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Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. essential oil and extract obtained by microwave extraction: Comparison with hydrodistillation

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Abstract

Origanum glandulosum Desf. essential oils obtained by hydrodistillation (HD), solvent-free microwave extraction (SFME) and the extract obtained by microwave-assisted extraction (MAE) were investigated by capillary gas chromatography and gas chromatography/mass spectrometry. The main components of both oils obtained by HD and SFME were thymol (41.6–81.1%) followed by γ-terpinene (27.0–3.1%), *p*-cymene (17.1–4.0%) and carvacrol (2.2–4.4%), respectively. In the same way, thymol (65.4%), γ-terpinene (13.1%), *p*-cymene (7.2%) and carvacrol (3.5%) were the main components of the extract obtained by hexane microwave extraction. The SFME method was most selective for the extraction of thymol. The examination of the antimicrobial activity of both essential oils against 10 bacteria, two yeasts and four moulds revealed that *O. glandulosum* oil is more antifungal than antibacterial. To our knowledge, the antifungal activity of the *O. glandulosum* oil obtained by HD and both antimicrobial and antifungal activities of *O. glandulosum* SFME oil were not yet reported. Our study suggests that *O. glandulosum* essential oil has the potential to be used as a food preservative and to prevent the growth of nosocomial bacteria.

Keywords: *Origanum glandulosum* Desf.; Lamiaceae; Essential oil composition; Extract composition; Microwave extraction; Thymol; γ-Terpinene; *p*-Cymene; Carvacrol; Antimicrobial activities

1. Introduction

Due to the increasing consumer demand for more natural foods, the abuse of toxic synthetic food substances and the increasing microbial resistance of pathogenic microorganisms against antibiotics, natural substances isolated from plants are considered as promising sources of food preservatives (Burt, 2004; Peschel et al., 2006; Smith-Palmer, Stewart, & Fyfe, 2001). In this context, aromatic plants, and especially spices of *Origanum* genus, have emerged as effective compounds to provide microbiological safety of foods (Aligiannis, Kalpoutzakis, Mitaku, & Chinou, 2001; Baydar, Sagdic, Ozkan, & Karadogan, 2004; Ipek et al., 2005; Sivropoulou et al., 1996; Souza, Stamford, Lima, & Trajano, 2007).

The genus *Origanum* (family Lamiaceae) comprises about 38 species widespread in the Mediterranean, Euro-Siberian and Irano-Siberain regions (Kokkini, 1996). *Origanum* plants are widely used as a culinary herb, to flavour food products and alcoholic beverages (Aligiannis et al., 2001; Sivropoulou et al., 1996). Among them, *Origanum vulgare* ssp. *glandulosum* (Desf.) Ietswaart, synonymous

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O. glandulosum Desf., is an endemic spontaneous plant, growing in North Africa (Algeria and Tunisia) (Ietswaart, 1980). In Algeria, *O. glandulosum* is an aromatic shrub called "zaâter" which is mostly used as a medicinal plant against whooping cough, cough, fever and bronchitis (Baba Aissa, 1991).

Due to their antibacterial, antifungal, insecticidal, antioxidant and anti-carcinogenic activities (Ipek et al., 2005), the essential oil composition of *Origanum* species were scarcely studied. *Origanum* taxa can be divided into three groups, according to the occurrence of the following main components: (i) linalool, terpinen-4-ol and sabinene (ii) carvacrol and/or thymol, (iii) sesquiterpenes (Kokkini, 1996). Three studies reported the chemical composition of Algerian *O. glandulosum* essential oil obtained by hydrodistillation (Belhattab et al., 2005; Ruberto, Baratta Tiziana, Sari, & Kaâbeche, 2002; Sari et al., 2006) and extraction–distillation (Belhattab et al., 2005) in which thymol and carvacrol were identified as the main components. The high level of thymol contained in *O. glandulosum* oil gives it strong biological activity. The Algerian *O. glandulosum* oil exhibited a good antioxidant effectiveness (Belhattab et al., 2005; Sari et al., 2006) and showed antimicrobial activities against bacteria, yeast and fungi such as, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*, *Candida albicans* and *Candida tropicalis* (Sari et al., 2006).

The essential oil of aromatic herbs is traditionally obtained by hydrodistillation or solvent extraction. Various novel techniques have been developed for the extraction of natural products from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield and enhance the quality of extracts. Among them, the microwave-assisted extraction and more recently, the solvent-free microwave extraction have been considered as alternatives for the extraction of essential oil or volatile compounds from aromatic plants (Craveiro, Matos, & Alencar, 1989; Flamini et al., 2007; Lucchesi, Chemat, & Smadja, 2004a, Lucchesi, Chemat, & Smadja, 2004b, 2007; Paré, Belanger, & Stafford, 1994).

Since the *Origanum* oil has been used widely as pharmaceuticals, flavouring and antimicrobial agents in the food industry, it is necessary to find the most suitable method for the improvement of the quality of *O. glandulosum* oil. In this study, to our knowledge, we investigated for the first time, the chemical composition of *O. glandulosum* extract and oil obtained by MAE and SFME, respectively. The chemical composition of *O. glandulosum* oil obtained by hydrodistillation (HD) was also studied and compared. Therefore, the comparison of the three techniques in terms of isolation times, yields and composition were reported. The antibacterial and antifungal activities of both HD and SFME oils were individually tested against 10 bacteria, two yeasts and four moulds. To our knowledge, the antimicrobial and antifungal activities of *O. glandulosum* SFME oil, the antifungal activity of the HD oil and the antimicrobial activity of the HD oil against five bacteria are reported for the first time.

2. Materials and methods

2.1. Plant material

Samples of *O. glandulosum* Desf. were collected in Sebdou region of Tlemcen city (western Algeria) in June 2005 during the flowering period. Identification of the species was confirmed by the Laboratory of Botanic Ecology of the University of Tlemcen. A voucher specimen was deposited at the herbarium of this laboratory under the code Lb.05.

2.2. Experimental apparatus and methods

2.2.1. Hydrodistillation apparatus and procedure

The dried aerial parts were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus, according to the Pharmacopée Européenne (1996), and yielded 4.8% of pale yellow oil. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4 °C until used.

2.2.2. Microwave-assisted extraction apparatus and procedure

Microwave-assisted extraction was performed at atmospheric pressure using an Arthur Martin multimode microwave oven operating at 2450 MHz and 850 W as described previously (Craveiro et al., 1989). In MAE procedure, 25 g of plant material was inserted into an extraction vessel and 200 ml of hexane was added. The extraction time was 2 min and the extraction temperature 60–80 °C, setting the microwave extractor at maximum power. After cooling, the vessel was opened and the supernatant was filtered; then the filtrate is deposited in the freezer during 12 h in order to precipitate fixed waxes and oils. After the second filtration, the extract was filtered trough a column containing activated carbon, in order to eliminate the pigments (Jean, Collin, & Lord, 1992). Finally, the filtrate was reduced by rotary evaporation. The extract was collected, dried under anhydrous sodium sulphate and stored at 4 °C until used. The extraction yield was 1.0%.

2.2.3. Solvent-free microwave extraction apparatus and procedure

An Arthur Martin multimode microwave oven operating at 2450 MHz and 850 W equipped with a external cooling system as described previously (Lucchesi et al., 2004a, 2004b), was used for the extraction of the essential oil of *O. glandulosum.* In SFME procedure performed at atmospheric pressure, 25 g of dry plant material was moistened prior to extraction by soaking in 60 ml of water for 1 h and then draining off excess water. The moistened material was placed in a reactor and heated by microwave irradiation for 20 min without adding any solvent. A refrigerant system outside the microwave cavity, condensed the distillate

continuously. The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4 °C until used. The method yielded 3.3% of deep yellow oil.

2.2.4. GC and GC/MS analysis

GC analyses were carried out using a Perkin Elmer Autosystem GC apparatus equipped with a single injector and two flame ionization detectors (FID). The apparatus was used for simultaneous sampling to two fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm) with different stationary phases: Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Temperature program: 60–230 °C at 2 °C/min and then held isothermal 230 °C (30 min). Carrier gas: helium (1 ml/min). Injector and detector temperatures were held at 280 °C. Split injection was conducted with a ratio split of 1:80. Injected volume: 0.1 μl. GC/MS analyses were carried out using a Perkin Elmer TurboMass detector, directly coupled to a Perkin Elmer Autosystem XL equipped with fused-silica capillary columns (60 m × 0.22 mm, film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane) and Rtx-Wax (polyethylene glycol). Other GC conditions were the same as described above. GC/MS (EI) conditions: ion source temperature: 150 °C; energy ionization: 70 eV; electron ionization mass spectra were acquired over the mass range 35–350 Da. Scan time: 1 s. Split injection was conducted with a ratio split of 1:80.

2.2.5. Components identification

Identification of the components was based (i) on the comparison of their GC retention indices (RI) on non-polar and polar columns, determined relative to the retention time of a series of *n*-alkanes with linear interpolation, with those of authentic compounds or literature data (Jennings & Shibamoto, 1980; Joulain & König, 1998); and (ii) on computer matching with commercial mass spectral libraries (Adams, 2001; Köning, Hochmunth, & Joulain, 2001; McLafferty & Stauffer, 1994; National Institute of Standards & Technology, 1999) and comparison of spectra with those of our personal library. Relative amount of individual components were performed on the basis of their GC peak areas on the two capillary Rtx-1 and Rtx-Wax columns, without FID response factor correction.

2.3. Antimicrobial activity

2.3.1. Microbial strains

The antimicrobial and antifungal activities of both HD and SFME oils were individually tested against a panel of microorganisms, including: (i) clinically isolated strains from the Microbiology Laboratory of Tlemcen University Hospital: three Gram-positive bacteria: *Bacillus subtilis* (Bs), *S. aureus* (Sa), *Listeria monocytogenes* (Lm), eight Gram-negative bacteria: *E. coli* (E_1), *E. coli* (E_2), *E. coli* (E_3), *Klebsiella pneumoniae* (Kp), *P. aeruginosa* (P_1), *P. aeruginosa* (P_2), *Citrobacter freundii* (Ci), *Salmonella typhimurium* (St); (ii) referenced strains: two yeasts: *C. albicans* (Ca) 444 (Pasteur Institut, Paris), *C. albicans* (Ca) 9036 (American Type Culture Collection) and (iii) four moulds: *Fusarium oxysporum* (Fus) 963917, *Cladosporium herbarum* (Cla) 3369, *Botrytis cinerea* (Bor) 843390, *Aspergillus flavus* (Asp) 994224 (Museum National d'Histoire Naturelle de Paris). All the strains were grown on Mueller–Hinton agar (MHA) for the bacteria and Saboureaud Dextrose Agar (SDA) with chloramphenicol for yeasts and SDA for moulds.

2.3.2. Preparation of the inoculums

Culture media: Bacteria were assayed on Mueller Hinton Agar (MHA, Pronadisa Hispanalab, g/l): beef infusion 2.0; acid casein peptone 17.5; starch 1.5; bacteriological agar 17.0. Sabouraud Dextrose Agar (SDA, Merck, g/l): peptone, 10.0; glucose, 20.0; aga-agar, 17.0 with chloramphenicol 0.5 g was used for yeasts and SDA for the moulds. The inoculum used for all the assays reached the microbial density of the order of 10^6–10^7 CFU/ml for the bacteria and yeasts and 10^8–10^9 spores/ml for the moulds.

Bacteria: The strains preserved in the nutrient agar at 4 °C, were revived in nutrient solution and incubated at 37 ± 1 °C during 18–24 h., 0.1 ml of each culture was added to 10 ml BHIB (Brain Heart Infusion Broth, Pronadisa Hispanalab, S.A.).

Yeasts: The strains preserved at 4 °C in the Sabouraud agar supplemented with chloramphenicol were revived in nutrient solution and incubated at 30 ± 1 °C for 24–48 h., 0.1 ml of each culture was added to 10 ml sterile physiological water.

Moulds: The inoculum was presented in the form of spores' suspension in sterile physiological water at 0.1% of Tween 80 (Tantaoui-Elaraki et al., 1992).

2.3.3. Antimicrobial screening

Two techniques were used to test the microbial activity of the *O. glandulosum* oils: the paper disc diffusion (Belaiche, 1979) and the dilution agar method (Benjilali, Tantaoui-Elaraki, Ismaili Alaoui, & Ayadi, 1986). The minimum inhibitory concentration (MIC) was determined by the latter method.

Paper disc diffusion: The agar plate containing the appropriate medium was spread with the inoculum containing 10^8 CFU/ml. The filter paper dishes (6 mm in diameter) were impregnated with 3 μl of the oil and then placed onto agar plates. After incubation at 37 ± 1 °C for 18–24 h for bacteria, at 30 ± 1 °C for 24–48 h for yeasts and 10 days at room temperature for moulds, the diameters of inhibition zones were measured in mm. Gentamycin (15 μg), Amphotericin B (100 μg) and Econazol (50 μg) were used as positive controls for bacteria, yeast and moulds, respectively. All the experiments were performed in triplicate.

Determination of the minimum inhibitory concentration (MIC): A dilution agar method was used to determine the MIC. A 10% aqueous solution of Tween 80 was added to 1 ml of oil under sterile conditions to give an oil/Tween

proportion of 80:20 (v/v). The mixture was strongly agitated over 2 min to disperse the oil. The obtained mother solution (MS) was carried in medium to obtain a concentration of 50 μg/ml to 120 μg/ml and poured into Petri dishes. Two controls were included in this test. Each dish contained a sterile solution of Tween 80 and the culture medium, respectively. After incubation at 37 ± 1 °C for 24 h for the bacteria, at 30 ± 1 °C for 48 h for the yeasts and 10 days at room temperature for moulds, the MIC was defined as the lowest concentration of the oil at which the microorganism did not demonstrate visible growth. Antibiotics used were the same as decribed above.

3. Results and discussion

3.1. Chemical composition of the oils

O. glandulosum essential oils obtained by hydrodistillation (HD), solvent-free microwave extraction (SFME) and extract obtained by microwave-assisted extraction (MAE), were investigated by capillary GC and GC/MS. The analysis allowed the identification of 31 components which accounted for 99.6%, 97.5% and 98.2% of HD and SFME oils and MAE extract, respectively. All these compounds were characterized by comparing their EI–mass spectra and their retention indices with those of our own library, except for thymoquinone and thymohydroquinone which were identified by comparison of their EI–mass spectra and their retention indices with those of commercial libraries (Köning et al., 2001) and literature data (Jukic & Milos, 2005). The retention indices of the oils and extract constituents and their relative percentages are listed in Table 1. In total, 24 monoterpenes, five sesquiterpenes and two non-terpenic compounds were identified. Among them, 18 were hydrocarbons and 13 were oxygenated compounds, in particular eight alcohols, two oxides, two quinones and one ketone.

The composition of *O. glandulosum* HD and SFME oils and MAE extract was very similar. The main components were thymol followed by γ-terpinene, *p*-cymene and carvacrol. However, relative concentrations of compounds differed according to the extraction method. SFME oil contained the highest percentage of thymol, followed by the MAE extract and the HD oil in which thymol amounted to 81.1%, 65.4% and 41.6%, respectively. In the same way, carvacrol, which is also an active antioxidant ingredient (Ruberto et al., 2002) amounted to 4.4%, 3.5% and 2.2% in the SFME oil, MAE extract and HD oil, respectively. Conversely, both HD oil and MAE extract contained the highest percentage of γ-terpinene (27.0 and 13.1%, respectively) and *p*-cymene (17.1 and 7.2%, respectively), while these two hydrocarbon monoterpenes, only amounted for 3.1% and 4.0%, respectively, in the SFME oil. The SFME oil, the MAE extract and the HD oil were dominated by the large amounts of oxygenated compounds which represented 87.4%, 72.0% and 45.2%, respectively. The higher abundance of oxygenated compounds in SFME oil than in HD oil is related to the rapid heating of polar substances by microwaves and to the smaller amount of water used, which prevented the decomposition of principal oxygenated constituents by thermal and hydrolytic reactions (Lucchesi, Smadja, Bradshaw, Louw, & Chemat,

Table 1
Chemical composition of hydrodistillation (HD) and solvent-free microwave extraction (SFME) oils and extract obtained by microwave extraction (MAE) of *Origanum glandulosum*

Constituents	LRI_a	RI_a	RI_p	% HD oil	% SFME oil	% MAE extract
α-Thujene	932	928	1023	1.0	tr	0.2
α-Pinene	936	931	1022	0.7	tr	0.1
Camphene	950	943	1066	0.1	–	–
1-Octen-3-ol	962	959	1446	0.2	0.1	0.2
3-Octanone	981	963	1253	0.1	0.0	0.2
β-Pinene	978	970	1110	0.2	0.0	tr
Myrcene	987	979	1159	2.0	0.3	0.7
α-Phellandrene	1002	997	1164	0.3	–	0.1
Δ-3-Carene	1010	1005	1147	0.1	–	tr
α-Terpinene	1013	1008	1178	2.8	0.3	1.0
p-Cymene	1015	1011	1268	17.1	4.0	7.2
Limonene	1025	1020	1199	0.6	0.3	0.4
(*Z*)-β-Ocimene	1029	1024	1230	0.1	–	0.1
(*E*)-β-Ocimene	1041	1034	1247	0.1	–	tr
γ-Terpinene	1051	1047	1243	27.0	3.1	13.1
trans-Sabinene hydrate	1053	1051	1451	0.2	0.2	0.6
Terpinolene	1082	1078	1280	0.1	–	tr
Linalool	1086	1081	1544	0.7	0.6	0.9
Borneol	1150	1148	1698	0.1	–	0.2
Terpinen-4-ol	1164	1161	1600	0.1	0.1	0.1
α-Terpineol	1176	1172	1697	0.2	0.4	0.4
Thymoquinone	1215	1216	–	–	–	tr
Carvacrol methyl ether	1226	1231	1603	–	0.2	0.2
Thymol	1267	1266	2189	41.6	81.1	65.4
Carvacrol	1278	1278	2219	2.2	4.4	3.5
β-Caryophyllene	1420	1424	1591	1.0	1.1	1.6
α-Humulene	1455	1456	1665	0.1	–	0.1
β-Bisabolene	1503	1500	1720	0.1	0.2	0.2
β-Sesquiphellandrene	1516	1516	1765	0.6	0.8	1.1
Thymohydroquinone	1518	1522	>2850	–	–	0.3
Caryophyllene oxide	1578	1576	1980	0.2	0.3	0.3
Total				99.6	97.5	98.2
Isolation (min)				240	20	2
Yields % (w/w)				4.8	3.3	1.0
Hydrocarbons compounds				54.2	10.1	25.9
Oxygenated compounds				45.6	87.4	72.0
Monoterpene hydrocarbons				52.2	8.0	22.9
Oxygenated monoterpenes				45.1	87.0	71.6
Sesquiterpene hydrocarbons				1.8	2.1	3.0
Oxygenated sesquiterpenes				0.2	0.3	0.3
Others compounds				0.3	0.1	0.4

Order of elution and percentages of individual components are given on RTX-1 column. RI_a and RI_p: retention index on RTX-1 apolar column and RT-Wax polar column, respectively. LRI_a: retention indices reported from literature (Jennings & Shibamoto (1980); Joulain & König (1998); Jukic & Milos (2005); Köning et al. (2001)). tr: trace (<0.05).

2007). It is noticeable that aromatic terpenes and derivatives such as *p*-cymene, thymol, carvacrol and carvacrol methyl ether thymoquinone and thymohydroquinone, represented 89.7%, 76.6% and 60.9% of SFME oil, MAE extract and HD oil, respectively. To our knowledge, thymohydroquinone was identified for the first time in the *O. glandulosum* extract, while thymoquinone was identified as glycosidic bound volatile in *O. glandulosum* from Algeria (Belhattab et al., 2005). These compounds are responsible for the strong antioxidant effect of the thyme essential oil obtained after catalytic oxidation (Jukic & Milos, 2005). Finally, the HD oil investigated in this study was qualitatively similar to those reported in the literature (Belhattab et al., 2005; Ruberto et al., 2002; Sari et al., 2006) but differed for the higher amount of γ-terpinene (27.0%) and for the lower percentage of carvacrol (2.2%) which amounted to 1.1–18.7% and 7.6–72.6%, respectively, in the Algerian samples reported in the literature.

Concerning the comparison of the three techniques in terms of isolation times and yields, both microwave extraction and distillation were clearly fast (2 and 20 min), while 3 h were required for hydrodistillation. Furthermore, it is noticeable that an extraction time of 20 min with SFME provided an appreciably high yield comparable with that obtained after 3 h by means of HD (3.3% versus 4.8%), which is the reference method in essential oil extraction. The ultimate yield of extract obtained by MAE was smaller 1.0%.

3.2. Antimicrobial activity

Due to the smaller yield of the solvent microwave extraction, we investigated the antimicrobial and antifungal activities of both oils obtained by HD and SFME on various germs (Table 2). The results obtained from the disc diffusion method, indicated that oils exhibited a stronger antimicrobial activity on the germs in comparison with the control antibiotic (Figs. 1 and 2). Oil obtained by SFME exerted more activity against fungi than bacteria. On bacteria, the HD and SFME oils were more active against *E. coli* (E_3), *S. aureus* (Sa) and *S. thyphimirium* (St) with inhibition zones measured at 24–27 mm, 23–24 mm and 25–26 mm, respectively. In contrast, *P. aeruginosa* (P_1), (P_2) and *K. pneumoniae* (Kp) were the most resistant strains to the oils while *L. monocytogenes*, an important food pathogen, shown a modest sensibility. The fungus *C. albicans* (Ca) 444, *C. herbarum* (Cla) 3369, *B. cinerea* (Bor) 843390 and *A. flavus* (Asp) 994224 were very sensitive to the oils.

The results of the MIC, show that *P. aeruginosa* (P_1) and (P_2) seemed to be resistant to the oils of *O. glandulosum* at 110.00 μg/ml. Maximum activity was observed

Table 2
Antimicrobial activities of both HD and SFME *Origanum glandulosum* oils expressed by the diameter inhibition zones and MIC (μg/ml) methods

Microorganisms	DD[a] (mm)					MIC[b] (μg/ml)				
	Essential oils		ATB			Essential oils		ATB		
	HD	SFME	GEN[c]	AmB[d]	EN[e]	HD	SFME	GEN	AmB	EN
Gram-positive bacteria										
Bacillus subtilis (Bs)	18	20	19	nd	nd	87.50	59.50	4	nd	nd
Staphylococcus aureus (Sa)	23	23	21	nd	nd	79.25	79.25	2	nd	nd
Listeria monocytogenes (Lm)	16	20	20	nd	nd	78.00	58.00	2	nd	nd
Gram-negative bacteria										
Echerichia coli 1 (E_1)	19	20	22	nd	nd	87.00	79.25	4	nd	nd
E. coli 2 (E_2)	21	22	23			87.00	79.25	4	nd	nd
E. coli 3 (E_3)	24	27	20			59.25	59.25	2	nd	nd
Klebsiella pneumoniae (Kp)	8	11	20	nd	nd	59.00	59.00	4	nd	nd
Pseudomonas aeruginosa (P_1)	8	10	10	nd	nd	120.50	120.50	4	nd	nd
P. aeruginosa (P_2)	7	10	14			110.00	100.00	4	nd	nd
Citrobacter freundii (Ci)	21	20	19					4		
Salmonella typhimurium (St)	25	26	22	nd	nd	64.00	64.25	4	nd	nd
Yeasts										
Candida albicans (Ca) 444	34	44	nd	18	25	36.00	36.25	nd	1	nd
C. albicans (Ca) 9036	26	35	nd	21	18	57.00	57.00	nd	1	nd
Moulds										
Fusarium oxysporum (Fus) 963917	20	24	nd	20	13	56.00	56.50	nd	nd	4
Cladosporium herbarum (Cla) 3369	28	32	nd	13	12	56.50	50.25	nd	nd	4
Botrytis cinerea (Bor) 843390	27	33	nd	12	11	57.50	57.25	nd	nd	4
Aspergillus flavus (Asp) 994224	33	39	nd	9	25	55.25	52.25	nd	nd	3

nd: not determined.

[a] DD: agar disc diffusion method. Diameter of inhibition zone (mm) including well diameter of 6 mm.

[b] MIC: minimum inhibitory concentration. Values given as μg/ml. HD and SFME: oils obtained by hydrodistillation and by solvent-free microwave extraction, respectively. ATB: antibiotics.

[c] GEN: Gentamicine (15 μg).

[d] AmB: Amphotericin B (100 μg).

[e] EN: Econasol (50 μg).

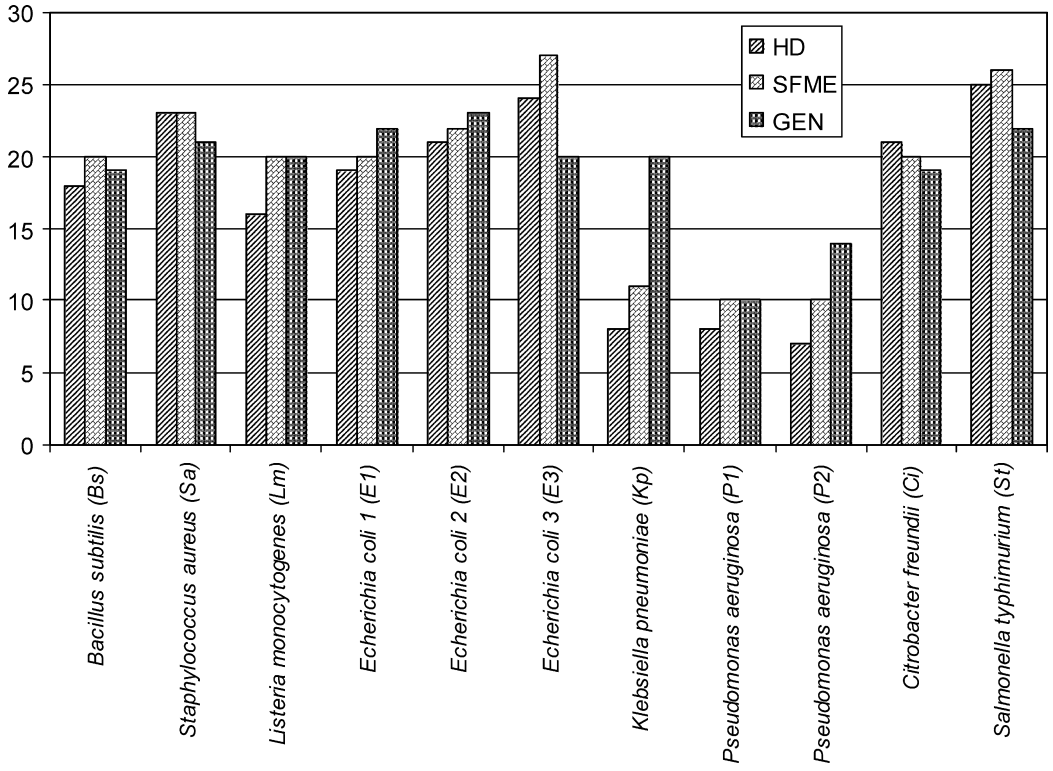

Fig. 1. Mean diameter (mm) of inhibition zones of bacteria versus hydrodistillation (HD) and solvent-free microwave extraction (SFME) oils and antibiotic Gentamicin (GEN).

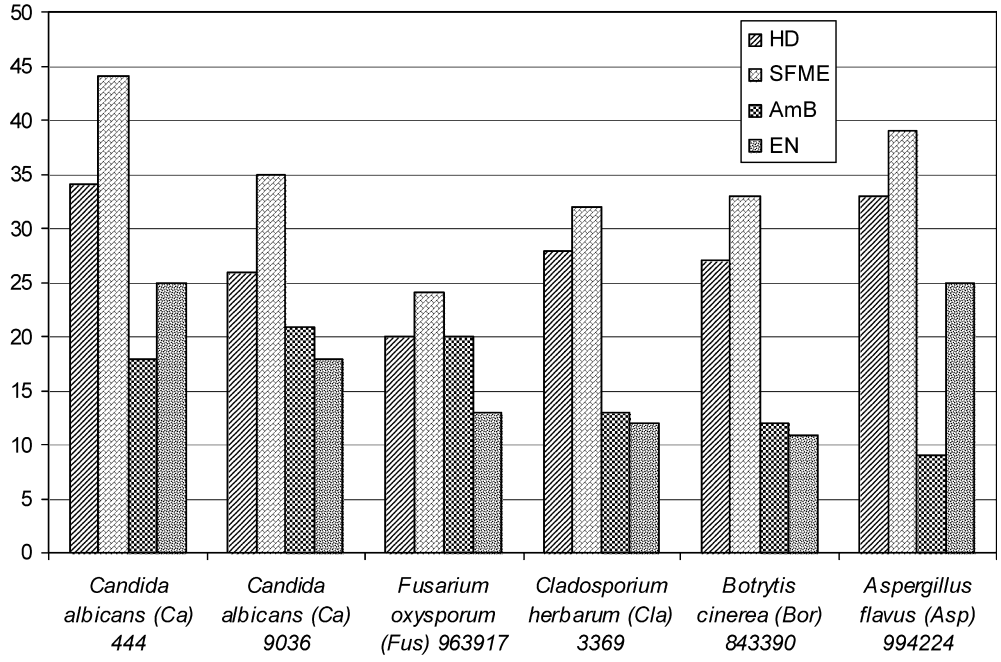

Fig. 2. Mean diameter (mm) of inhibition zones of fungi versus hydrodistillation (HD) and solvent-free microwave extraction (SFME) oils and antibiotics: Amphthericin B (AmB) and Econazol (EN).

against the fungi than the bacteria, such as *C. albicans* (Ca) 444, *C. herbarum* (Cla) 3369 and *A. flavus* (Asp) 994224 with MICs 56.00–56.50 μg/ml, 56.50–50.25 μg/ml and 55.25–52.25 μg/ml to the oils obtained by HD and SFME, respectively.

The antimicrobial nature of *O. glandulosum* essential oils investigated in this study, was apparently related to its phenolic components, such as thymol, carvacrol, carvacrol methyl ether and its precursors *p*-cymene and γ-terpinene. Especially, the large amount of thymol in SFME and HD

oil (81.1% and 41.6%, respectively), was responsible for the high activity. It has been suggested that phenolic derivatives can cause membrane-disturbing activities (Ipek et al., 2005). All micro-organisms tested, showed a variable degree of susceptibility to the SFME and HD oils, except for *P. aeruginosa*, which was resistant, as reported in the literature (Sari et al., 2006).

It is noticeable, that the results of antimicrobial investigation on *O. glandulosum* HD oil were similar to the ones obtained by Sari et al. (2006) on only four bacteria (*E. coli*, *P. aeruginosa*, *S. aureus*, *E. hirae*) and two yeasts (*C. albicans* and *C. tropicalis*). In contrast, the oil obtained by SFME investigated in our study, showed better antifungal activity with less MICs and higher inhibition zones than the HD oil tested by only the disc method diffusion tested by Sari et al. (2006) which zones varied from 8 mm to 18 mm. For the first time, we reported the antimicrobial activity of *O. glandulosum* essential oils against five bacteria: *B. subtilis*, *L. monocytogenes*, *K. pneumoniae*, *C. freundii*, *S. typhimurium* and four moulds: *F. oxysporum*, *C. herbarum*, *B. cinerea* and *A. flavus*.

4. Conclusion

O. glandulosum oils obtained by hydrodistillation and solvent-free microwave extraction and the extract obtained by microwave extraction were investigated by capillary GC and GC/MS and compared in terms of isolation times, yields and chemical composition. The antimicrobial activities of both HD and SFME oils against microorganisms, involved in nosocomial infections and food borne illnesses were investigated. Our data, confirm the antimicrobial potential of *O. glandulosum* essential oil. In addition, our results support the possibility to use solvent-free microwave extraction as alternative method to produce active essential oils. SFME offers many important advantages, including higher extraction yield, shorter extraction time and the highest percentage of the active component thymol. Both HD and SFME thymol-rich oils showed a good antimicrobial activity. However, the largest inhibition zones and the smallest MIC values of *O. glandulosum* SFME oil against bacteria, yeasts and moulds, reported as causal agents of foodborne diseases and/or food spoilage, promoted a potential antifungal agent in food. Further experiments are required in order to establish the real application of solvent-free microwave extraction in foods, phytotherapy, cosmetics and/or pharmaceuticals.

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