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Chemistry, Antioxidant, Antibacterial and Antifungal Activities of Volatile Oils and their Components

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The present paper reports the chemical composition, antioxidant and antibacterial activities of several essential oils and their components. Analysis showed that three oils (*Carum carvi* L., *Verbena officinalis* L. and *Majorana hortensis* L.) contained predominantly oxygenated monoterpenes, while others studied (*Pimpinella anisum* L., *Foeniculum vulgare* Mill.) mainly contained anethole. *C. carvi*, *V. officinalis* and *M. hortensis* oils exhibited the most potent antioxidant activity, due their contents of carvacrol, anethole and estragol. Antibacterial action was assessed against a range of pathogenic and useful bacteria and fungi of agro-food interest. *V. officinalis* and *C. carvi* oils proved the most effective, in particular against *Bacillus cereus* and *Pseudomonas aeruginosa*. Carvacrol proved most active against *Escherichia coli*, and completely inhibited the growth of *Penicillium citrinum*. The oils proved inactive towards some Lactobacilli strains, whereas single components showed an appreciable activity. These results may be important for use of the essential oils as natural preservatives for food products.

Keywords: Antimicrobial activity, chemical composition, food preservation, monoterpenes, natural antioxidants.

Essential oils arise from plant secondary metabolism, [1] and they are widely used in cosmetics as scent components, and in the food industry to improve the flavor and organoleptic properties of different foods [2]. Essential oils have interesting biological properties [3] and several investigations have demonstrated their effectiveness as natural antioxidants, prompting experimental work aimed at identifying the most bioactive compounds. Generally, in order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. However, side-effects of some synthetic antioxidants used in food processing have been documented [4].

Literature reports have described natural antioxidants with radical-scavenging activity from fruits, vegetables, herbs and cereal extracts. Due to the versatile content of essential oils they should be considered as natural agents for food preservation due to their antimicrobial and potential antioxidant activity [5].

The antimicrobial activity of the essential oils is often attributed to the presence of terpenoid and phenolic components [6]. The available literature reports carvacrol, citral, 1,8-cineole, limonene, α- and β-pinene and linalool as active compounds [7] that exhibit significant antimicrobial activities when tested separately [8]. In a previous work, we reported that some essential oils from the family Labiatae exhibited a good antimicrobial activity against different pathogenic bacteria and fungi [9].

In this paper, we report the results of a study aimed to evaluate the chemical composition of the essential oils of *Pimpinella anisum* L. (anise), *Carum carvi* L. (caraway), *Foeniculum vulgare* Miller (fennel) (Apiaceae), *Majorana hortensis* L. (marjoram) (Lamiaceae), *Verbena officinalis* L. (vervain) (Verbenaceae), and to evaluate their antioxidant and antimicrobial activities, as well as those of their main components.

Table 1 outlays the chemical composition of the investigated oils. The main constituent of *P. anisum* and *F. vulgare* (Apiaceae) essential oils was *cis*-anethole, which represented 97.1% and 76.3% of the whole composition, respectively.

Table 1: Chemical composition of essential oils of *Pimpinella anisum* (anise), *Carum carvi* (caraway), *Foeniculum vulgare* (fennel), *Majorana hortensis* (marjoram), and *Verbena officinalis* (vervain).

Compound	Ki[a]	Ki[b]	Anise	Caraway	Fennel	Marjoram	Vervain	Identification [d]
			%[c]	%	%	%	%	
α-Thujene	930	1035	---	0.2±0.0	T	0.1±0.0	---	RI, MS
α-Pinene	938	1032	0.3±0.0	0.5±0.2	1.8±0.1	9.0±0.1	0.2±0.0	RI, MS, Co-GC
(-)-Camphene	953	1076	---	---	---	0.3±0.0	---	RI, MS, Co-GC
Sabinene	973	1132	T	1.0±0.1	T	1.1±0.1	0.5±0.0	RI, MS, Co-GC
Hepten-3-one	975		---	---	---	T	0.2±0.1	RI, MS
β-Pinene	978	1118	---	7.4±0.4	0.5±0.1	3.8±0.9	T	RI, MS, Co-GC
cis-Pinane	980		---	0.1±0.0	---	---	---	RI, MS
Verbenene	982		---	T	T	T	---	RI, MS
Myrcene	993	1174	---	0.7±0.1	0.2±0.1	0.7±0.3	---	RI, MS, Co-GC
α-Phellandrene	995	1176	0.1±0.0	T	0.3±0.0	0.2±0.0	---	RI, MS, Co-GC
Δ3-Carene	997	1153	0.1±0.0	---	0.3±0.1	0.3±0.0	---	RI, MS, Co-GC
α-Terpinene	1012	1188	---	T	T	0.1±0.0	T	RI, MS, Co-GC
o-Cymene	1020	1187	0.1±0.0	0.2±0.0	0.7±0.1	2.6±0.9	0.1±0.0	RI, MS, Co-GC
p-Cymene	1024	1280	---	0.1±0.1	0.3±0.0	0.4±0.1	---	RI, MS, Co-GC
β- Phellandrene	1029	1218	T	0.6±0.2	0.4±0.1	9.1±0.5	0.7±0.2	RI, MS, Co-GC
Limonene	1030	1203	---	14.3±0.5	1.5±0.5	6.4±0.5	2.3±0.9	RI, MS, Co-GC
1,8-Cineole	1034	1213	---	0.1±0.0	T	33.5±0.3	0.4±0.1	RI, MS
(*Z*)-β-Ocimene	1038	1246	T	0.1±0.0	T	0.1±0.0	T	RI, MS, Co-GC
(*E*)-β-Ocimene	1049	1280	---	0.3±0.1	T	0.2±0.1	0.3±0.1	RI, MS, Co-GC
γ-Terpinene	1057	1255	T	T	0.1±0.0	0.8±0.3	0.1±0.0	RI, MS, Co-GC
Fenchone	1067	1392	0.2±0.0	---	14.2±0.4	---	---	RI, MS
Terpinolene	1086	1265	T	T	T	0.2±0.1	T	RI, MS
Linalol	1097	1553	0.4±0.1	0.5±0.1	T	9.8±0.7	0.1±0.0	RI, MS, Co-GC
trans-Thujone	1115	1449	---	0.1±0.0	T	T	---	RI, MS, Co-GC
trans-Pinocarveol	1138	1654	---	T	T	0.1±0.0	T	RI, MS
iso-Borneol	1144	1633	---	---	---	0.1±0.0	---	RI, MS, Co-GC
Camphor	1145	1532	---	T	T	0.2±0.0	---	RI, MS, Co-GC
iso- Pinocamphone	1153	1566	---	T	T	0.2±0.0	0.2±0.0	RI, MS
trans-Pinocamphone	1159		---	4.3±0.9	---	T	T	RI, MS
Pinocarvone	1165	1587	---	---	---	T	T	RI, MS
Borneol	1167	1719	---	---	---	2.0±0.5	0.1±0.0	RI, MS, Co-GC
Terpinen-4-ol	1176	1611	---	T	T	0.4±0.1	0.2±0.0	RI, MS, Co-GC
dihydro-Carveol	1177		---	---	0.3±0.1	0.8±0.1	---	RI, MS
p-Cymen-8-ol	1185	1864	---	---	T	0.1±0.0	T	RI, MS
α-Terpineol	1189	1706	T	T	---	0.7±0.1	0.3±0.1	RI, MS, Co-GC
Myrtenal	1193	1648	---	0.1±0.0	0.1±0.0	0.7±0.1	---	RI, MS
Estragole	1195	1670	---	65.0±0.9	0.8±0.1	0.1±0.0	---	RI, MS, Co-GC
Myrtenol	1196	1804	---	---	---	0.2±0.1	---	RI, MS
Isobornyl formate	1228		---	---	---	---	45.4±0.9	RI, MS
Linalyl acetate	1248	1565	---	---	---	3.3±0.6	---	RI, MS, Co-GC
Geraniol	1255	1857	---	---	---	0.6±0.1	---	RI, MS
cis-Anethole	1262		97.1±0.4	T	76.3±0.9	---	0.2±0.0	RI, MS
Bornyl acetate	1264	1591	---	0.1±0.0	---	1.2±0.5	T	RI, MS
(*E*)-Citral	1270		---	---	---	---	44.5±0.9	RI, MS, Co-GC
Isobornyl acetate	1277		---	0.1±0.0	---	0.6±0.1	T	RI, MS
Thymol	1290	2198	---	---	---	0.7±0.1	---	RI, MS, Co-GC
Carvacrol	1297	2239	---	---	T	4.1±0.90	---	RI, MS, Co-GC
Myrtenyl acetate	1313		---	T	---	T	---	RI, MS
Terpinyl acetate	1333		---	---	---	0.5±0.0	---	RI, MS
Methyl Eugenol	1369	2023	---	0.6±0.1	T	---	T	RI, MS
α-Copaene	1377	1497	---	T	T	0.1±0.0	0.2±0.1	RI, MS
Isoledene	1382		---	T	T	T	0.1±0.0	RI, MS
β-Elemene	1387	1600	---	0.2±0.0	T	T	0.2±0.1	RI, MS
Longifolene	1411	1576	---	---	T	0.1±0.0	T	RI, MS
β-Caryophyllene	1418	1612	T	0.1±0.0	T	0.3±0.1	0.1±0.1	RI, MS
β-Cedrene	1424	1638	---	---	---	0.5±0.1	0.4±0.1	RI, MS
Aromadendrene	1437	1628	T	0.2±0.0	T	T	---	RI, MS
α-Humulene	1455	1689	---	T	T	0.3±0.1	0.2±0.0	RI, MS
allo-Aromadendrene	1463	1661	---	T	T	T	0.1±0.0	RI, MS
γ-Gurjunene	1473	1687	---	---	T	0.1±0.0	T	RI, MS
cis-*β*-Guaiene	1490	1694	---	0.4±0.2	---	---	---	RI, MS
Bicyclogermacrene	1491	1756	---	T	---	0.1±0.0	0.1±0.0	RI, MS
cis-Muurola-4(14),5-diene	1510	1675	---	0.1±0.0	T	0.1±0.0	0.2±0.1	RI, MS
α-7-*epi*-Selinene	1518	1740	---	T	T	0.1±0.0	0.2±0.1	RI, MS
α-Cadinol	1652	2255	---	0.6±0.1	---	---	---	RI, MS
Total compounds			**98.3**	**98**	**97.8**	**97.0**	**97.6**	
Monoterpenes hydrocarbons			0.6	25.5	6.1	35.4	4.2	
Oxigenated monoterpenes			0.6	70.9	15.4	59.9	91.2	
Total Monoterpenes			1.2	96.4	21.5	95.3	95.4	
Sesquiterpenes hydrocarbons			0	1	0	1.7	1.8	
Oxigenated sesquiterpenes			0	0.6	0	0	0	
Total Sesquiterpenes			0	1.6	0	1.7	1.8	
Non terpenes			97.1	0	76.3	0	0.4	
Oxigenated compounds			0.6	71.5	15.4	59.9	91.2	

[a] = Ki = Retention Index on a HP-5 column, [b] = Ki = Retention Index on a HP Innowax column, [c] = --- = absent, T = traces, less than 0.05%, [d] = RI= Retention index identical to bibliography, MS = identification based on comparison of mass spectra, Co-GC = retention time identical to authentic compound.

The dominant components in *C. carvi* oil were estragole (65.0%), limonene (14.3%), β-pinene (7.4%) and *trans*-pinocamphone (4.3%). In the Labiatae family, marjoram essential oil was mainly constituted by 1,8-cineole (33.5%), α-pinene (9.0%) and limonene (6.4%). The vervain (Verbenaceae) essential oil was mainly represented by citral and isobornyl formate, in approximately equal proportions.

Monoterpenes were the most abundant components of the oils analysed, representing a percentage ranging between 95.4%, in vervain oil, and 96.4% in caraway oil. They were constituted mainly of oxygenated monoterpenes, present in amounts ranging between 59.9% (marjoram oil) and 91.2% (vervain). On the other hand, the oils of anise and fennel were mainly constituted of non terpenes ranging between 97.1%, in the anise oil, and 76.3%, in fennel. Our data on anise oil composition agrees with the available literature. Tabanca *et al.* [10] reported that anise oil was constituted predominantly of *E*-anethole (94.2%). Fennel oil contains mainly anethole [11], and limonene and carvone have been reported [12] as the main components of caraway oil; our study confirmed limonene as one of the most abundant components of this oil. However, for marjoram oil, our results disagree with those reported [13], in which terpinene-4-ol, *trans*-sabinene hydrate, and *cis*-sabinene hydrate acetate were the main components with limonene only a minor component. A previous study reported a different composition for vervain oil: Ardakani *et al.* [14] identified 3-hexen-1-ol, 1-octen-3-ol, linalool, verbenone and geranial as its major components.

Anti-radical scavenging activity was tested by the DPPH model system and expressed as absolute percentage of DPPH inhibition (I_{DPPH}, Table 2 and Figure 1, respectively) [15].

All the essential oils showed antioxidant activity, with marjoram and caraway exhibiting the highest activity, with values for I_{DPPH} of 84.9% and 54%, respectively. Conversely, the essential oil of anise (in which the percentage of monoterpenes was as low as 1.2%) was the least effective antioxidant (I_{DPPH}= 19%). Vervain, although containing a very high percentage of monoterpenes, exhibited an intermediate level of antioxidant activity, similar to that of fennel essential oil (I_{DPPH}= 32.3%). This latter containing 21.5% of monoterpenes, showed almost double the radical scavenging potency to anise. Vervain oil showed the same antioxidant activity as fennel oil.

Table 2: The antioxidant activity, expressed as absolute percentage of DPPH inhibition, of *Pimpinella anisum* (anise), *Carum carvi* (caraway), *Foeniculum vulgare* (fennel), *Majorana hortensis* (marjoram), and *Verbena officinalis* (vervain).

	6 min	30 min	60 min
Anise	3.7±0.6	13.0±1.7	19.0±1.8
Caraway	10.6±0.9	34.9±1.8	54.0±2.5
Fennel	7.2±1.1	23.4±1.1	32.3±1.8
Marjoram	46.4±6.3	76.1±4.7	84.9±5.2
Vervain	8.0±1.0	21.0±1.4	32.7±2.2
Control	1.2±1.2	2.4±2.1	2.8±2.5

Our results are in agreement with a previous study [16], which demonstrated for 98 pure essential oils, strong correlation between the chemical composition and antioxidant activity. The authors indicated that antioxidant activity seems directly related to the presence of monoterpenes. In our samples of marjoram and caraway, such compounds reached percentages of 95.3% and 96.4%, respectively. The appreciable antioxidant activity found in the marjoram oil is probably ascribable to carvacrol, a well known antioxidant component [17] with positive synergism with other components. The radical scavenging activity of caraway oil agrees with the literature [18] and it is possible that the strong antioxidant activity is due to estragol (a major component at 65.0%).

The essential oils and their main constituents were tested also for their antimicrobial activity against some food-borne pathogenic bacterial strains, both Gram-positive and -negative. In addition, they were tested against different useful Lactobacilli strains. The antimicrobial activity of the essential oils is reported in Table 3.

The oils appeared more effective against the Gram-positive bacteria (both *B. cereus* strains, *Ent. faecalis* and *S. aureus*) than against the Gram-negative *Ps. aeruginosa* and *E. coli* strains. The most sensitive microorganisms were the two *B. cereus* strains and *Ent. faecalis* and, to a lesser extent, *P. aeruginosa*. On the other hand, *S. aureus* and, in particular, *E. coli* were the least sensitive ones. Among the essential oils, vervain exhibited the strongest antimicrobial activity against almost all the strains tested, in particular against *B. cereus* 4384 and *P. aeruginosa* (with inhibition zones of 18.7 and 15.3 mm, respectively). A strong activity was also exhibited against *Ent. faecalis*, where a zone of about 10 mm was observed in the presence of a 445 µg/paper disc of the essential oil. Caraway oil displayed, at the highest concentration assessed, an antibacterial

Table 3: Inhibition of bacterial growth provoked by essential oils of *Pimpinella anisum* (anise), *Carum carvi* (caraway), *Foeniculum vulgare* (fennel), *Majorana hortensis* (marjoram), and *Verbena officinalis* (vervain). Data are expressed in mm and do not include the diameter of paper disc. Results are shown as mean±standard deviation (SD) of the inhibition zone (n=3).

Essential oil	*Bacillus cereus* 4313 IZ(±SD)	*Bacillus cereus* 4384 IZ(±SD)	*Pseudomonas aeruginosa* IZ(±SD)	*Escherichia coli* IZ(±SD)	*Enterococcus faecalis* IZ(±SD)	*Staphylococcus aureus* IZ(±SD)
Anise 98µg	0	0	0	0	0	0
Anise 196µg	0	0	0	0	0	0
Anise 490µg	5.7(±0.3)	6.0(±0.0)	0	0	6.8(±0.8)	0
Caraway 91µg	0	0	0	0	0	0
Caraway 182µg	5.5(±0.0)	8.8(±0.3)	7.5(±0.9)	0	8.7(±1.1)	0
Caraway 455µg	6.7(±0.6)	9.8(±0.3)	9.3(±1.1)	0	11.7(±2.9)	7.8(±0.3)
Fennel 96 µg	0	0	0	0	0	0
Fennel 193 µg	5.7(±0.3)	0	0	0	0	0
Fennel 482 µg	6.5(±0.7)	5.7(±0.3)	0	0	0	0
Marjoram 90µg	0	0	0	0	0	0
Marjoram 180µg	0	0	7.0(±0.0)	0	6.8(±0.3)	0
Marjoram 450µg	6.0(±0.0)	6.3(±0.1)	0	0	9.5(±0.9)	7.0(0.0)
Vervain 89µg	0	9.7(±0.6)	6.7(±0.6)	0	0	0
Vervain 178µg	0	12.0(±2.6)	10.3(±1.1)	0	7.3(±0.6)	7.3(±0.6)
Vervain 445µg	7.0(±0.0)	18.7(±1.5)	15.3(±1.5)	0	10.3(±0.6)	8.7(±1.1)
Gentamycin 8 µg	22.7(±1.1)	20.7(±1.1)	20.3(±0.6)	20.7(±1.1)	24.7(±0.6)	10.7(±1.1)
Chloramphenicol 66 µg	16.3(±0.6)	18.7(±0.6)	11.7(±0.6)	15.3(±0.6)	26.3(±1.1)	13.3(±2.9)
Tetracycline 7 µg	15.3(±0.6)	13.3(±0.6)	14.7(±0.6)	17.7(±1.1)	18.7(±1.1)	9.3(±0.6)

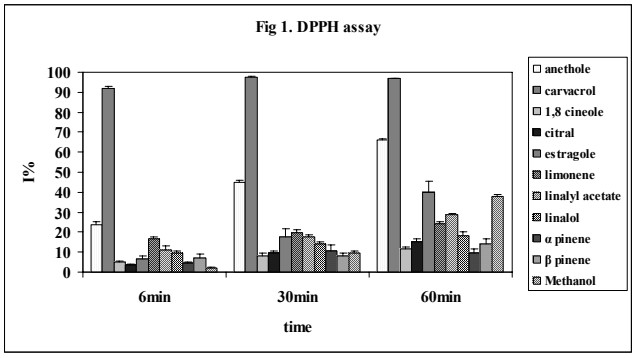

Figure 1: The antioxidant activity (DPPH assay) of main components of essential oils.

activity against almost all the strains tested, in particular *Ent. faecalis* (inhibition zone 11.7 mm), *B. cereus* 4384 and *P. aeruginosa* (inhibition zones 9.8 and 9.3 mm, respectively). An intermediate level of antimicrobial activity was reported for the marjoram essential oil, which displayed antimicrobial activity against almost all the pathogen strains, although only at the highest concentration (450 µg/paper disc). This oil appeared particularly effective against *Ent. faecalis*, with an inhibition zone of about 9.5 mm. Fennel essential oil only seemed to be selectively effective against two strains of *B. cereus* at the highest concentration tested (482 µg/paper disc).

Table 4 summarizes the antimicrobial activity of the individual oil components. Carvacrol had the widest spectrum of activity, followed by citral, linalool, estragole, limonene and linalyl acetate. Anethole, β-pinene and α-pinene were the least effective. In our experiment, carvacrol exhibited the strongest antimicrobial activity, with inhibition zones ranging from 7.3 mm (at 97.6 µg/paper disc, *versus B. cereus* 4313) to 29.7 mm (at 488 µg/paper disc, *versus E. coli*). Estragole displayed an intermediate antimicrobial activity, mainly against *S. aureus* (12.3 mm at a dose of 473 µg/paper disc). Linalyl acetate showed a weak activity only against *B. cereus* 4313 and 4384, and *Ent. faecalis*.

The essential oils appear not to inhibit significantly the Lactobacilli growth (data not reported). However, in contrast, the isolated components, with the exception of 1,8-cineole, citral, and α-pinene, were found to possess effective antimicrobial activity (Table 5) both against starters (*L. sakei*, *L. casei*) and pro-biotic microorganisms (*L. rhamnosus, L. bulgaricus* and *L. acidophilus*). Our results confirm the antimicrobial performance exhibited by vervain oil. The loss of activity exhibited by caraway oil against *E. coli* disagrees with other studies, in which a good antimicrobial action was reported [19]. The divergent results might be due to a different chemical composition of the oil, as reported by Suppakul *et al.* [20].

Hammer *et al.* [19] demonstrated, for fennel oil, an activity, at concentrations above 1%, only against *P. aeruginosa*, while *E. coli* and *S. aureus* were more sensitive. A weak activity was also observed for anise

Table 4: Inhibition of bacterial growth provoked by main components of essential oils. Data are expressed in mm and do not include the diameter of paper disc. Results are shown as mean±standard deviation (SD) of the inhibition zone (n=3).

	***Bacillus cereus* 4313**	***Bacillus cereus* 4384**	***Pseudomonas aeruginosa***	***Escherichia coli***	***Enterococcus faecalis***	***Staphylococcus aureus***
	IZ(±SD)	**IZ(±SD)**	**IZ(±SD)**	**IZ(±SD)**	**IZ(±SD)**	**IZ(±SD)**
anethole 99.8 µg	6.7(±1.1)	0	0	0	0	9.3(±0.6)
anethole 199.6 µg	7.3(±0.6)	0	0	0	0	9.0(±0.0)
anethole 499 µg	6.7(±0.6)	0	5.3(±0.6)	0	0	10.7(±1.1)
carvacrol 97.6 µg	7.3(±0.6)	7.7(±1.1)	8.3(±1.1)	8.0(±1.7)	12.0(±2.0)	20.0(±0.0)
carvacrol 195.2 µg	13.0(±1.7)	15.7(±2.9)	9.3(±0.6)	12.3(±0.6)	17.0(±1.7)	20.7(±1.1)
carvacrol 488 µg	21.7(±2.9)	23.0(±3.6)	16.7(±2.1)	29.7(±0.6)	21.7(±2.9)	23.7(±1.5)
citral 88µg	6.8(±3.2)	9.3(±0.6)	6.7(±0.6)	0	6.7(±3.2)	6.0(±0.0)
citral 176µg	6.0(±0.0)	11.3(±3.2)	12.0(±1.7)	0	6.7(±0.6)	6.3(±0.6)
citral 440µg	10.7(±1.1)	15.7(±4.0)	22.3(±4.6)	9.7(±0.6)	9.7(±0.6)	9.7(±0.6)
1,8-cineole 92.2 µg	0	6.7(±1.1)	0	0	6.3(±0.6)	8.7(±0.6)
1,8-cineole 184.4 µg	6.3(±0.6)	7.3(±1.1)	0	0	6.0(±0.0)	10.3(±0.6)
1,8-cineole 461 µg	7.0(±0.0)	7.3(±2.3)	0	0	8.3(±0.6)	11.0(±1.7)
estragole 94.6 µg	7.0(±0.0)	6.7(±1.1)	0	6.3(±1.1)	6.7(±0.6)	10.3(±0.6)
estragole 189.2 µg	7.0(±0.0)	6.7(±0.6)	7.0(±0.0)	6.7(±1.5)	5.3(±0.6)	11.3(±0.6)
estragole 473 µg	6.3(±0.6)	6.3(±0.6)	6.7(±0.6)	6.3(±1.1)	7.3(±1.5)	12.3(±0.6)
limonene 84 µg	0	0	0	0	0	9.7(±0.6)
limonene 168 µg	0	6.7(±1.1)	6.3(±1.1)	0	0	11.7(±1.5)
limonene 420 µg	6.3(±0.6)	6.7(±0.5)	6.7(±2.5)	0	6.7(±0.6)	12.0(±2.6)
linalyl acetate 89.5 µg	2.0(±0.0)	1.3(±2.3)	0	0	2.3(±0.6)	0
linalyl acetate 179 µg	3.0(±0.0)	1.3(±2.3)	0	0	2.7(±0.6)	0
linalyl acetate 447.5 µg	3.0(±0.0)	4.3(±0.6)	0	0	3.7(±0.6)	0
linalol 85.8 µg	4.0(±0.0)	2.0(±0.0)	2.3(±0.6)	2.0(±0.0)	4.3(±0.6)	9.3(±0.6)
linalol 171.6 µg	6.0(±1.0)	3.3(±0.6)	3.7(±1.5)	4.0(±0.0)	6.3(±0.6)	10.0(±0.0)
linalol 429 µg	9.3(±0.6)	4.7(±0.6)	8.7(±0.6)	5.7(±1.1)	9.3(±0.6)	14.7(±0.6)
α pinene 86 µg	0	0	0	0	0	8.7(±0.6)
α pinene 172 µg	0	0	0	0	0	10.7(±1.1)
α pinene 430 µg	0	0	0	0	0	13.0(±1.7)
β pinene 86 µg	0	0.7(±1.1)	0	0	0	11.0(±1.7)
β pinene 172 µg	0.7(±1.1)	1.3(±1.1)	0	0	0	12.7(±2.5)
β pinene 430 µg	1.3(±1.1)	2.0(±1.7)	5.7(±0.6)	0	5.7(±0.6)	13.0(±1.7)
Gentamycin 8 µg	22.7(±1.1)	20.7(±1.1)	20.3(±0.6)	20.7(±1.1)	24.7(±0.6)	10.7(±1.1)
Chloramphenicol 66 µg	16.3(±0.6)	18.7(±0.6)	11.7(±0.6)	15.3(±0.6)	26.3(±1.1)	13.3(±2.9)
Tetracycline 7 µg	15.3(±0.6)	13.3(±0.6)	14.7(±0.6)	17.7(±1.1)	18.7(±1.1)	9.3(±0.6)

essential oil, with inhibition zones not exceeding 6.8 mm (against *Ent. faecalis*), in agreement with Hammer *et al*. [19].

Phenols, like carvacrol, are well-known active substances, acting both against Gram-negative and Gram-positive microorganisms. The phenolic hydroxyl group of carvacrol seems essential also for the antimicrobial activity against the food-borne pathogen *B. cereus*, and slightly less against the other pathogens tested. In all cases, as demonstrated for *B. cereus*, it could cause the destabilization of the membrane and a depletion of the microbial ATP pools that lead to impairment of essential processes and finally to cell death [21]. The activity of carvacrol against *B. cereus* could let us hypothesise its use as a natural food preservative against this strain, which is strictly linked to food-borne illnesses and which contaminates several food products.

The strong antimicrobial activity exhibited by citral agrees with literature data [22]. However, its antimicrobial effects on lactic acid bacteria could prove problematic when they are required for a fermentative process.

Estragole was the main component in caraway oil. This showed lower activity against *S. aureus* and a stronger effect against *Ent. faecalis*.

The results, presented in Table 6, show that the tested essential oils exhibited variable degrees of antifungal activity. Marjoram and caraway oils were active against all fungal strains, with inhibition zones ranging from 9.3 mm (exhibited by caraway essential oil against *P. citrinum*) to 13.7 mm (marjoram essential oil against *D. hansenii*), at the highest concentration used in our experiments.

Table 5: Inhibition of bacterial lactic growth provoked by main components of essential oils (Data are expressed in mm and do not include the diameter of paper disc. Results are shown as mean±standard deviation (SD) of the inhibition zone (n=3).

	L. sakei IZ(±SD)	*L. rhamnosus* IZ(±SD)	*L. casei* IZ(±SD)	*L. bulgaricus* IZ(±SD)	*L. acidophilus* IZ(±SD)
anethole 99.8 µg	0	2.0(±0.0)	7.3(±0.6)	7.7(±0.6)	4.0(±0.0)
anethole 199.6 µg	1.3(±2.3)	3.7(±0.6)	11.3(±0.6)	15.3(±0.6)	6.3(±0.6)
anethole 499 µg	10.0(±0.0)	5.0(±0.0)	14.3(±2.1)	19.7(±0.6)	10.3(±0.6)
carvacrol 97.6 µg	10.0(±0.0)	9.0(±1.0)	7.3(±0.6)	8.7(±1.1)	6.3(±0.6)
carvacrol 195.2 µg	13.3(±1.1)	11.0(±0.0)	12.7(±0.6)	10.7(±1.1)	9.7(±0.6)
carvacrol 488 µg	20.3(±1.5)	15.0(±1.0)	17.3(±0.6)	17.3(±1.1)	14.0(±1.7)
citral 88 µg	0	0	0	0	0
citral 176µg	0	0	0	0	0
citral 440µg	0	0	0	0	0
1,8-cineole 92.2 µg	0	0	0	0	5.3(±0.6)
1,8-cineole 184.4 µg	0	0	10.0(±0.0)	6.7(±0.6)	5.7(±1.1)
1,8-cineole 461 µg	0	0	12.3(±0.6)	11.3(±1.1)	10.0(±0.0)
estragole 94.6 µg	6.3(±0.6)	4.3(±0.6)	8.7(±1.1)	7.7(±0.6)	5.0(±0.0)
estragole 189.2 µg	8.7(±1.1)	5.0(±0.0)	13.3(±2.9)	9.7(±0.6)	5.7(±0.6)
estragole 473 µg	10.0(±0.0)	9.3(±1.1)	14.0(±1.7)	14.7(±0.6)	10.3(±0.6)
limonene 84 µg	0	2.0(±0.0)	7.3(±2.1)	4.3(±3.8)	3.7(±0.6)
limonene 168 µg	0	4.0(±0.0)	11.3(±1.1)	9.7(±0.6)	5.0(±0.0)
limonene 420 µg	1.3(±2.3)	5.3(±2.1)	13.7(±1.5)	14.7(±0.6)	6.7(±0.6)
linalyl acetate 89.5 µg	0	2.8(±1.0)	5.3(±0.6)	0	4.7(±0.6)
linalyl acetate 179 µg	0	3.7(±0.6)	8.7(±1.1)	4.3(±3.8)	5.0(±0.0)
linalyl acetate 447.5 µg	1.3(±2.3)	5.7(±0.6)	12.3(±0.6)	13.3(±2.1)	8.0(±0.0)
linalol 85.8 µg	0	8.7(±0.6)	8.3(±1.5)	10.0(±0.0)	22.0(±0.0)
linalol 171.6 µg	0	10.7(±1.1)	10.3(±0.6)	14.3(±0.6)	22.0(±0.0)
linalol 429 µg	10.0(±0.0)	13.7(±1.5)	13.3(±1.5)	18.7(±1.1)	22.0(±0.0)
α pinene 86 µg	0	0	6.3(±2.1)	0	0
α pinene 172 µg	0	0	9.3(±0.6)	0	0
α pinene 430 µg	0	0	13.7(±1.5)	8.7(±1.1)	0
β pinene 86 µg	0	2.3(±0.6)	9.7(±0.6)	0	4.0(±0.0)
β pinene 172 µg	0	3.3(±0.6)	13.7(±1.5)	0	6.7(±0.6)
β pinene 430 µg	0	4.0(±0.0)	19.3(±1.1)	10.7(±0.6)	9.7(±0.6)

Table 6: Inhibition of fungal growth provoked by essential oils of *Pimpinella anisum* (anise), *Carum carvi* (caraway), *Foeniculum vulgare* (fennel), *Majorana hortensis* (marjoram), and *Verbena officinalis* (vervain). Data are expressed in mm and do not include the diameter of paper disc. Results are shown as mean±standard deviation (SD) of the inhibition zone (n=3).

	Penicillium simplicissimum IZ(±SD)	*Aureobasidium pullulans* IZ(±SD)	*Penicillium citrinum* IZ(±SD)	*Penicillium expansum* IZ(±SD)	*Debaryomyces hansenii* IZ(±SD)	*Penicillium aurantiogriseum* IZ(±SD)
Anise 98µg	5.0(±0.0)	2.7(±2.3)	2.3(±2.1)	0	0	0
Anise 196µg	6.0(±1.0)	6.3(±0.6)	4.7(±0.6)	0	0	1.7(±2.9)
Anise 490µg	9.7(±0.6)	7.0(±0.0)	6.7(±0.6)	0	0	6.3(±0.6)
Caraway 91µg	4.7(±0.6)	4.7(±4.0)	0	5.0(±0.0)	0	2.3(±4.0)
Caraway 182µg	7.3(±1.4)	7.0(±0.0)	7.0(±0.0)	7.0(±0.0)	10.0(±1.0)	5.7(±1.1)
Caraway 455µg	10.8(±1.0)	10.3(±1.5)	9.3(±0.6)	10.3(±1.5)	11.0(±1.7)	10.0(±0.0)
Fennel 96 µg	3.7(±0.6)	2.7(±2.3)	0	0	0	0
Fennel 193 µg	4.0(±0.0)	3.3(±2.9)	0	0	0	1.3(±2.3)
Fennel 482 µg	5.7(±0.6)	5.3(±0.6)	4.0(±3.5)	0	1.7(±2.9)	6.0(±0.0)
Marjoram 90µg	7.3(±2.1)	7.7(±1.1)	6.7(±2.9)	6.3(±0.6)	9.0(±1.0)	7.2(±0.3)
Marjoram 180µg	9.8(±0.3)	8.7(±1.5)	9.3(±2.1)	8.5(±1.3)	9.0(±1.7)	10.0(±2.0)
Marjoram 450µg	11.3(±1.1)	12.7(±1.1)	11.0(±1.0)	11.7(±2.9)	13.7(±2.3)	11.7(±2.9)
Vervain 89µg	5.3(±0.6)	3.7(±3.2)	3.7(±0.6)	0	5.2(±1.3)	0
Vervain 178µg	6.7(±0.6)	9.0(±1.7)	6.3(±0.6)	7.0(±0.0)	8.7(±1.5)	0
Vervain 445µg	11.3(±1.1)	15.0(±0.0)	11.8(±0.3)	14.0(±1.7)	12.7(±0.6)	0

Fennel and vervain essential oils exhibited different activity against the fungi tested; in particular, fennel showed a weaker activity (about 50%) than vervain and in addition, the two oils were ineffective against some strains. Fennel oil did not show activity against *P. expansum*, while vervain oil was ineffective against *P. aurantiogriseum*. On the other hand, vervain oil exhibited the highest activity against

Table 7: Inhibition of fungal growth provoked by main components of essential oils Data are expressed in mm and do not include the diameter of paper disc. Results are shown as mean±standard deviation (SD) of the inhibition zone (n=3).

	Penicillium simplicissimum **IZ(±SD)**	***Aureobasidium pullulans*** **IZ(±SD)**	***Penicillium citrinum*** **IZ(±SD)**	***Penicillium expansum*** **IZ(±SD)**	***Debaryomyces hansenii*** **IZ(±SD)**	***Penicillium aurantiogriseum*** **IZ(±SD)**
anethole 99.8 µg	4.8(±0.3)	4.8(±0.3)	4.3 (± 0.6)	0	0	0
anethole 199.6 µg	8.2(±1.0)	5.8(±0.3)	5.0(±0.0)	0	0	0
anethole 499 µg	9.3(±0.6)	10.0(±0.0)	8.3(±1.5)	0	7.3(±1.5)	0
carvacrol 97.6 µg	no growth	10.3(±0.6)	no growth	no growth	7.7(±0.6)	5.7(±0.6)
carvacrol 195.2 µg	no growth	14.3 (±0.6)	no growth	no growth	12.0(±0.0)	9.7(±0.6)
carvacrol 488 µg	no growth	16.3(±3.2)	no growth	no growth	15.7(±1.1)	15.0(±0.0)
citral 88µg	8.7(±0.6)	5.2(±1.26)	6.8(±2.0)	5.8(±0.3)	0	5.7(±0.6)
citral 176µg	10.0(±3.6)	10.0(±0.0)	8.8(±1.1)	8.3(±1.5)	8.3(±2.9)	8.3(±1.1)
citral 440µg	12.7(±3.06)	14.0(±1.7)	12.0(±1.3)	13.0(±1.7)	11.7(±2.9)	13.3(±1.1)
1,8-cineole 92.2 µg	4.0(±0.0)	7.7(±0.6)	5.2(±0.3)	0	0	4.7(±0.6)
1,8-cineole 184.4 µg	4.7(±0.6)	7.3(±1.5)	8.5(±1.3)	0	6.7(±1.1)	5.7(±1.1)
1,8-cineole 461 µg	10.0(±0.0)	14.3 (±0.6)	10.3(±0.6)	0	9.7(±0.6)	9.3(±0.6)
estragole 94.6 µg	5.0(±0.0)	5.0(±0.0)	5.0(±0.0)	9.7(±0.6)	4.3 (±0.6)	0
estragole 189.2 µg	8.5(±0.5)	10.7(±1.1)	7.7(±1.1)	10.3(±0.6)	6.3(±1.1)	0
estragole 473 µg	14.0(±1.7)	15.3 (±0.6)	15.7(±1.1)	12.3(±0.6)	12.7(±2.1)	0
limonene 84 µg	4.0(±0.0)	4.0(±0.0)	3.3(±0.6)	0	5.3(±1.1)	6.3(±2.3)
limonene 168 µg	6.8(±0.8)	6.3(±0.6)	4.0(±0.0)	0	8.0(±1.7)	9.5(±0.9)
limonene 420 µg	8.7(±0.4)	8.0(±1.7)	4.3(±0.6)	0	10.7(±1.1)	13.3(±2.9)
linalyl acetate 89.5 µg	4.7(±0.6)	4.7(±0.6)	2.0(±0.0)	0	3.3(±2.9)	2.3 (± 4.0)
linalyl acetate 179 µg	8.3(±1.1)	9.8(±1.3)	2.0(±0.0)	0	7.3(±0.6)	4.7 (± 4.0)
linalyl acetate 447.5 µg	11.0(±1.0)	12.7(±2.1)	2.0(±0.0)	0	11.3(±1.1)	4.7 (± 4.5)
linalol 85.8 µg	8.7(±3.2)	9.7(±2.5)	6.3(±0.6)	0	7.7(±0.6)	9.3(±0.6)
linalol 171.6 µg	12.3(±1.5)	13.7(±1.5)	7.3(±2.1)	10.7(±1.1)	10.7(±1.1)	15.7(±3.2)
linalol 429 µg	14.3(±2.1)	16.7(±2.9)	13.3(±1.5)	11.7(±2.9)	16.3(±1.1)	18.7(±1.5)
α pinene 86 µg	0	3.3(±0.6)	0	0	0	0
α pinene 172 µg	0	4.3(±0.6)	0	0	0	0
α pinene 430 µg	0	5.7(±1.1)	0	0	8.7(±2.3)	0
β pinene 86 µg	4.3(±0.6)	5.3(±0.6)	0	0	3.7(±0.6)	5.3(±0.6)
β pinene 172 µg	5.7(±0.6)	8.3(±1.1)	0	0	7.3(±2.3)	8.3(±1.1)
β pinene 430 µg	8.8(±0.8)	13.3(±1.5)	3.3(±2.9)	0	9.7(±0.6)	13.3(±1.5)

A. pullulans (inhibition zone of 15 mm). The growth of *P. citrinum* was appreciably reduced by the essential oils tested, with inhibition zones ranging from 4.0 mm (fennel oil), to about 12 mm (vervain oil).

Table 7 shows the antifungal activity of the components. The compounds with the strongest spectrum of activity appeared to be citral and linalool, which were effective against all fungi assayed. 1,8-Cineole, estragole, limonene and linalyl acetate acted against almost all the microorganisms. The weakest activity was exhibited by α-pinene, the best result for which was recorded against *D. hansenii* (8.7 mm inhibition zone). This compound showed activity against all the fungi assayed, producing inhibition zones always above 11 mm. Linalyl acetate, present only in the marjoram essential oil, was more active on fungi than on bacteria. However, its action was less effective than the marjoram essential oil against *P. citrinum* and *P.aurantiogriseum*. *cis*-Anethole, the main component of anise and fennel essential oils, was differently effective against the strains used in the test. It displayed an antifungal effect against *P. simplicissimum* and *P. citrinum*, as well as against *A. pullulans* and, at the highest concentration used, against *D. hansenii*. The different percent composition of anise and fennel oils, in which anethole represents 97.1% and 76.3% of the total oil, respectively could help to explain the different biological activity. 1,8-Cineole was effective against all fungal strains, except *P. expansum*. The maximum activity of anethole was recorded against *A. pullulans* (inhibition zone 10 mm). Carvacrol was the most active compound tested. It was highly effective against *A. pullulans* and *D. hansenii*, and, tested at the same concentration used in the antimicrobial assay, it did not permit any growth of almost all *Penicillium* strains tested, in particular against *P. expansum*, the agent of the blue mould which causes one of the principal postharvest diseases in agriculture, and against *P. citrinum*. The genus *Penicillium* is an

important contaminant of foods and agricultural commodities. Many *Penicillium* species are also known producers of a number of very dangerous mycotoxins. *Aureobasidium pullulans* is a saprophyte species distributed widely throughout the environment. Clinically, it has been reported to cause a variety of localized infections, including peritonitis, cutaneous infection, pneumonia, meningitis, corneal and scleral infection, as well as abscesses in the spleen and jaw. *Debaryomyces hansenii* is a hemiascomycetous yeast, often associated with the food and drink processing industries. This strain can be commonly found in freshwater and seawater or as a parasitic, opportunistic organism in humans, fish and vegetable matter [23]. The antifungal activity of caraway oil has also been reported in previous studies, particularly against several *Aspergillus* strains [24]. The activity exhibited by the essential oils against *P. citrinum* is notable due to the well known capability of this fungus to produce the toxic metabolite citrinin, a hepatonephrotoxic mycotoxin involved in different diseases in animals and human [25]. Generally, essential oils can exert their toxic effect against fungi through the disruption of the fungal membrane integrity [26], and, thereby, inhibit respiration and ion transport processes. Citral has been recently used as an ingredient for the production of edible films capable of improving shelf life and food quality by serving as selective barriers against different pathogenic bacteria [27]. The generally high antifungal activity exhibited by the essential oils could indicate, as for the antimicrobial activity, a synergistic interaction among their chemical components.

Data obtained clearly showed the inhibitory activity of the essential oils tested against pathogenic bacterial and fungal strains. On the other hand, these oils showed no inhibitory activity against lactic acid bacteria. These findings, considered together, suggest the future use of these essential oils as natural preservatives for food products, due to their positive effect on their safety and shelf life.

Experimental

Essential oils: Essential oils of *Pimpinella anisum* L., *Carum carvi* L., *Foeniculum vulgare* Miller, *Majorana hortensis* L., and *Verbena officinalis* L. were purchased from the Azienda Chimica E Farmaceutica (A.C.E.F.) Spa (Fiorenzuola d´Arda, Italy). The densities of the oils were: *P. anisum* (0.981g/mL), *C. carvi* (0.913 g/mL), *F. vulgare* (0.964 g/mL), *M. hortensis* (0.903 g/mL), and *V. officinalis* (0.889 g/mL). Anethole, carvacrol, citral, 1,8-cineole, estragole, limonene, linalyl acetate, linalol, α-pinene and β-pinene were purchased from Sigma Aldrich, Co (Milan, Italy). All samples were kept at -20°C until analysis.

Gas chromatography (GC): GC analyses were carried out using a Perkin-Elmer Sigma-115 gas chromatograph with a data handling system and a flame ionization detector (FID). Separation was achieved by a fused-silica capillary column HP-5 MS, 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness. The operating conditions were as follows: injector and detector temperatures, 250°C and 280°C, respectively; oven temperature programme: 5 min isothermal at 40°C, subsequently at 2°C/min up to 250°C and finally raised to 270° at 10°C/min. Analysis was also run by using a fused silica HP Innowax polyethylene glycol capillary column (50 m x 0.20 mm i.d., 0.20 μm film thickness). In both cases, helium was used as the carrier gas (1 mL/min). Diluted samples (1/100 v/v, in *n*-hexane) of 1 μL were manually injected at 250°C, and in the splitless mode. The percentage composition of the oils was determined by normalization of the GC peak areas, calculated as mean values of 3 injections from each oil, without using correction factors.

Gas chromatography–mass spectrometry (GC-MS): GC–MS analysis was performed using an Agilent 6850 Ser. A apparatus, equipped with a fused silica HP-1 capillary column (30 m x 0.25 mm i.d.; film thickness 0.33 μm), linked on line with an Agilent Mass Selective Detector MSD 5973; ionization voltage 70 electrons, multiplier energy 2000 V. Gas chromatographic conditions were as given above, transfer line was kept at 295°C. The oil components were identified from their GC retention indices by comparison with either literature values [28] or with those of authentic compounds available in our laboratories. The identity of the components was assigned by comparing their retention indices, relative to C_8–C_{24} *n*-alkanes under the same operating conditions. Further identification was made by comparison of their MS on both columns with those stored in NIST 02 and Wiley 275 libraries, those from the literature [29], and from an 'in house' library.

Free-radical scavenging method: The free-radical scavenging activity of the essential oils and their main components was measured by using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [30]. The analysis was performed in microplates, by

adding 7.5 µL of sample (previously diluted 1:1 in DMSO) to 303 µL of a methanol solution of DPPH (153 mM). Then, the absorbance was measured in a UV-Vis spectrophotometer (Varian Cary 50 MPR, USA). The absorbance of DPPH radical without antioxidant, i.e., the control, was measured as basis. All determinations were in triplicate. Inhibition of free radical by DPPH in percent (I%) was calculated in following way: I% $[(A_{blank} - A_{sample}/A_{blank})] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound read at 517 nm until 60 min. Tests were carried out in triplicate.

Antimicrobial assay: The inhibition zone test on agar plates was employed to investigate the antimicrobial activity. Samples were tested against the following bacteria: non-pathogenic strains (*Lactobacillus acidophilus* DSM 20079; *L. casei* DSM 9595; *L. bulgaricus* DSM 20081; *L. sakei* DSM 20494; and *L. rhamnosus* DSM 20711); pathogenic Gram-positive strains *Bacillus cereus* (DSM 4313 and DSM 4384), *Staphylococcus aureus* DSM 25923 and *Enterococcus faecalis* DSM 2352; Gram-negative strains *Escherichia coli* DSM 8579 and *Pseudomonas aeruginosa* ATCC 50071. All strains were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ Germany). Each strain was incubated at 37°C for 18 h in its own specific growth medium. Lactic acid bacteria were grown in Man de Rogosa Sharpe (MRS) broth (Oxoid, UK), and *E. coli*, *Ent. faecalis*, *S. aureus*, *P. aeruginosa* and *B. cereus* in Nutrient Broth (Oxoid, UK). The microbial suspensions (1x 10^8 Colony Forming Units-CFU/mL) were uniformly spread onto the specific solid media plates (Ø=90 mm dishes). Sterile Whatman N° 1 paper filter discs (Ø=5 mm) were individually placed on the inoculated plates and impregnated with different doses of either essential oils or of their main compounds, previously diluted 1:10 (v/v) in dimethylsulfoxide (DMSO) (final amount ranging from 84 to 499 µg/paper disc). After 30 min under sterile conditions at room temperature, plates were incubated at 37°C for 24-48 h, depending on the strain. The diameter of the clear zone shown on plates was accurately measured and the antimicrobial activity expressed in mm (not including disc diameter of 5 mm). Sterile deionised water and pure DMSO (10 µL/paper disc) were used as negative control. Gentamycin (8 µg/paper disc), chloramphenicol (66 µg/paper disc) and tetracycline (7 µg/paper disc), in physiological solution, served as positive controls. Samples were tested in triplicate and results are expressed as mean ± standard deviation.

Antifungal activity: The inhibition zone test on agar plates was employed to investigate the antifungal activity of the essential oils and their main compounds. Six fungal strains of agro-food interest, *Penicillium citrinum* DSM 1997, *P. simplicissimum* DSM 1097, *Aureobasidium pullulans* DSM 62074, *P. expansum* DSM 1994, *P. aurantiogriseum* DSM 2429, and *Debaryomyces hansenii* DSM 70238 were used. All strains were purchased from DSMZ. Different amounts of essential oils and their components, previously diluted 1:10 (v/v) in DMSO (final doses ranging from 84 to 499 µg/paper disc), were used. A cell suspension of fungi was prepared in sterile distilled water and plated onto Potato Dextrose Agar (PDA) (Oxoid). Sterile Whatman N° 1 paper filter discs (Ø=5 mm) were individually placed on the inoculated plates and impregnated with different doses of either essential oils or of their main compounds, previously diluted 1:10 (v/v) in dimethylsulfoxide (DMSO) (final amount ranging from 84 to 499 µg/paper disc). After 20 min under sterile conditions at room temperature, plates were incubated at 28°C until the mycelium of fungi reached the edges of the control plate (negative control without the sample added extracts); the resulting clear zones of inhibition were measured in mm, expressing the antifungal activity. DMSO (10 µL) was used as negative control. Samples were tested in triplicate and the results are expressed as mean ± standard deviation.

References

[1] Di Pasqua R, De Feo V, Villani F, Mauriello G. (**2005**) *In vitro* antimicrobial activity of essential oils from Mediterranean Apiaceae, Verbenaceae and Lamiaceae against foodborne pathogens and spoilage bacteria. *Annals of Microbiology*, ***55***, 139-142.

[2] Madsen HL, Bertelsen G. (**1995**) Spices as antioxidants. *Trends in Food Science and Technology*, ***6***, 271–277.

[3] Evandri MG, Battinelli L, Daniele C, Mastrangelo S, Bolle P, Mazzanti G. (**2005**) The antimutagenic activity of *Lavandula angustifolia* (lavender) essential oil in the bacterial reverse mutation assay. *Food and Chemical Toxicology*, ***43***, 1381–1387.

[4] Ames BM. (**1983**) Dietary carcinogens and anticarcinogens: Oxygen radical and degenerative diseases. *Science*, ***221***, 1256–1263.

[5] Burt S. (**2004**) Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, ***94***, 223–253.

[6] Conner DE. (**1993**) Naturally occuring compounds. In *Antimicrobials in foods.* Davidson P, Branen AL (Eds) Marcel Dekker, New York, NY, 441–468.

[7] Demetzos C, Perdetzoglou DK. (**2001**) Composition and antimicrobial studies of the oils of *Origanum calcaratum* Juss. and *O. scabrum* Boiss. et Heldr. from Greece. *Journal of Essential Oil Research*, ***13***, 460–462.

[8] Lambert RJ, Skandamis PN, Coote PJ, Nychas GJ. (**2001**) A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, ***91***, 453-462.

[9] De Martino L, De Feo V, Nazzaro F. (**2008**) Chemical composition and biological activity of some Labiatae essential oils. In: *Proceedings of the 21st International ICFMH Symposium "Food Micro 2008"*, p 488.

[10] Tabanca N, Demirci B, Ozek T, Kirimer N, Can Baser KH, Bedir E, Khand IA, Wedge DE. (**2006**) Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella* species gathered from Central and Northern Turkey. *Journal of Chromatography A*, ***1117***, 194-205.

[11] Singh G, Maurya S, de Lampasona MP, Catalan C. (**2006**) Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. *Food Control*, ***17***, 745-752.

[12] Bailer J, Aichinger T, Hackl G, de Hueber K, Dachler M. (**2001**) Essential oil content and composition in commercially available dill cultivars in comparison to caraway. *Industrial Crops and Products*, ***14***, 229–239.

[13] Omidbaigi R, Bastan MR. (**2005**) Essential oil composition of marjoram cultivated in north of Iran. *Journal of Essential Oil-Bearing Plants*, ***8***, 56-60.

[14] Ardakani MS, Mosaddegh M, Shafaati A. (**2003**) Volatile constituents from the aerial parts of *Verbena officinalis* L. (vervain). *Iranian Journal of Pharmaceutical Research*, ***2***, 39-42.

[15] Gulçin I, Sat IG, Beydemir S, Elmastas M, Kufrevioglu OI. (**2004**) Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). *Food Chemistry*, ***87***, 393–400.

[16] Ruberto G, Baratta MT. (**2000**) Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, ***69***, 167-174.

[17] Aeschbach R, Loliger J, Scott BC, Murcia A, Butler J, Halliwell B, Aruoma OI. (**1994**) Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food and Chemical Toxicology*, ***32***, 31–36.

[18] Yu LL, Zhou KK, Parry J. (**2005**) Antioxidant properties of cold-pressed black caraway, carrot, cranberry, and hemp seed oils. *Food Chemistry*, ***91***, 723-729.

[19] Hammer KA, Carson CF, Riley TV. (**1999**) Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, ***86***, 985-990.

[20] Suppakul P, Miltz J, Sonneveld K, Bigger SW. (**2003**) Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry*, ***51***, 3197-3207.

[21] Ultee A, Bennik MHJ, Moezelaar R. (**2002**) The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, ***68***, 1561-1568.

[22] Dorman HJD, Deans SG. (**2000**) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, ***88***, 308-316.

[23] Maggi RG, Govind NS. (**2004**) Regulated expression of green fluorescent protein in *Debaryomyces hansenii*. *Journal of Industrial Microbiology and Biotechnology*, ***31***, 301-310.

[24] Pawar VC, Thaker VS. (**2006**) *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses*, ***49***, 316–323.

[25] Xu B-J, Jia X-Q, Gu L-J, Sung C-K. (**2006**) Review on the qualitative and quantitative analysis of the mycotoxin citrinin. *Food Control*, ***17***, 271–285.

[26] Knoblock K, Pauli A, Iberl B, Weis N, Weigand H. (**1988**) Antibacterial activity and antifungal properties of essential oil components. *Journal of Essential Oil Research*, ***1***, 119-128.

[27] Rojas-Graü MA, Avena-Bustillos RJ, Olsen C, Friedman M, Henika PR, Martin-Belloso O, Pan Z, McHugh TH. (**2007**) Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. *Journal of Food Engineering*, ***81***, 634-641.

[28] Davies NW. (**1990**) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases. *Journal of Chromatography*, ***503***, 1-24.

[29] Adams RP. (**2001**) *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Allured Publishing, Carol Stream, IL.

[30] Brand-Williams W, Cuvelier ME, Berset C. (**1995**) Use of free radical method to evaluate antioxidant activity. *Food Science and Technology*, ***28***, 25-30.