Antifungal Activities of *Origanum vulgare* subsp. *hirtum, Mentha spicata, Lavandula angustifolia,* and *Salvia fruticosa* Essential Oils against Human Pathogenic Fungi

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The essential oils of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* exhibited antifungal properties against the human pathogens *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii*. Of the four oils, *O. vulgare* subsp. *hirtum* oil showed the highest fungicidal activity and at a dilution of 1/50000 caused a 95% reduction in the number of metabolically active cells within 6 h of exposure. Among the main components of the four oils, carvacrol and thymol exhibited the highest levels of antifungal activity. The therapeutic efficacy of the *O. vulgare* subsp. *hirtum* essential oil was tested in rats experimentally infected with *T. rubrum* and yielded promising results. Furthermore, the above essential oils were tested with the Ames test and did not exhibit any mutagenic activity.

Keywords: *Essential oils; Origanum vulgare; Mentha spicata; Lavandula angustifolia; Salvia fruticosa; Malassezia furfur; Trichophyton rubrum; Trichosporon beigelii; Dermatophytosis; antifungal; mutagenic; in vivo studies*

INTRODUCTION

Aromatic plants have been widely used to extend the shelf life of foods and in folk medicine. It is known that most of their properties are due to the essential oils they contain as products of their secondary metabolism.

Recently many studies on the antifungal activities of the essential oils have been reported (Garg and Siddiqui, 1992; Daouk et al., 1995, Shimoni et al., 1993; Muller-Riedau et al., 1995; Thompson, 1989; Mishra and Dubey, 1994; Kishore et al., 1993). Most of these have focused on the antifungal activities of essential oils against soilborne pathogens (Shimoni et al., 1993; Muller-Riedau et al., 1995) and food storage fungi (Thompson, 1989; Mishra and Dubey, 1994; Kishore et al., 1993). Essential oils of some plants have proved their potential for use as natural fumigants in controlling the fungal deterioration of some foods during storage (Dwivedi and Dubey, 1993; Mishra and Dubey, 1994). However, there is only limited information in the literature on the antifungal activity of essential oils toward human fungal pathogens. In the present study the antifungal activities of four essential oils [*Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart, *Mentha spicata* L., *Lavandula angustifolia* Miller, and *Salvia fruticosa* Miller (syn.: *S. triloba* L.)] were examined against three widely spread pathogenic fungal strains that cause superficial skin infections in humans.

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MATERIALS AND METHODS

Plant Material: GC and GC/MS Analyses of Essential Oils. Wild growing, fully flowered *O. vulgare* subsp. *hirtum* plants were collected from Mt. Iti (central Greece). *M. spicata* and *S. fruticosa* were collected from Sithonia Peninsula (northern Greece), whereas *L. angustifolia* was of commercial origin, bought in the market of Thessaloniki. Voucher specimens of the collected plants are kept in the Herbarium of the Laboratory of Systematic Botany and Phytogeography, Aristotle University of Thessaloniki.

The air-dried plant material (leaves only) was cut in small pieces, and the essential oils were isolated after hydrodistillation for 2 h. The three essential oils were chromatographed using a Shimadzu GC-14A gas chromatograph equipped with a Supelcowax 10 (Supelco, Bellefonte, PA) capillary column (60 m × 0.25 mm i.d.) and a flame ionization detector (FID). The carrier gas was helium, and the linear gas velocity was 20.4 cm s^{-1}. The injection and FID temperature was initially 70 °C, increased at a rate of 2 °C/min to 180 °C, then increased at a rate of 4 °C/min to 200 °C, and finally isothermal for 10 min. GC/MS analyses were conducted using a Shimadzu GC/MS QP2000 system equipped with a Supelcowax 10 capillary column (60 m × 0.25 mm i.d.) under the same GC conditions. For GC/MS detection an electron impact (EI) quadropolar system was used with ionization energy of 70 eV. The essential oil components were identified by comparing their relative retention times and mass spectra with those of authentic samples, the *Wiley Registry of Mass Spectral Data* (McLafferty, 1994), and literature citations (Cornu and Massot, 1979; Masada, 1976; Jennings and Shibamoto, 1980). It should be noted that all experiments in this study were carried out with the same lot of essential oil to ensure reproducibility due to the inherent variability observed from lot to lot.

Fungal Strains and Media. The following reference strains of fungi, purchased from the National Collection of Pathogenic Fungi (Public Health Laboratory, Mycology Reference Laboratory, Bristol), were used as test organisms in all antifungal assays: *Malassezia furfur* (yeast, NCPF No. 3349),

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Table 1. Qualitative and Quantitative Composition (Percent) of Essential Oils Obtained from *O. vulgare* subsp. *hirtum*, *M. spicata*, *L. angustifolia*, and *S. fruticosa*

	compound	*O. vulgare* subsp. *hirtum*	*M. spicata*	*L. angustifolia*	*S. fruticosa*
1	α-thujene	0.04		0.03	0.30
2	α-pinene	0.65	0.91	0.58	6.85
3	camphene		0.04	0.04	0.02
4	*β*-pinene	0.98	0.09	0.25	8.20
5	sabinene	0.11	1.15	0.08	4.81
6	*δ*-3-carene			0.04	0.03
7	myrcene	0.12	0.84	0.45	3.16
8	α-phellandrene	0.06			0.03
9	α-terpinene	1.63	0.45		0.06
10	limonene	1.21	5.07	0.25	1.50
11	1,8-cineole	0.21	5.42	13.10	43.10
12	*β*-phellandrene		0.07		
13	(*Z*)-*β*-ocimene	0.16	0.14	3.11	
14	*γ*-terpinene	5.54	0.04	0.07	0.09
15	(*E*)-*β*-ocimene	0.04	0.03	2.84	0.06
16	*p*-cymene	7.35		0.09	0.93
17	terpinolene	0.05		0.09	0.03
18	α-thujone				0.95
19	*β*-thujone				0.93
20	3-octanol			0.08	
21	*cis*-linalool oxide (furanoid)			0.04	
22	1-octen-3-ol		0.08	0.53	0.19
23	*trans*-linalool oxide (furanoid)			0.05	
24	*trans*-sabinene hydrate	0.38	0.53		0.13
25	camphor			5.50	18.34
26	*cis*-sabinene hydrate	0.53	0.38		0.13
27	linalool	0.18	0.06	20.18	0.12
28	linalyl acetate			18.60	0.07
29	bornyl acetate				0.82
30	terpinen-4-ol	0.03	0.60	1.05	1.45
31	lavandulyl acetate			16.01	
32	*β*-caryophyllene	0.97	0.90	2.15	1.53
33	lavandulol			3.09	
34	*trans*-dihydrocarvone		0.12		
35	*cis*-dihydrocarvone		4.90		
36	isoborneol	0.06			1.52
37	α-humulene	0.20		0.25	0.16
38	α-terpineol + borneol		0.83	1.29	1.03
39	dihydrocarvyl acetate		0.19		
40	germacrene D		1.76	0.80	
41	neodihydrocarveol		0.18		
42	neoisodihydrocarvyl acetate		0.90		
43	carvone		59.12		
44	dihydrocarveol		6.27		
45	neoisodihydrocarveol		2.11		
46	*trans*-carvyl acetate		0.24		
47	*trans*-carveol		0.68		
48	*cis*-carveol		3.90		
49	neryl aceate			0.87	
50	geranyl acetae			0.45	
51	*cis*-jasmone		0.16		
52	*p*-cymen-8-ol	0.50			
53	caryophyllene oxide			0.15	1.34
54	spathulenol	0.13			
55	thymol	45.22			0.03
56	carvacrol	33.05			

Trichophyton rubrum (keratinophilic fungus, NCPF No. 192), and *Trichosporon beigelii* (yeast, NCPF No. 3067). *T. rubrum* and *T. beigelii* were grown and maintained in Sabouraud agar or in potato dextrose agar (PDA), while *M. furfur* was grown in a medium containing 0.5% (w/v) glucose, 1.5% (w/v) bacteriological agar, 0.01% (w/v) yeast extract, 0.4% (w/v) ox bile, 0.1% (v/v) glycerol, 2% (v/v) sterile olive oil, 0.05% (v/v) Tween 60, and 1% (v/v) whole cow's milk. All cultures were incubated at 27 (±1) °C for 7 days.

Antifungal Assays. *(a) Poisoned Food Technique.* The toxicity of the essential oils against the three fungi was tested by using the poisoned food technique described by Grover and Moore (1962). The oils were diluted in 95% ethanol and were then mixed and homogenized by ultrasonication with the appropriate fungus growth medium to achieve final concentrations of 4, 2, 1, 0.5, 0.25, or 0.125 μL/mL of medium. Control growth medium contained equivalent amounts of 95% ethanol. Fungal disks, 7 mm in diameter, taken from a 7-day-old culture, were placed in the center of the Petri dish and incubated at 27 ±1 °C for 7 days. At the end of the incubation period the minimum inhibitory concentration (MIC) that caused complete inhibition of the mycelial growth was determined. The fungistatic or fungicidal nature of the oils was determined by testing revival of growth following transfer of the mycelial disk to an appropriate agar plate without oil. Finally, the lowest concentration of the oil that caused fungal death (minimum lethal concentration; MLC) was determined.

(b) Disk Diffusion Assay. Filter paper disks (Whatman No. 1, 5 mm in diameter) containing 5 μL of essential oil or isolated compound (authentic samples, Aldrich Chemical Co., Milwaukee, WI) were applied to the surface of agar plates previously seeded with the test fungus. The fungal inoculum was taken from a 7-day-old culture. The plates were incubated for 3 days, and the net zone of inhibition was determined. The results

Table 2. Antifungal Activities of Essential Oils of *O. vulgare* subsp. *hirtum*, *M. spicata*, *L. angustifolia*, and *S. fruticosa* As Determined by the Disk Diffusion Assay[a]

essential oil	tested organism		
	M. furfur	*T. rubrum*	*T. beigelii*
O. vulgare	21	40	60
M. spicata	NI[b]	40	25
L. angustifolia	3.5	40	9
S. fruticosa	NI	40	15

[a] The diameter (mm) of the inhibition zone represents the mean of three independent experiments. The diameter of the paper disk, 5 mm, is not included. [b] No inhibition observed.

Table 3. Antifungal Activity of the *O. vulgare* subsp. *hirtum*, *M. spicata*, *L. angustifolia*, and *S. fruticosa* Essential Oils As Determined by the Poisoned Food Technique

essential oil	activity	*M. furfur*	*T. rubrum*	*T. beigelii*
O. vulgare	MIC[a1]	1	0.25	0.25
	MLC[b]	NI[c]	1	1
M. spicata	MIC	NI	0.25	0.25
	MLC	NI	4	2
L. angustifolia	MIC	NI	1	2
	MLC	NI	4	NI
S. fruticosa	MIC	NI	2	4
	MLC	NI	NI	NI

[a] Minimum inhibitory concentration (expressed as μL of essential oil/mL of culture medium) of the essential oil that caused complete inhibition of growth (see Materials and Methods). [b] Minimum lethal concentration (expressed as ìL of essential oil/mL of culture medium) of the essential oil that killed the fungal inoculum. [c] No inhibition observed.

indicated in Tables 2 and 4 and in the text represent the net zone of inhibition after subtraction of the diameter (5 mm) of the paper disk.

(c) MTT Assay. The MTT assay is based on the reduction of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO) by mitochondrial dehydrogenases in metabolically active cells to a blue formazan, which can be measured spectrophotometrically (Hansen et al., 1989). A mycelial disk taken from a 7-day-old culture of *T. beigelii* was transferred in Sabouraud broth and incubated overnight in a shaking incubator at 30 °C and 220 rpm, until its optical density at 530 nm became 0.6 unit. The oils were initially diluted 1/100 in 95% ethanol, and appropriate amounts were then mixed and homogenized by ultrasonication in Sabouraud broth (25 mL) so as to yield 1:5000, 1:10000, and 1:50000 dilutions of essential oil. Control growth medium contained equivalent amounts of 95% ethanol. These broths were inoculated with 1 mL of the above overnight culture and were incubated at 30 °C and 220 rpm for 30 h. At 6-h intervals during the incubation period, triplicate 1-mL samples were removed from each culture and centrifuged at 14000*g* for 5 min. The cell pellets were redissolved in 250 μL of Sabouraud broth, 50 μL of MTT solution (5 mg/mL in phosphate-buffered saline) was added, and the incubation was continued under the same conditions for a further 2 h. The cells were collected by centrifugation at 14000*g* for 5 min, and the formazan crystals formed were solubilized in 500 μL of 10% (v/v) Triton X-100 in acidified 2-propanol (2 mL of concentrated HCl/500 mL of solvent). Finally, the optical density of each culture was measured using an automated plate reader (Bio-Tech, EL311SX) at a test wavelength of 570 nm and a reference wavelength of 630 nm.

Animals. Two-month-old male Wistar rats were maintained at 21 °C and were allowed access to feed and water ad libitum.

In Vivo Fungitoxicity Assay. The in vivo evaluation of the antifungal activity of the *O. vulgare* subsp. *hirtum* essential oil was made according to the method of Kishore et al. (1993). On the back of each animal four areas (4 cm^2 each) were cleaned and depilated. The infectious inoculum was prepared from a 7-day-old culture of *T. rubrum* grown on PDA by mixing with 500 mg of sterile white sand (SiO_2) and 2.5 mL of autoclaved bee honey. The inoculum was applied on the animals' backs immediately after depilation and left for 2 days. The establishment of active infection was confirmed on the third day by isolation of the pathogen from skin scales cultured from infected loci on PDA plates containing 100 units/mL penicillin and streptomycin. In the animals in which active infection was confirmed, treatment was initiated on the sixth day post inoculation and continued until complete recovery from infection was achieved. Two ointments were prepared containing 1% (v/v) of essential oil mixed either in petroleum jelly or in a pharmaceutical lotion (Medi-Pak, General Medical Corp., Richmond, VA). The animals were divided into four treatment groups: group 1 was treated with petroleum jelly, group 2 with 1% essential oil in petroleum jelly, group 3 with lotion, and group 4 with 1% essential oil in lotion. The treatments were applied once daily, and the infected areas were scored visually for inflammation and scaling as well as for the presence of the pathogen by cultivating skin scales from infected loci in PDA plates containing 100 units/mL penicillin and streptomycin.

Table 4. Antifungal Activity of the Main Components of the Essential Oils *O. vulgare* subsp. *hirtum*, *M. spicata*, *L. angustifolia*, and *S. fruticosa* As Determined by the Disk Diffusion Assay[a]

main component	tested organism		
	M. furfur	*T. rubrum*	*T. beigelii*
γ-terpinene	1	6	4
p-cymene	1	1	3
thymol	19	TI[c]	TI
carvacrol	15	TI	TI
limonene	NI[b]	3	3
carvone	7	TI	TI
dihydrocarveol	9	3	33
trans-carveol	11	60	39
linalool	NI	NI	16
linalyl acetate	NI	11	10
α-pinene	1	3	NI
β-pinene	2	3	2
1,8-cineole	2	3	3
camphor	2	3	NI

[a] The diameter (mm) of the inhibition zone represents the mean of three independent experiments. The diameter of the paper disk, 5 mm, is not included. [b] No inhibition observed. [c] Total inhibition.

Ames Test. The possible mutagenicity of the essential oils was tested as described by Maron and Ames (1983), using four *Salmonella typhimurium* strains, TA97, TA98, TA100, and TA102, and different amounts of essential oils.

RESULTS AND DISCUSSION

The qualitative and quantitative compositions of the four essential oils analyzed are shown in Table 1. The essential oil obtained from *O. vulgare* subsp. *hirtum* plants (commercially known as Greek oregano) is characterized by a high phenol content (carvacrol + thymol = 78.27% of the total oil). Other main oregano oil constituents are the two monoterpene hydrocarbons, γ-terpinene and *p*-cymene (5.54 and 7.35% of the total oil, respectively). The high content of carvacrol, thymol, and their precursors, γ-terpinene and *p*-cymene (Poulose and Croteau, 1978), characterizes all "oregano" type essential oils (Kokkini, 1994, 1997). Carvone (59.12% of the total oil) and the related ketones, alcohols, and esters characterize the composition of *M. spicata* essential oil. *M. spicata* plants with carvone-rich essential oils are widely used commercially under the name

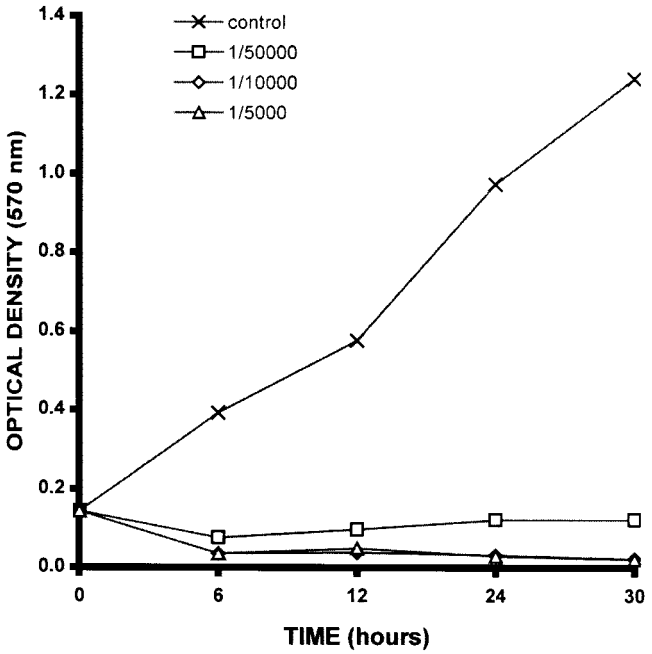

Figure 1. Time- and concentration-dependent effect of *O. vulgare* subsp. *hirtum* essential oil on the rate of growth of *T. beigelii*. Equal aliquots of overnight cultures were inoculated in Sabouraud broth supplemented with various concentrations of essential oil (1/5000, 1/10000, and 1/50000). Cell growth was determined by using the MTT assay (see Materials and Methods).

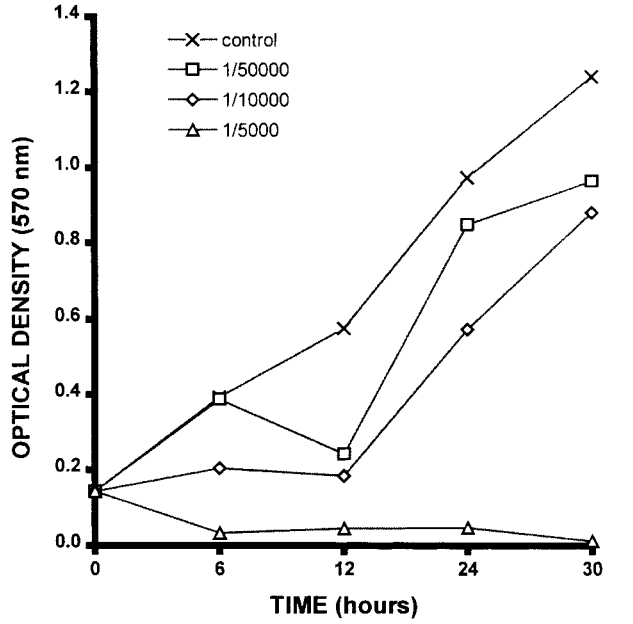

Figure 2. Time- and concentration-dependent effect of *M. spicata* essential oil on the rate of growth of *T. beigelii*. Equal aliquots of overnight cultures were inoculated in Sabouraud broth supplemented with various concentrations of essential oil (1/5000, 1/10000, and 1/50000). Cell growth was determined by using the MTT assay (see Materials and Methods).

spearmint (Kokkini, 1992, and references cited therein). The essential oil of *L. angustifolia* has a typical lavender composition (Lawrence, 1995, and references cited therein). Among its main components are 1,8-cineole, camphor, linalool and its acetate (both accounting for 38.78% of the total oil), and lavandulyl acetate. Finally, the essential oil of *S. fruticosa* (known commercially as Greek sage) is characterized by the high content of 1,8-cineole and camphor (43.10 and 18.34% of the total oil,

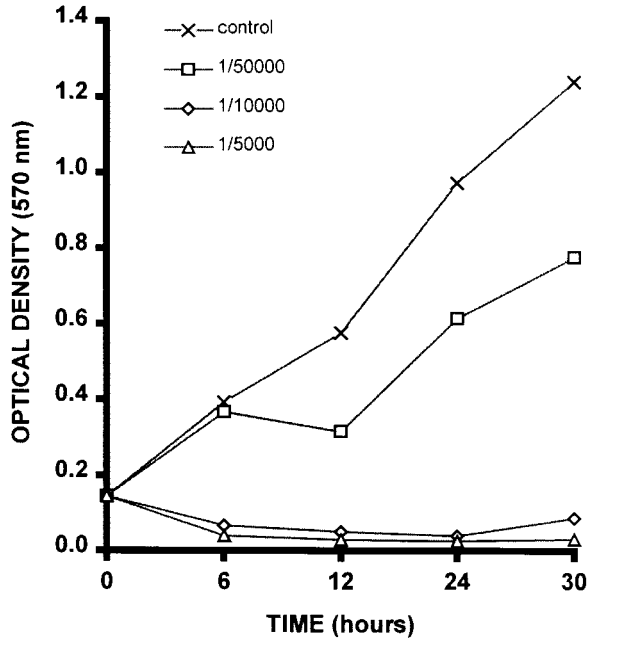

Figure 3. Time- and concentration-dependent effect of *L. angustifolia* essential oil on the rate of growth of *T. beigelii*. Equal aliquots of overnight cultures were inoculated in Sabouraud broth supplemented with various concentrations of essential oil (1/5000, 1/10000, and 1/50000). Cell growth was determined by using the MTT assay (see Materials and Methods).

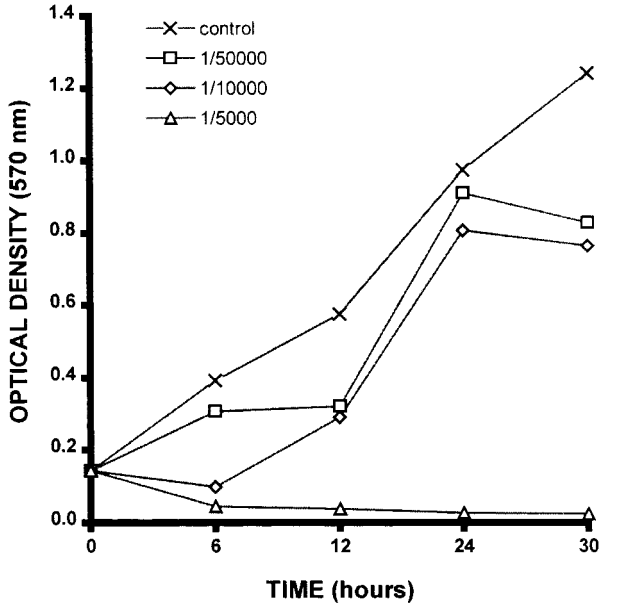

Figure 4. Time- and concentration-dependent effect of *S. fruticosa* essential oil on the rate of growth of *T. beigelii*. Equal aliquots of overnight cultures were inoculated in Sabouraud broth supplemented with various concentrations of essential oil (1/5000, 1/10000, and 1/50000). Cell growth was determined by using the MTT assay (see Materials and Methods).

respectively) and relatively high amounts of α- and β-pinene. These compounds have also been reported as main constituents of *S. fruticosa* plants collected from different areas of Greece (Catsiotis and Iconomou, 1984; Harvala et al., 1987; Karousou, 1995).

The antifungal properties of the four essential oils were initially evaluated by the disk diffusion assay in parallel with the poison food technique, against a panel of three human pathogenic fungal strains. The results presented in Tables 2 and 3 show that the essential oils

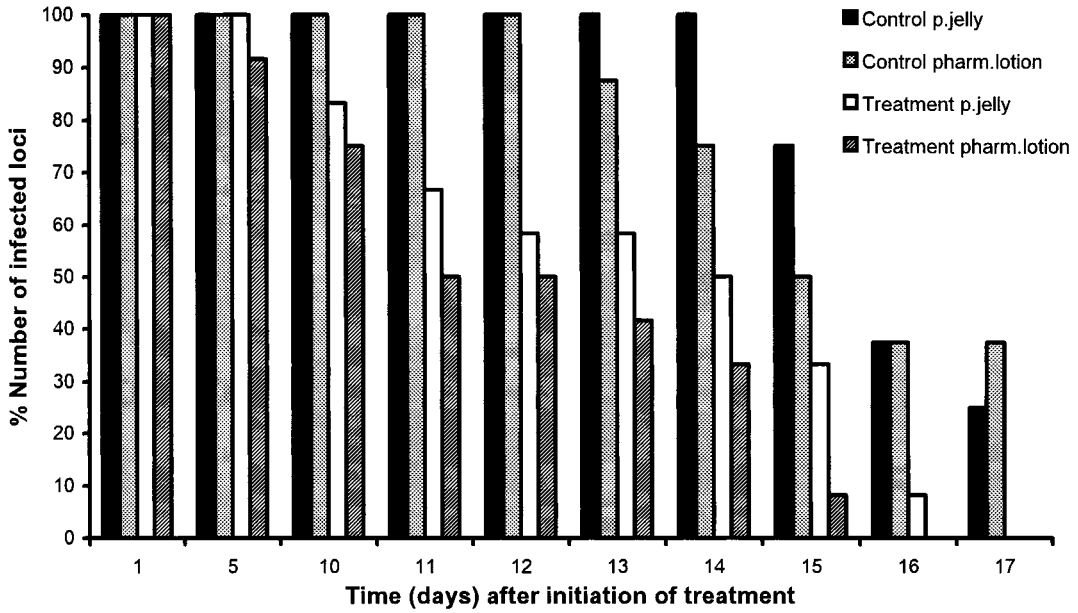

Figure 5. Efficacy of ointments containing 1% (v/v) *O. vulgare* subsp. *hirtum* essential oil in petroleum jelly or in pharmaceutical lotion, in rats experimentally infected with *T. rubrum*.

exhibited variable degrees of antifungal activity against the strains tested with the exception of *M. furfur*, which appeared to be sensitive only to *O. vulgare* subsp. *hirtum* oil and, to a lesser extent, *L. angustifolia* oil. Both oils, at the concentrations tested, exhibited only fungistatic and not fungicidal activity against *M. furfur*. Higher resistance of *M. furfur* to antifungal agents relative to other fungi has been previously reported by Kubo and Himejima (1992). The four oils exhibited similar activities against *T. rubrum* when tested by the disk diffusion assay, whereas their MLC values varied considerably. Thus, although *S. fructicosa* oil did not show any fungicidal activity against *T. rubrum*, the *O. vulgare* subsp. *hirtum* oil was very potent, with an MLC value of 1 μL/mL.

Of the four oils tested *O. vulgare* subsp. *hirtum* exhibited the highest overall antifungal properties because it showed the lowest MLC values (1 μL/mL for *T. rubrum* and *T. beigelii*). In agreement, Shimoni et al. (1993) showed that of four oils tested, *Satureja thymbra*, *Micromeria fruticosa*, *Salvia triloba*, and *Majorana syriaca*, the oregano type oils (*S. thymbra* and *M. syriaca*) exhibited the highest antifungal activity against soil-borne and foliar pathogenic fungi. Antifungal properties have also been detected in other oregano oils against food storage fungi (*Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium* species; Daouk et al., 1995) and soil-borne phytopathogenic fungi (*Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Phytophthora capsici*; Muller-Riebau et al., 1995). In addition, high cytotoxic activity of *O. vulgare* subsp. *hirtum* essential oil against several eukaryotic cell lines has been reported (Sivropoulou et al., 1996). The essential oil of *M. spicata* exhibited moderate antifungal and fungicidal activities against *T. rubrum* and *T. beigelii*. In agreement, other *Mentha* essential oils have been reported to exhibit variable degrees of antifungal activities against phytopathogenic (Muller-Riebau, 1995) and keratinophilic fungi (Kishore and Mishra, 1993). *L. angustifolia* and *S. fruticosa* showed moderate to low antifungal activities, as has been previously reported using other fungal stains (Muller-Riebau et al., 1995; Shimoni et al., 1993).

In an effort to evaluate the individual contributions of the main components of the four oils to the antifungal property, we evaluated the activity of the main compounds by the disk diffusion assay, using authentic commercially available preparations. The results presented in Table 4 show that most compounds exhibited a variable degree of antifungal activity. γ-terpinene, *p*-cymene, limonene, linalool, linalyl acetate, α- and β-pinene, 1,8-cineole, and camphor showed very low antifungal activities. This result is in agreement with those of Muller-Riebau et al. (1995), who reported low antifungal activities for the above compounds, while Garg and Siddiqui (1992) reported that 1,8-cineole exhibited a moderate but variable degree of antifungal activity against human and plant pathogenic fungi. Furthermore, camphor has been shown to be cytotoxic for eukaryotic cells (Sivropoulou et al., 1997). Thymol, carvacrol, and carvone showed high antifungal activity. Carvone has been shown to possess fungistatic activity against *Penicillium hirsutum* (Smid et al., 1995). Interestingly, carvacrol and thymol showed much greater antifungal activities than their biosynthetic precursors γ-terpinene and *p*-cymene, respectively (see Table 4). High antifungal activity for carvacrol and thymol against food storage (Thompson, 1989) and phytopathogenic fungi (Muller-Riebau et al., 1995) was also reported. Moreover, these phenolic compounds showed higher inhibition against the plant pathogen *P. capsici* than the soil-applied systemic fungicide Previcur N. Synergistic (partial or total) antimicrobial activity of thymol and carvacrol against some bacteria involved in upper respiratory tract infections has been reported (Didry et al., 1993). Consequently, the high content of thymol and carvacol may account for the high antifungal activity of *O. vulgare* subsp. *hirtum* oil.

The next set of experiments was designed to examine the effect of the essential oils on the viability of fungal cells in relation to cumulative time of exposure and oil concentration. The method applied was based on the reduction of the tetrazolium salt MTT by mitochodrial dehydrogenases in metabolically active cells to a blue formazan, which can be measured spectrophotometrically. This method has been extensively used to mea-

sure cell toxicity, proliferation, and activation (Hansen et al., 1989) and in the screening of antiviral compounds (Takeuchi et al., 1991). Among the three fungi used in the initial experiments, we selected *T. beigelii* due to its good growth characteristics in liquid culture. The results are presented in Figures 1−4 and show that treatment of *T. beigelli* with the four oils resulted in a dose-dependent growth inhibition. All four oils at 1/5000 dilution caused 95% reduction in the number of metabolically active cells within 6 h of exposure. In the case of *L. angustifolia*, this effect was attained at 1/10000 dilution and in the case of *O. vulgare* subsp. *hirtum* at a dilution as high as 1/50000.

The four oils were tested for their potential mutagenic activity by the Ames test (Maron and Ames, 1983). The oils were tested at concentrations of 250, 500, 1000, and 2000 ppm with no mutagenic activity observed at any concentration for all of the oils. Since the essential oil of *O. vulgare* subsp. *hirtum* showed the highest antifungal activity of the four oils tested, we examined its therapeutic potency against experimentally induced dermatophytosis in rats. *T. rubrum* was chosen as the infectious agent for two reasons: (i) it is the causative agent of ringworm and is associated with well-defined clinical parameters (scaly, erythematous to tawny brown, bilateral and asymmetric lesions, exhibiting a sharply marginated border frequently studded with small vesicles): and (ii) infection with *T. rubrum* has been achieved in laboratory animals (guinea pigs; Kishore and Mishra, 1993). Two ointments containing 1% (v/v) of oil were prepared in petroleum jelly or in a pharmaceutical lotion (Medi-Pak). The therapeutic efficacy of the two ointments was evaluated daily in two ways: (i) macroscopic examination of lesions and (ii) screening for the presence of the infectious agent by culturing skin scales from the infected area. A lesion was scored as cured only when the infected area was free of macroscopic lesions and the cultures were negative. The therapeutic efficacy of the oil appeared higher in the pharmaceutical cream preparation than in the petroleum jelly. At the 11th day of treatment, a 50% reduction in the number of fungus-positive areas was observed in the animal group treated with 1% oil in pharmaceutical lotion relative to the control animals treated with the pharmaceutical lotion alone (see Figure 5). This reduction was accelerated as treatment time increased. During the 17-day observation period the oil-treated animals were cured completely, whereas the fungus could still be recovered from the infected areas of the control-treated animals. It should be noted that in many cases macroscopic lesions disappeared long before elimination of the infectious agent, indicating that long treatment periods of application and evaluation are necessary.

The results presented here should stimulate studies on improved formulations, determination of optimal concentrations for clinical applications, and comparative studies of the therapeutic efficacy of essential oils with conventional drugs currently applied to control ringworm.

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