method is used for the evaluation of the antibacterial activity of essential oils, there are three main factors that influence the outcome of the results: (i) the composition of the sample tested (type of plant, geographical location, and time of the year), (ii) the microorganism (e.g., inoculum size, etc.), and (iii) the method used for growing and enumerating the surviving bacteria. Although there are abundant studies in the literature regarding the subjective assessment of inhibition of the essential oils, the aforementioned limitations have not been discussed. More specifically, in the disk diffusion method, the inhibition area depends on the ability of the essential oil to diffuse uniformly through the agar as well as on the released oil vapors. Other factors that may influence the experimental results involve the presence of multiple active components. These active compounds at low concentrations may interact antagonistically, additively, or synergistically with each other. Some of the differences in the antimicrobial activity of oils observed in complex foods as compared to their activity when used alone in laboratory media could be due to the partitioning of active components between lipid and aqueous phases in foods (*34*). An alternative route to overcome these problems refers to the use of impedance-based methods that allow the monitoring of the microbial inhibition in real time mode. Thus, the impedimetric method is recognized as a rapid way to screen the biocide activity of novel antimicrobial agents and to simultaneously estimate their growth kinetics via mathematical modeling (*34*). The technique refers to the use of a medium that provides a sharp detectable impedimetric change as the bacterial population grows and converts the low conductivity nutrients into highly charged products. During the preliminary screening by the disk diffusion technique, all *Satureja* plants were shown to possess significant antimicrobial activities, and we decided to study more thoroughly their inhibitory activities by performing instrumental microbiology experiments (impedance). The respective results are shown in **Tables 5**−**9**. Essential oils, either mixed in the growth media or acting as volatiles, inhibited significantly the growth of all pathogens in both inocula sizes tested (**Tables 5**−**9**). It was observed that growth media supplemented with essential oil (1% mixture) were more effective than the use of volatile fractions against the same organisms after 48 h of incubation (**Tables 5**−**9**). The volatile compounds of essential oils increased the lag phase (given as detection time) of the Gram-negative tested bacteria (*E. coli* O157:H7 and *S. enteritidis*). On the other hand, their populations as well as the maximum conductances were lower as compared to the control (**Tables 5** and **6**). The effect of volatile compounds was also evident among the Gram-positive bacteria *S. aureus* and *B. cereus*. These bacteria were completely inhibited in both treatments (e.g., essential oils diluted or used as volatiles; **Tables 7** and **9**). Finally, in the case of *L. monocytogenes*, the inhibition was only evident in the samples tested for the SSPPS and SSPDI specimens (both Dikti and Psiloritis; **Table 7**, samples with high inoculum).

It is further noticeable that the volatile compounds were shown to possess an effect on the metabolic activity, as it was expressed as final conductance measurements for the Gram-negative bacteria (*Salmonella* and *E. coli*). This is evident considering the results of **Tables 5**−**9**, which indicate that there is no correlation between counts (number of cells) and conductance (μS) of these two pathogens (**Tables 6** and **7**).

In conclusion, all tested Gram-negative and Gram-positive foodborne pathogenic bacteria were inhibited from the essential oils of all *Satureja* plants examined. The inhibition was evident in concentrations of essential oils up to 1% in the mix with broth. Volatile compounds had a strong inhibitory effect against

Table 8. Growth of *L. monocytogenes* ScottA in BH Broth and the Effect of Essential Oil on Detection Time, Maximum Conductance, and Final Viable Count

inoculum (cfu/mL)	10^5			10^2		
essential oil	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)
control	11.6	120	9.73	16	130	9.81
SSPPS 1%[a]			<1			<1
SSPDI 1%[a]			<1			<1
SAPP 1%[a]			<1			<1
ST 1%[a]			<1			<1
SSPPS 5 μL[b]	19	70	9.68			<1
SSPDI 5 μL[b]	18.6	55	9.18			<1
SAPP 5 μL[b]			<1			<1
ST 5 μL[b]			<1			<1

[a] Essential oil mixed in 1% concentration with BHI broth. [b] Sterile Whatman disks dipped with 5 μL of the essential oil (effect of volatiles).

Table 9. Growth of *B. cereus* FSS 134 in BH Broth and the Effect of Essential Oil on Detection Time, Maximum Conductance, and Final Viable Count

inoculum (cfu/mL)	10^5			10^2		
essential oil	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)	detection time (h)	maximum conductance (μS)	final viable count (log cfu/mL)
control	8	110	9.46	15	110	9.94
SSPPS 1%[a]			<1			<1
SSPDI 1%[a]			<1			<1
SAPP 1%[a]			<1			<1
ST 1%[a]			<1			<1
SSPPS 5 μL[b]			3.56			<1
SSPDI 5 μL[b]			<1			<1
SAPP 5 μL[b]			<1			<1
ST 5 μL[b]			<1			<1

[a] Essential oil mixed in 1% concentration with BHI broth. [b] Sterile Whatman disks dipped with 5 μL of the essential oil (effect of volatiles).

Gram-positive bacteria while in the case of Gram-negative bacteria, a differentiation on the rate of their metabolic activity was observed. Thus, the volatile compounds of ST have the most notable effects against the tested bacteria. Generally, it can be suggested that the essential oils of all *Satureja* species possess strong antibacterial activities of different extents against organisms of importance to food spoilage and/or poisoning, as well as to those of interest to the medical field such as *Salmonella*, *Listeria*, and *Staphyloccocus*. In view of their broad activity, these essential oils may find industrial applications as natural preservatives and conservation agents in the cosmetic and/or food industries and as active ingredients in medical preparations.

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