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Antimicrobial and antiviral effects of essential oils from selected Umbelliferae and Labiatae plants and individual essential oil components

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Abstract: The essential oils obtained from *Anethum graveolens*, *Foeniculum vulgare* collected at fully-mature and flowering stages, *Mentha piperita*, *Mentha spicata*, *Lavandula officinalis*, *Ocimum basilicum* (green- and purple-leaf varieties), *Origanum onites*, *O. vulgare*, *O. munitiflorum*, *O. majorana*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Satureja cuneifolia*, as well as the widely encountered components in essential oils (γ-terpinene, 4-allylanisole, (-)-carvone, dihydrocarvone, *D*-limonene, (-)-phencone, cuminyl alcohol, cuminyl aldehyde, cuminol, *trans*-anethole, camphene, isoborneol, (-)-borneol, *L*-bornyl acetate, 2-decanol, 2-heptanol, methylheptane, farnesol, nerol, isopulegol, citral, citronellal, citronellol, geraniol, geranyl ester, linalool, linalyl oxide, linalyl ester, α-pinene, β-pinene, piperitone, (-)-menthol, isomenthone, carvacrol, thymol, vanillin, and eugenol), were screened for their antiviral activity against *Herpes simplex* type-1 (*HSV-1*) and parainfluenza type-3 (PI-3). Cytotoxicity was expressed as cytopathogenic effect. Most of the oils and compounds displayed strong antiviral effects against *HSV-1*, ranging between 0.8 and 0.025 μg mL^{-1}. However, the samples tested were less effective against PI-3, with results ranging between 1.6 and 0.2 μg mL^{-1}. The essential oil of *A. graveolens* was the most active. Most of the tested oils and compounds exhibited good antibacterial and antifungal effects.

Key words: Essential oil, antiviral, antibacterial, antifungal, cytotoxicity

Seçilmiş Umbelliferae ve Labiatae bitkilerinin uçucu yağları ve tek uçucu yağ bileşiklerinin antimikrobiyal ve antiviral etkileri

Özet: *Anethum graveolens*, tam-olgun ve çiçeklenme dönemlerinde toplanan *Foeniculum vulgare*, *Mentha piperita* ve *M. spicata*, *Lavandula officinalis*, *Ocimum basilicum* (yeşil ve mor-yapraklı varyeteler), *Origanum onites*, *O. vulgare*, *O. munitiflorum* ve *O. majorana*, *Rosmarinus officinalis*, *Salvia officinalis* ve *Satureja cuneifolia*'dan elde edilen uçucu yağlar ve yanı sıra uçucu yağlarda sıklıkla rastlanan bileşenler (γ-terpinen, 4-allilanisol, (-)-karvon, dihidrokarvon, *D*-limonen, (-)-fenkon, kuminil alkol, kuminil aldehit, kuminol, *trans*-anetol, kamfen, izoborneol, (-)-borneol, *L*-bornil asetat, 2-dekanol, 2-heptanol, metilheptan, farnesol, nerol, izopulegol, sitral, sitronellal, sitronellol, geraniol, geranil ester, linalol, linalil oksit, linalil ester, α-pinen, β-pinen, piperiton, (-)-mentol, izomenton, karvakrol, timol, vanilin ve öjenol) *Herpes simplex* tip-1 (*HSV-1*) ve parainfluenza tip-3'e (PI-3) karşı antiviral aktiviteleri için taranmıştır. Sitotoksisite,

sitopatojenik etki olarak ifade edilmiştir. Yağların ve bileşiklerin çoğu, 0,8-0,025 μg mL^{-1} arasında değişmek üzere, *HSV-1*'e karşı yüksek antiviral aktivite göstermiştir. Ancak, test edilen örnekler 1,6-0,2 μg mL^{-1} arasında değişen oranda PI-3'e karşı daha az etkilidirler. *A. graveolens* uçucu yağı en aktiftir. Yağların çoğu iyi antibakteriyel ve antifungal aktivite göstermişlerdir.

Anahtar sözcükler: Uçucu yağ, antiviral, antibakteriyel, antifungal, sitotoksisite

Introduction

Antiviral and antimicrobial drugs are subject to microbial resistance, and this has become a growing public problem all over the world. Therefore, ample research to discover potent new antibiotics is compulsory. Since many essential oils have been reported to possess strong antimicrobial effects (1-3), we examined the antiviral, antibacterial, and antifungal activities of various essential oils obtained from the cultivated plants of Umbelliferae and Labiatae: *Anethum graveolens* L., *Foeniculum vulgare* Mill. (collected in fully-mature and flowering periods), *Mentha piperita* L., *M. spicata* L., *Lavandula officinalis* Chaix ex Villars, *Ocimum basilicum* L. (green- and purple-leaf varieties), *Origanum onites* L., *O. vulgare* L., *O. munitiflorum* Hausskn., *O. majorana* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., and *Satureja cuneifolia* Ten., as well as the widely found essential oil constituents *γ*-terpinene, 4-allylanisole, (-)-carvone, dihydrocarvone, *D*-limonene, (-)-phencone, cuminyl alcohol, cuminyl aldehyde, cuminol, *trans*-anethole, camphene, isoborneol, (-)-borneol, *L*-bornyl acetate, 2-decanol, 2-heptanol, methylheptane, farnesol, nerol, isopulegol, citral, citronellal, citronellol, geraniol, geranyl ester, linalool, linalyl oxide, linalyl ester, α-pinene, β-pinene, piperitone, (-)-menthol, isomenthone, carvacrol, thymol, vanillin, and eugenol. Antiviral activity was tested against the DNA virus *Herpes simplex* type-1 (*HSV-1*) and the RNA virus parainfluenza type-3 (PI-3). Cytotoxicity was determined using Madin-Darby bovine kidney (MDBK) and Vero (African green monkey kidney) cell lines, and their cytopathogenic effects (CPEs) were expressed as maximum nontoxic concentrations (MNTCs). Antibacterial activity of the essential oils and individual components was screened against a number of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*), and they were tested for their antifungal activity against 2 fungi, *Candida albicans* and *C. parapsilosis*, by broth microdilution method.

Materials and methods

Plant materials

The plant materials used in this study were cultivated in 2006 at the experimental farm of the Faculty of Agriculture, Selçuk University, under the ecological conditions of Konya Province (Turkey). The plants were harvested during the 2006 harvest season and used in the experiments.

Individual essential oil components

The pure essential oil components tested in this study were purchased from Carl Roth Chemical Company (Karlsruhe, Germany).

Distillation of the essential oils

Air-dried and powdered samples (100 g) of material from each plant were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the essential oils; the obtained essential oils were used in the activity assays.

Antiviral activity assays

In order to determine the antiviral activity of the samples, *HSV-1*, as a representative of DNA viruses, and PI-3, as a representative of RNA viruses, were used. The isolated strains of the test viruses and the Vero cell line used in this study were obtained from the Department of Virology, Veterinary Faculty, Ankara University (Ankara, Turkey). The cell culture was grown in Eagle's Minimal Essential Medium (EMEM; Seromed, Biochrom, Berlin, Germany) enriched with 10% fetal calf serum (Biochrom), 100 mg mL^{-1} of streptomycin, and 100 IU mL^{-1} of penicillin in a humidified atmosphere of 5% carbon dioxide at 37 °C. All antiviral activity assay conditions were the same as described in our previous study, in which the tissue culture infecting dose ($TCID_{50}$) was calculated (4).

Maximum CPE concentrations, as the indicator of the antiviral activities of the samples, were determined.

Cytotoxicity

The MNTC of each sample was determined based on cellular morphologic alteration as described in our previous study (5). MNTCs were determined by comparing treated and untreated control cultures.

Antibacterial and antifungal activity assays

Antibacterial activity tests were carried out against standard (American Type Culture Collection (ATCC) and Culture Collection of Refik Saydam Central Hygiene Institute (RSKK)) and isolated strains (clinical isolate; Department of Microbiology, Faculty of Medicine, Gazi University). To determine antibacterial activity, the gram-negative strains of *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 10145, *Proteus mirabilis* ATCC 7002, *Klebsiella pneumoniae* RSKK 574, and *Acinetobacter baumannii* RSKK 02026 and the gram-positive strains of *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* ATCC 6633 were used as standards. The isolated strains of *E. coli*, *P. mirabilis*, and *K. pneumoniae* with extended spectrum β-lactamase (ESBL) enzyme and *P. aeruginosa* isolate (resistant to gentamicin), *A. baumannii* isolate (resistant to cephalosporin), gram-positive isolated strains of *S. aureus* (resistant to methicillin; MRSA), *E. faecalis* (resistant to cephalosporin), and *B. subtilis* (resistant to ceftriaxone) were used to determine antibacterial activity. The fungi *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 were used to evaluate antifungal activity. Culture suspensions, stock solutions, and inoculums were prepared according to the methods of the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards (NCCLS)), and all other conditions were applied as described in our earlier studies (6,7).

Preparation of the samples and standards

For stock solution preparation, the samples were dissolved in an ethanol-hexane solution (1:1, v/v) using 1% Tween 80 at the final concentrations of 512 and 1024 μg mL^{-1} and sterilized by filtration (0.22 μm; Millipore, USA). Ampicillin (AMP), gentamicin (GM), ofloxacin (OFX), levofloxacin (LFX), ketoconazole (KET), and fluconazole (FLU) were used as the standard antibacterial and antifungal drugs. Reference antibacterial agents of AMP (Fako), GM (Fako), OFX (Hoechst Marion Roussel), and LFX (Fako) and the reference antifungal agents of KET (Bilim) and FLU (Pfizer) were obtained from their respective manufacturers; they were then dissolved in phosphate buffer solution (AMP; pH 8.0, 0.1 mol mL^{-1}), dimethyl sulfoxide (DMSO) (KET), or in water (GM, OFX, LFX, and FLU). The microdilution method employed for antibacterial and antifungal activity tests according to CLSI methods was applied as described in our previous study (8).

Results and discussion

In the antiviral assays, all of the essential oils and constituents displayed notable inhibition against *HSV-1*, ranging between 0.8 and 0.025 μg mL^{-1} as compared to acyclovir (from 1.6 to <0.012 μg mL^{-1}), while some of them had antiviral activity against PI-3 of between 1.6 and 0.2 μg mL^{-1} (Table 1). However, the tested samples were found to be less effective against PI-3; only the essential oils of *A. graveolens*, *F. vulgare* (fully mature), *M. piperita*, *M. spicata*, *O. munitiflorum*, *O. vulgaris*, and *S. cuneifolia* inhibited this virus. Among the compounds, (-)-carvone, dihydrocarvone, cuminol, cuminyl alcohol, camphene, and carvacrol were not active against PI-3; *L*-bornyl acetate and *D*-limonene were completely inactive against both viruses. The essential oils had almost the same cytotoxicity (0.8-1.6 μg mL^{-1}) against MDBK cells as acyclovir (1.6 μg mL^{-1}); only citral, citronellol, isopulegol, farnesol, linalool, (-)-carvone, piperitone, isomenthone, methylheptane, α-pinene, γ-terpinene, geraniol, and thymol showed MNTC values of 3.2 μg mL^{-1} (Table 1). The essential oils and single components exerted very similar cytotoxicity as the expressed MNTCs on Vero cells (0.8-3.2 μg mL^{-1}) when compared with oseltamivir (1.6 μg mL^{-1}). As seen in Table 2, most of the essential oils and components tested showed remarkable antibacterial and antifungal activity in a wide range.

Many excellent reviews have mentioned the strong antibacterial and antifungal activities of essential oils and their components (9-13). Nevertheless, there have been few reports on the antiviral activity of

Table 1. Antiviral activity and cytotoxicity of the essential oils, pure compounds, and references.

Essential oils	MDBK[a] cells (µg mL^{-1})			Vero cells (µg mL^{-1})		
	MNTC[b] (µg mL^{-1})	CPE[c] inhibitory concentration *HSV-1*[d]		MNTC (µg mL^{-1})	CPE inhibitory concentration PI-3[e]	
		Maximum	Minimum		Maximum	Minimum
Anethum graveolens	1.6	0.8	0.025	1.6	0.8	0.4
Foeniculum vulgare (fully mature)	0.8	0.4	0.2	1.6	0.8	0.4
Foeniculum vulgare (flowering)	1.6	0.8	-[f]	3.2	-	-
Lavandula officinalis	1.6	0.8	-	3.2	-	-
Mentha piperita	0.8	0.4	0.2	1.6	0.8	0.4
Mentha spicata	0.8	0.4	0.2	1.6	0.8	0.4
Ocimum basilicum (green variety)	1.6	0.8	-	3.2	-	-
Ocimum basilicum (purple variety)	1.6	0.8	-	3.2	-	-
Origanum onites	1.6	0.8	-	3.2	-	-
Origanum majorana	1.6	0.8	-	3.2	-	-
Origanum munitiflorum	0.8	0.4	0.2	1.6	0.8	0.4
Origanum vulgaris	0.8	0.4	0.2	1.6	0.8	0.4
Rosmarinus officinalis	1.6	0.8	-	3.2	-	-
Salvia officinalis	1.6	0.8	-	3.2	-	-
Satureja cuneifolia	0.8	0.4	0.2	1.6	0.8	0.4
Components						
Citral	3.2	0.8	0.5	1.6	1.6	0.4
Citronellol	3.2	0.8	0.5	3.2	1.6	0.4
Citronellal	1.6	0.4	0.025	0.8	0.2	-
Isoborneol	1.6	0.8	0.2	1.6	0.8	-
L-Bornyl acetate	-	-	-	-	-	-
(-)-Borneol	1.6	0.8	0.2	1.6	0.4	0.2
Isopulegol	3.2	0.8	0.05	3.2	1.6	0.4
Farnesol	3.2	0.8	0.5	1.6	1.6	0.4
Linalool	3.2	0.8	0.5	3.2	1.6	0.4
Linalyl oxide	1.6	0.4	0.025	0.8	0.2	-
Linalyl ester	0.8	0.4	0.2	0.8	0.1	-
(-)-Carvone	3.2	0.8	0.8	3.2	-	-
Dihydrocarvone	1.6	0.8	-	3.2	-	-
Piperitone	3.2	0.4	0.05	1.6	1.6	0.4
(-)-Menthol	1.6	0.8	0.4	1.6	0.4	0.2
Isomenthone	3.2	0.8	0.5	3.2	1.6	0.4
2-Heptanol	1.6	0.4	0.025	0.8	0.2	-
2-Decanol	1.6	0.4	0.025	0.8	0.2	-
Methylheptane	3.2	0.8	0.5	1.6	1.6	0.4
α-Pinene	3.2	0.8	0.5	1.6	1.6	0.4
β-Pinene	0.8	0.4	0.2	0.8	0.1	-
γ-Terpinene	3.2	0.8	0.05	3.2	1.6	0.4
D-Limonene	-	-	-	-	-	-
Geraniol	3.2	0.8	0.5	1.6	1.6	0.4
Nerol	1.6	0.4	0.025	0.8	0.2	-
Geranyl ester	0.8	0.4	0.2	0.8	0.1	-
(-)-Phencone	0.8	0.4	0.2	0.8	0.2	-
4-Allylanisole	0.8	0.4	0.2	0.8	0.2	-
trans-Anethole	1.6	0.8	0.1	0.8	0.2	-
Cuminyl aldehyde	1.6	0.8	0.1	1.6	0.1	-
Cuminol	1.6	0.8	-	3.2	-	-
Cuminyl alcohol	1.6	0.8	-	3.2	-	-
Camphene	1.6	0.8	0.2	1.6	-	-
Carvacrol	1.6	0.8	-	3.2	-	-
Eugenol	1.6	0.4	0.025	0.8	0.1	-
Vanillin	1.6	0.4	0.1	1.6	0.4	0.2
Thymol	3.2	0.8	0.05	3.2	1.6	0.4
References						
Acyclovir	1.6	1.6	<0.012	-	-	-
Oseltamivir	-	-	-	1.6	1.6	<0.012

[a] MDBK: Madin-Darby bovine kidney; [b] MNTC: maximum nontoxic concentrations; [c] CPE: cytopathogenic effect; [d] *HSV-1*: *Herpes simplex* virus (type-1); [e] PI-3: Parainfluenza (type-3); [f] - : no activity observed.

Table 2. Antimicrobial activity of the essential oils, pure compounds, and references against tested standard and isolated strains of microorganisms expressed as minimum inhibitory concentrations (MICs; μg mL^{-1}).

Essential oils	*E. coli*		*P. aeruginosa*		*P. mirabilis*		*K. pneumoniae*		*A. baumannii*		*S. aureus*		*E. faecalis*		*B. subtilis*		***C. albicans* ATCC 10231**	*C. parapsilosis* **ATCC 22019**
	ATCC 35218	Isolated strain ESBL+	ATCC 10145	Isolated strain	ATCC 7002	Isolated strain ESBL+	RSKK 574	Isolated strain ESBL+	RSKK 02026	Isolated strain	ATCC 25923	Isolated strain MRSA	ATCC 29212	Isolated strain	ATCC 6633	Isolated strain		
A. graveolens	4	64	2	32	4	64	8	128	8	64	1	>128	1	128	1	4	16	8
F. vulgare (fully mature)	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
F. vulgare (flowering)	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
L. officinalis	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
M. piperita	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
M. spicata	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
O. basilicum (green variety)	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
O. basilicum (purple variety)	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
O. onites	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
O. majorana	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
O. munitiflorum	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
O. vulgaris	4	64	2	32	4	64	8	128	8	64	1	>128	1	128	1	4	16	8
R. officinalis	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
S. officinalis	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
S. cuneifolia	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
Compounds																		
Citral	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Citronellol	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Citronellal	4	32	2	64	8	64	8	64	4	32	8	>128	4	128	4	16	8	16
Isoborneol	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	4	8
L-Bornyl acetate	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
(-)-Borneol	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	4	8
Isopulegol	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Farnesol	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
Linalool	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Linalyl oxide	4	32	2	64	8	64	8	64	4	32	8	>128	4	128	8	16	8	16
Linalyl ester	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
(-)-Carvone	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Dihydrocarvone	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
Piperitone	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16

Table 2. (Continued).

Essential oils	*E. coli*		*P. aeruginosa*		*P. mirabilis*		*K. pneumoniae*		*A. baumannii*		*S. aureus*		*E. faecalis*		*B. subtilis*		***C. albicans* ATCC 10231**	*C. parapsilosis* **ATCC 22019**
	ATCC 35218	Isolated strain ESBL+	ATCC 10145	Isolated strain	ATCC 7002	Isolated strain ESBL+	RSKK 574	Isolated strain ESBL+	RSKK 02026	Isolated strain	ATCC 25923	Isolated strain MRSA	ATCC 29212	Isolated strain	ATCC 6633	Isolated strain		
(-)-Menthol	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	8	8
Isomenthone	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
2-Heptanol	4	32	2	64	8	64	8	64	4	32	8	>128	4	128	8	16	8	16
2-Decanol	4	32	2	64	8	64	8	64	4	32	8	>128	4	128	4	16	8	16
Methylheptane	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
α-Pinene	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
β-Pinene	4	64	2	32	4	64	8	128	8	64	1	>128	1	128	1	4	16	8
γ-Terpinene	4	128	2	32	4	128	8	128	2	64	16	>128	8	128	8	16	8	16
D-Limonene	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Geraniol	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
Nerol	4	32	2	64	8	64	8	64	4	32	8	>128	4	128	8	16	8	16
Geranyl ester	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
(-)-Phencone	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
4-Allylanisole	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
trans-Anethole	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
Cuminyl aldehyde	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
Cuminol	16	64	4	64	16	64	8	128	8	64	4	>128	4	128	2	4	16	16
Cuminyl alcohol	4	64	2	32	4	64	8	128	8	64	1	>128	1	128	1	4	16	8
Camphene	4	32	4	32	4	32	4	64	8	64	2	64	2	64	2	64	8	8
Carvacrol	4	64	2	32	4	64	8	128	8	64	4	>128	4	128	2	4	16	8
Eugenol	4	32	2	64	8	64	8	64	4	32	8	>128	4	128	8	16	8	16
V Vanillin	8	64	4	32	8	64	8	64	8	64	2	64	2	64	2	64	4	8
Thymol	8	128	4	32	8	128	8	128	2	64	16	>128	8	128	8	16	8	16
References																		
AMP[a]	2	>128	-	-	2	>128	2	>128	2	>128	<0.12	>128	0.5	>128	0.12	0.5	-	-
GM[b]	-[h]	-	0.5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OFX[c]	0.12	0.5	1	64	<0.12	1	<0.12	0.5	0.12	64	0.25	64	1	32	-	-	-	-
LVX[d]	<0.12	0.5	1	64	<0.12	1	<0.12	1	0.12	64	0.25	128	0.5	32	-	-	-	-
KET[f]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
FLU[g]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4

[a] AMP: ampicillin; [b] GM: gentamicin; [c] OFX: ofloxacin; [d] LFX: levofloxacin; [f] KET: ketoconazole; [g] FLU: fluconazole; [h] -: not tested.

essential oils and individual components. Knowledge of the antimicrobial components in essential oils is of prime importance. In aromatherapy, one of the key points is the discovery of the active molecules that make up the chemotype of essential oils; in most cases there are hundreds in each type of oil. We also tested commercially available pure essential oil components, as phytochemical content is strongly associated with the occurrence of biological activity.

For instance, we demonstrated that 2 fennel oils at the fully mature and flowering stages displayed different degrees of inhibitory effects against the viruses tested (Table 1); this may result from their chemical compositional differences. Chemical composition of the fennel oils was shown to vary drastically during the preripening, ripening, and flowering periods of the plant (14), which is in accordance with our present findings.

In another study by Schuhmacher et al. (15), the essential oil of *M. piperita*, also known as peppermint oil, was tested against *HSV* type-1 and type-2; it showed strong virucidal effects, which is consistent with our data. In a recent study by Oke et al. (16), *S. cuneifolia* essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS); it was dominated by carvacrol (44.99%). However, in our antiviral assay, carvacrol was not as active as *S. cuneifolia* essential oil against the viruses tested. Considering the antiviral activity of the essential oil of the plant, the monoterpenes tested here (borneol and β-pinene) were more active than the essential oil. This finding was also supported by data from a study of the essential oils of eucalyptus, tea tree, and thyme and their major monoterpene compounds: α-terpinene, γ-terpinene, α-pinene, *p*-cymene, terpinen-4-ol, α-terpineol, thymol, citral, and 1,8-cineole (17).

Armaka et al. (18) reported a strong antiviral effect of isoborneol against *HSV-1*, but it did not show significant cytotoxicity on human and monkey cell lines, which is consistent with our results. In another study (19), *Melissa officinalis* essential oil was shown to strongly inhibit *HSV* type-1 and type-2, and the main components in the oil were identified as citral and citronellal. In our assays, citral, citronellal, and citronellol strongly inhibited *HSV* type-1; these components may be contributing to the antiviral effect of the essential oil of *M. officinalis*. On the other hand, the essential oil of *Chrysanthemum trifurcatum* was tested against *HSV* type-1, and it did not exhibit antiviral activity (20). The major constituents of the oil are limonene (20.89%), γ-terpinene (19.13%), 1,8-cineole (10.64%), β-pinene (8.67%), and α-pinene (5.32%), according to GC-MS. We also tested the antiviral effects of limonene against *HSV* type-1 and found it to be completely inactive. The ineffectiveness of oil against *HSV* type-1 could be associated with the ineffectiveness of limonene as the chief component in the oil.

Soković et al. studied the antibacterial effects of several essential oils, some of which we have studied, such as *M. piperita*, *M. spicata*, *O. basilicum*, and *O. vulgaris* (21). They stated that *O. vulgaris* oil and carvacrol displayed the highest activity among the tested materials. In our study, these oils and components were also among the most effective.

Essential oils contain a large number of components, and it is likely that their mode of action involves several targets in the bacterial cell (9,10,13,17). However, in most cases, a synergy exists in the occurrence of antimicrobial activity. In conclusion, these examples show that terpene derivatives found in major quantities in essential oils have the potential to affect the bioactivity of their respective essential oils. Therefore, the chemical composition of essential oils and the amount of individual oil compounds are very important and related to each other in terms of biological activity.

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