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Chemical Composition and Antibacterial Activity of Essential Oils from *Thymus spinulosus* Ten. (Lamiaceae)

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The chemical composition of essential oils from aerial parts of *Thymus spinulosus* Ten. (Lamiaceae) is reported. Four oils from plants growing in different environmental conditions were characterized by GC and GC-MS methods; the oils seem to indicate a new chemotype in the genus *Thymus*. Influences of soil and altitude characteristics on the essential oil composition are discussed. The oils showed antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, and *Bacillus cereus*) and Gram-negative (*Proteus mirabilis*, *Escherichia coli*, *Salmonella typhimuium* Ty2, and *Pseudomonas aeruginosa*) bacteria.

KEYWORDS: ***Thymus spinulosus* Ten.; essential oil composition; antibacterial activity; terpenes; chemotypes; environmental conditions**

INTRODUCTION

Thymus (Lamiaceae) is a large genus divided in eight sections, comprising ∼200 species in the temperate and cold regions of the Old World and diffused in particular in the Mediterranean area. The genus shows a high variability in the shape and form of its species; in addition, a large number of endemics occur, and hybrids are frequently formed between species of different sections as well as between species with different levels of polyploidy. As a result, the taxonomy of this polymorphic genus is complex.

Some species of thyme are widely used for their aromatic and medicinal values, and the uses of thyme species largely depend on the composition of the essential oil (*1*−*11*).

Many species of *Thymus* have been extensively studied for the composition of essential oil, and >20 essential oil chemotypes have been observed (*12*−*16*).

Studies on the composition of essential oils have proved to be a valuable tool in the resolution of some taxonomic problems and can help in determining the relationship between different species in the genus and also in clarifying infraspecific classification, together with chromosomal, geographical, morphological, and hybridization studies (*17*).

In this paper we report a study on essential oils from *Thymus spinulosus* Ten. (= *T. zygis* Ucria non L.; = *T. acicularis* Ten., Guss. non W. et K.) (*18*), a species endemic to southern Italy and Sicily. The plant is a little shrub, growing on arid slopes, from 0 to 1000 m above sea level, and flowering in May−June. The aerial parts are sometimes used as a food flavoring. No chemical or pharmacological data are available in the literature on this plant. However, the essential oil composition of *T. zygis* L. subsp. *sylvestris* is reported (*19*, *20*).

Our study deals with the chemical composition and antimicrobial activity of essential oils from *T. spinulosus* collected at different heights above sea level and/or from different soils.

MATERIALS AND METHODS

Aerial parts of *T. spinulosus*, at flowering stage, were collected in four different areas on Rocca Busambra mountain, Corleone (Palermo, Sicily), at 1140 m above sea level from calcareous (*1*) and siliceous soils (*2*) and at 1060 m above sea level from siliceous (*3*) and calcareous (*4*) soils.

The plants were identified by Dr. F. Raimondo of the Dipartimento di Scienze Botaniche, University of Palermo.

Voucher specimens of different *T. spinulosus* plants are stored in the herbarium of the School of Pharmacy, University of Naples.

Oil Isolation and Analysis. Three lots of 25 g of air-dried aerial parts of each specimen, cut into small pieces, were submitted to hydrodistillation for 3 h, according to the standard procedure reported in the *European Pharmacopoeia* (*21*). The oil contents were 0.22, 0.25, 0.21, and 0.19% (v/w), on a fresh weight basis, respectively, for samples 1, 2, 3, and 4.

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Table 1. Percent Composition of the Essential Oils of Aerial Parts of *T. spinulosus*, Collected at Various Conditions of Soil and Height above Sea Level[a]

RI[a]	compound	1[b]	2[b]	3[b]	4[b]	identification[c]	RI[a]	compound	1[b]	2[b]	3[b]	4[b]	identification[c]
906	tricyclene	0.3	0	0	0	RI, MS	1323	pinocarvyl acetate	0	7.8	0	0	RI, MS
917	α-thujene	0.7	0	0.1	0.3	RI, MS	1336	α-cubebene	0.2	0	0.2	0	RI, MS
922	α-pinene	5.7	0.6	5.5	3.1	RI, MS, Co-GC	1360	α-copaene	0.4	0.5	0.4	0.3	RI, MS
932	camphor	2.0	0.3	0.3	0.5	RI, MS, Co-GC	1368	β-bourbonene	3.6	2.2	2.2	1.7	RI, MS
957	sabinene	0.4	0.2	0.7	0.2	RI, MS, Co-GC	1372	β-cubebene	0.2	0.3	0.3	0.2	RI, MS
960	β-pinene	0.7	0	0	0.3	RI, MS, Co-GC	1375	β-elemene	0.5	0.4	0.6	0.3	RI, MS
964	1-octen-3-ol	0.4	0.3	0.3	0	RI, MS, Co-GC	1392	α-gurjunene	0	0	0.1	0	RI, MS
978	octanol	0.1	0	0.1	0	RI, MS, Co-GC	1400	caryophyllene	9.2	10.8	8.3	11.1	RI, MS
981	myrcene	13.3	1.0	9.5	15.7	RI, MS, Co-GC	1410	β-gurjunene	0.7	1.0	0.7	0.6	RI, MS
989	α-phellandrene	0.1	0	0.2	0	RI, MS, Co-GC	1426	α-cadinene	0.2	0.4	0.3	0.2	RI, MS
1002	α-terpinene	0.6	0.6	0.3	0.3	RI, MS, Co-GC	1431	α-humulene	0.7	0.9	0.7	0.7	RI, MS
1005	*p*-cymene	4.3	0.6	0.2	0.5	RI, MS, Co-GC	1437	*allo*-aromadendrene	0.5	0.6	1.0	0.2	RI, MS
1011	1,8-cineole	3.3	1.2	1.8	0	RI, MS, Co-GC	1440	acoradiene[d]	0.5	0.3	0.5	0.4	RI, MS
1013	limonene	10.2	2.3	5.5	13.2	RI, MS, Co-GC	1442	*cis*-β-farnesene	2.2	8.2	3.3	3.6	RI, MS
1026	(*E*)-β-ocimene	0.3	0	0	0.6	RI, MS, Co-GC	1457	γ-muurolene	7.3	11.4	15.9	12.9	RI, MS
1036	(*E*)-β-ocimene	1.7	0	0	0	RI, MS, Co-GC	1462	β-selinene	0	0	0.1	0	RI, MS
1044	γ-terpinene	9.1	2.0	4.5	1.4	RI, MS, Co-GC	1473	viridiflorene[d]	0	1.6	2.1	1.0	RI, MS
1045	*cis*-sabinene hydrate	0	0	0	4.3	RI, MS, Co-GC	1480	α-muurolene	0.3	0.5	0.6	0.4	RI, MS
1057	*n*-octanol	0	0	0	0.2	RI, MS, Co-GC	1490	γ-cadinene	0.8	0.8	0.6	1.9	RI, MS
1064	1-nonen-3-ol	0.5	0	0.3	0	RI, MS	1491	β-bisabolene	0	1.8	1.2	0.5	RI, MS
1071	terpinolene	0.2	0.2	0.2	0	RI, MS	1494	(*Z*)-γ-bisabolene	0	2.3	0.7	0	RI, MS
1074	*trans*-sabinene hydrate	0.5	0	0.9	0.7	RI, MS	1500	δ-cadinene	0.7	1.5	1.1	0.9	RI, MS
1081	*n*-nonanol	0.5	0.6	0.4	0	RI, MS	1503	*trans*-β-farnesene	0.9	1.6	2.8	0.5	RI, MS
1083	linalool	0.9	0	3.3	0.9	RI, MS, Co-GC	1547	germacrene B	0.8	2.8	1.1	0	RI, MS
1105	camphor	0	0.9	0.1	0	RI, MS, Co-GC	1579	1*R*-*trans*-calamenene[e]	0	0.1	0	0	RI, MS
1137	borneol	2.9	0.8	0.2	1.1	RI, MS, Co-GC	1547	caryophyllene oxide	0.1	0	1.3	1.4	RI, MS
1149	terpinen-4-ol	0.6	0.4	1.2	0.9	RI, MS, Co-GC	1602	guaiol	0	0	1.1	0	RI, MS
1152	*p*-cymen-8-ol	0	0	0.4	0	RI, MS	1606	T-cadinol	0.4	1.4	0.7	4.3	RI, MS
1161	α-terpineol	0	0.6	0.7	0	RI, MS, Co-GC	1611	T-muurolol[d]	0	0.7	0.2	0.6	RI, MS
1162	*trans*-dihydrocarvone	0.5	0	0	0	RI, MS	1618	α-cadinol	0.5	2.2	0	1.3	RI, MS
1189	*cis*-carveol	0.1	0	0	0	RI, MS	1624	*cis*,*cis*-farnesol	0.3	1.4	0.4	0	RI, MS
1200	*p*-anisaldehyde	0	0.2	0.1	0	RI, MS	1627	germacra-1(10),4-dien-6-ol	1.3	11.3	8.9	4.0	RI, MS
1213	methylthymol	0.2	0	0	0	RI, MS, Co-GC	1635	*cis*-lanceol[d]	0	0.3	0	0	RI, MS
1237	geraniol	0.4	0	0	0	RI, MS	1645	14-hydroxy-α-muurulol	0	2.1	0.7	0	RI, MS
1269	thymol	0.1	0.2	0	0	RI, MS, Co-GC	1956	hexadecanoic acid	0	1.4	0.1	0	RI, MS
1278	carvacrol	0.4	0.7	0.9	0	RI, MS, Co-GC	2900	nonacosane	0	1.1	0	0	RI, MS

[a] Retention index on a DB-1 column, 30 m. Compounds are listed in the order of their elution on the DB-1 column. [b] 1 = *T. spinulosus* collected at 1140 m, calcareous soil; 2 = *T. spinulosus* collected at 1140 m, siliceous soil; 3 = *T. spinulosus* collected at 1060 m, siliceous soil; 4 = *T. spinulosus* collected at 1060 m, calcareous soil. [c] RI = retention index identical to bibliography; MS = identification based on comparison of mass spectra; Co-GC = retention time identical to authentic compounds. [d] Acoradiene is spiro[4,5]dec-7-ene, 1,8-dimethyl-4-(1-methylethenyl)-, (1*R*,4*S*,5*S*)-; viridiflorene is 1*H*-cycloprop[*e*]azulene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,4,7-tetramethyl-(1a*R*,7*R*,7a*S*,*bR*); T-muurolol is 1-naphthalenol-1,2,3,4,4a,7,8,8a-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, [1*S*-(1α,4α,4aα,8aα)]-; *cis*-lanceol is 2,6-heptadien-1-ol, 2-methyl-6-[(1*S*)-4-methyl-3-cyclohexen-1-yl]-, (2*Z*)-. [e] Tentative.

The oils were analyzed by GC and CG-MS. GC analyses were performed using a Perkin-Elmer Sigma-115 gas chromatograph with a data handling system and FID. The analysis was carried out using a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness = 0.25 μm). The operating conditions were as follows: injector and detector temperatures, 250 and 280 °C, respectively; carrier gas, He at a flow rate of 2 mL/min; oven temperature program, 5 min isothermal at 40 °C, raised at 2 °C/min to 250 °C, and finally held isothermally for 20 min. GC-MS analyses were performed using a Hewlett-Packard 5890 A apparatus, equipped with a DB-5 fused-silica column (30 m × 0.25 mm i.d., film thickness = 0.25 μm); linked on-line with an HP mass selective detector MSD 5970; ionization energy, 70 eV. Gas chromatographic conditions were as given above; transfer line temperature, 295 °C. The identity of oil components was established from their GC retention indices (*22*), by comparison of their MS spectra with those reported in the literature (*23*−*25*), and by computer matching with the Wiley 5 mass spectra library, as well as, whenever possible, by co-injection with standards available in our laboratories (Sigma-Aldrich Co., Milan, Italy). The linear retention indices were determined in relation to a homologous series of *n*-alkanes (C_8−C_{22}). Component relative concentrations were calculated from GC peak areas without using correction factors.

Bioassays. The antibacterial activity was evaluated in vitro by the paper-disk diffusion method (*26*) against eight bacteria strains, selected as representatives of the classes of Gram-positive (+) and Gram-negative (−). The microorganisms used were *Staphylococcus aureus* (ATCC 25923), *Streptococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (PCI 213), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933), *Escherichia coli* (ATCC 25922), and *Salmonella typhimurium* Ty2 (ATCC 19439). Aliquots of the samples were dissolved in *n*-hexane to give solutions in the concentration range of 10−0.62 mg/mL. Standardized inoculum was obtained by enriched culture of the microorganism in 0.5 mL of Mueller−Hinton broth, incubated at 37 °C for 6 h. Sterile disks (Whatman no. 1, 6 mm in diameter) were impregnated with 20 μL of each dilution of essential oil and then laid on the surface of agar plates so prepared: 9 mL of Muller−Hinton agar (*27*) medium and 1 μL of standardized inoculum of the microorganism. Each oil was performed in triplicate. At the end of the incubation time (24 h at 37 °C) the inhibition zones were measured. Control disks containing only 20 μL of *n*-hexane showed no inhibition in a preliminary test. Gentamycin (10 μg/disk, solvent DMSO) and tetracycline (10 μg/disk, solvent DMSO) were included in each assay as positive controls on Gram-positive and Gram-negative bacteria, respectively.

RESULTS AND DISCUSSION

The percent composition of the essential oils obtained from aerial parts of *T. spinulosus* is reported in **Table 1**. Seventy-two components were identified in the four oils.

The essential oil composition from the plants growing at 1140 m on calcareous soil is reported in column 1. This oil shows

Table 2. Antibacterial Activity of the Essential Oil of Aerial Parts of *T. spinulosus*, Collected at 1140 m, Calcareous Soil[a]

		inhibitory zone (mm)						
		at concentration of					gentamycin	tetracycline
bacteria	Gram	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	(10 μg/disk)	(30 μg/disk)
Sta. aureus	+	18	15	11	8	na	27	nt
Str. faecalis	+	16	13	10	7	na	29	nt
B. subtilis	+	15	11	8	na	na	30	nt
B. cereus	+	15	11	8	na	na	29	nt
Pr. mirabilis	–	12	8	na	na	na	nt	27
E. coli	–	17	12	9	7	na	nt	29
Sal. typhimurium Ty2	–	11	8	na	na	na	nt	26
Ps. aeruginosa	–	12	8	na	na	na	nt	20

[a] Values are the diameter of inhibitory zone (mm) at indicated concentration (mg/mL). nt = not tested; na = not active.

Table 3. Antibacterial Activity of the Essential Oil of Aerial Parts of *T. spinulosus*, Collected at 1140 m, Siliceous Soil[a]

		inhibitory zone (mm)						
		at concentration of					gentamycin	tetracycline
bacteria	Gram	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	(10 μg/disk)	(30 μg/disk)
Sta. aureus	+	13	9	7	na	na	27	nt
Str. faecalis	+	12	8	na	na	na	29	nt
B. subtilis	+	12	9	na	na	na	31	nt
B. cereus	+	11	9	na	na	na	29	nt
Pr. mirabilis	–	10	7	na	na	na	nt	28
E. coli	–	12	8	na	na	na	nt	27
Sal. typhimurium Ty2	–	10	7	na	na	na	nt	26
Ps. aeruginosa	–	9	na	na	na	na	nt	19

[a] Values are the diameter of inhibitory zone (mm) at indicated concentration (mg/mL). nt = not tested; na = not active.

the presence of 53 identified components, accounting for 93.3% of the whole oil. Monoterpenes predominate in this oil (58.5%), myrcene (13.3%), limonene (10.2%), and γ-terpinene (9.1%) being the main constituents. Oxygenated monoterpenes are represented by 8 components, accounting for 10.7% of the oil. Sesquiterpenes represent 32.3% of the oil, 5 of which are oxygenated derivatives accounting for 2.6% of the oil. The most abundant constituents of this fraction were caryophyllene (9.2%), γ-muurolene (7.3%), β-bourbonene (3.6%), and germacra-1(10),4-dien-6-ol (1.3%). The content of phenols is low (0.7%).

The percent composition of the essential oil of *T. spinulosus* collected at 1140 m from siliceous soil is reported in column 2. Fifty components were identified, representing 93.4% of the oil. Sesquiterpenes predominate in this oil (69.4%), with γ-muurolene (11.4%), germacra-1(10),4-dien-6-ol (11.3%), caryophyllene (10.8%), and *cis*-β-farnesene (3.6%) as the main constituents. Oxygenated sesquiterpenes of this fraction (seven) account for 19.4%. Monoterpenes represent 19.5% of the oil, 12.0% of which are oxygenated compounds (seven). Pinocarvyl acetate (7.8%), limonene (2.3%), γ-terpinene (2.0%), 1,8-cineole (1.2%), and myrcene (1.0%) are the main monoterpenes. Phenols account for 1.1% of the oil.

Fifty-six components of the essential oil from *T. spinulosus* growing at 1060 m on siliceous soil were identified (column 3), accounting for 95.9% of the oil. Sesquiterpenes (58.1%) predominate in the oil, with 13.3% represented by seven oxygenated compounds. γ-Muurolene (15.9%), germacra-1(10),4-dien-6-ol (8.9%), and caryophyllene (8.3%) were the main constituents of the sesquiterpene fraction. Monoterpenes represent 35.6% of the oil, with myrcene (9.5%), α-pinene (5.5%), limonene (5.5%), and γ-terpinene (4.5%) as the main constituents. Nine oxygenated monoterpenes constitute 8.0% of the oil. Phenols account for 1.0% of the oil.

Column 4 shows the composition of the essential oil of *T. spinulosus* collected at 1060 m from calcareous soil. Forty components were identified accounting for 97.2% of the oil. Sesquiterpenes (49.0%) represent the main fraction, with γ-muurolene (12.9%), caryophyllene (11.1%), T-cadinol (4.3%), and germacra-1(10),4-dien-6-ol (4.0%) as the main components. Oxygenated sesquiterpenes (five) account for 11.6% of the oil. Among monoterpenes (44.0%), four oxygenated compounds account for 3.4%. Myrcene (15.7%), limonene (13.2%), *cis*-sabinene hydrate (4.3%), and α-pinene (3.1%) are the main constituents of this fraction. Phenols are absent.

T. spinulosus collected in different environmental conditions gave oils with different compositions. Because the identity of plant material was checked repeatedly, any misidentification is ruled out. Data on the chemical composition of essential oils of *T. spinulosus* show no consistency with data available in the literature on *Thymus* chemotypes, and it can be safely suggested that these essential oil compositions indicate a new chemotype in the genus, characterized by myrcene−limonene among monoterpenes and γ-muurolene−caryophyllene−germacra-1(10),4-dien-6-ol among sesquiterpenes as main constituents.

The reported composition pattern is permanently maintained, although the relative amounts of mono- and sesquiterpenes in each sample are very variable. Moreover, considering the ratio of monoterpenes to sesquiterpenes (M/S), it seems that calcareous soils can be related with oils characterized by high monoterpene contents (M/S = 1.81 and 0.90 for plants growing on calcareous soils, at 1140 and 1060 m, respectively); siliceous soil are related to oils with a major percentage of sesquiterpenes (M/S = 0.31 and 0.66 for plants growing on siliceous soils, at 1140 and 1060 m, respectively).

The total quantity of oxygenated compounds appears to be related more to soil composition than to altitude: in calcareous soil the percentage of these constituents varies between 13.3 and 15.0%, for plants growing at 1140 and 1060 m, respectively. Samples collected on siliceous soils present higher percentages of oxygenated terpenes, from 31.4 to 31.3%, for plants growing

Table 4. Antibacterial Activity of the Essential Oil of Aerial Parts of *T. spinulosus*, Collected at 1060 m, Siliceous Soil[a]

		inhibitory zone (mm)						
		at concentration of					gentamycin	tetracycline
bacteria	Gram	10 mg/mL	5 mg/mL	2.5mg/mL	1.25mg/mL	0.62mg/mL	(10 μg/disk)	(30 μg/disk)
Sta. aureus	+	16	12	9	na	na	27	nt
Str. faecalis	+	15	11	7	na	na	28	nt
B. subtilis	+	14	10	8	na	na	30	nt
B. cereus	+	15	12	9	na	na	29	nt
Pr. mirabilis	–	13	9	na	na	na	nt	27
E. coli	–	18	14	10	7	na	nt	28
Sal. typhimurium Ty2	–	11	8	na	na	na	nt	27
Ps. aeruginosa	–	12	9	na	na	na	nt	20

[a] Values are the diameter of inhibitory zone (mm) at indicated concentration (mg/mL). nt = not tested; na = not active.

Table 5. Antibacterial Activity of the Essential Oil of Aerial Parts of *T. spinulosus*, collected at 1060 m, Calcareous Soil[a]

		inhibitory zone (mm)						
		at concentration of					gentamycin	tetracycline
bacteria	Gram	10 mg/mL	5 mg/mL	2.5 mg/mL	1.25 mg/mL	0.62 mg/mL	(10 μg/disk)	(30 μg/disk)
Sta. aureus	+	15	12	9	7	na	29	nt
Str. faecalis	+	14	10	7	na	na	28	nt
B. subtilis	+	14	11	8	na	na	29	nt
B. cereus	+	12	9	7	na	na	30	nt
Pr. mirabilis	–	12	9	na	na	na	nt	27
E. coli	–	16	12	9	na	na	nt	28
Sal. typhimurium Ty2	–	10	8	na	na	na	nt	27
Ps. aeruginosa	–	11	7	na	na	na	nt	20

[a] Values are the diameter of inhibitory zone (mm) at indicated concentration (mg/mL). nt = not tested; na = not active.

at 1140 and 1060 m, respectively. In all samples, the presence of phenols, characteristic of the majority of *Thymus* oils, is very low, varying from 0 to 1.1%. Another important chemical marker in *T. spinulosus* oils seems to be the presence of germacra-1(10),4-dien-6-ol. This compound has been previously reported as a constituent of the essential oil of *T. praecox* Opiz subsp. *polytrichus* (Kern. ex Borb.) Ronn. collected in the Tyrolean Alps (*28*) and in some populations of *T. serpyllum* L. subsp. *serpyllum* and *T. serpyllum* subsp. *tanaensis* (Hyl.) Jalas collected in Finland (*29*).

Soil chemical properties and altitude seem to be important factors in determining the composition of the essential oil of *T. spinulosus*. Our data agree in part with previous studies that correlate the environmental conditions with chemical features of essential oil in other *Thymus* species (*30*−*34*). On the other hand, other studies underscore the importance of genetic and reproductive characteristics in determining the composition of essential oil in *Thymus* ssp. (*35*). The ecological functions of the oil, such as protection from herbivores, interaction with microorganisms in the decomposition process, patterning of vegetation through allelopathic action (*36*), and physiological stage of the plant (*1*), have also been implicated in determining the essential oil composition.

It is important to note that the essential oils of *T. spinulosus* present a very low percentage of phenols but a high level of their biogenetic precursors, γ-terpinene and *p*-cymene (*37*, *38*). This could be due to the flowering time of *T. spinulosus*, early in the year, between May and June. This situation has been reported also for *T. hyemalis* Lange from Spain (*39*).

Moreover, other *Thymus* species present γ-terpinene as the main constituent, for example, *T. revolutus* Celak from Turkey (*40*) and *T. serpyllum* L. from Iran (*41*).

Tables 2−**5** show the inhibition of the four essential oil on the growth of four Gram-positive (+) and four Gram-negative (−) bacteria strains. The oil affected the tested organisms (*B. subtilis*, *Sta. aureus*, *Str. faecalis*, *B. cereus* var. *micoides*, *Pr. mirabilis*, *E. coli*, *Sal. typhimurium* Ty2, *Ps. aeruginosa*) to different degrees. *Sta. aureus* seems to be the most sensitive strain, whereas *Ps. aeruginosa* appears to be the most resistant one.

The good antibacterial activities of the essential oils of *T. spinulosus* in comparison with reference drugs gentamycin and tetracycline were also expected by considering their main constituents. In fact, essential oils with high monoterpene hydrocarbon contents have been reported to be very active against bacteria (*42*−*44*). Generally, thyme oils with high phenol contents were shown to possess considerable antibacterial and/or antifungal activities (*45*). Moreover, it is reasonable to suppose that differential sensitivities to antimicrobial compounds might be in relation to differences in cell envelope constitution and permeability (*7*) and that the lower susceptibility of *Ps. aeruginosa* can be considered to be due to its particular outer membrane (*46*).

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LITERATURE CITED

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