Antimicrobial Effect of Essential Oil Isolated from *Eucalyptus globulus* Labill. from Montenegro

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Abstract

Damjanović-Vratnica B., Đakov T., Šuković D., Damjanović J. (2011): **Antimicrobial effect of essential oil isolated from** *Eucalyptus globulus* Labill. **from Montenegro**. Czech J. Food Sci., **29**: 277–284.

Chemical composition of the essential oil of *Eucalyptus globulus* Labill., grown in Montenegro, was analysed by gas chromatography-mass spectrometry and its antimicrobial activity was evaluated against 17 microorganisms, including food poisoning and spoilage bacteria and human pathogens.The *Eucalyptus* essential oil yield was 1.8% (w/w) on the fresh weight basis, whereas the analysis resulted in the identification of a total of 11 constituents, 1.8 cineole (85.8%), α-pinene (7.2%), and β-myrcene (1.5%) being the main components. Other compounds identified in the oil were β-pinene, limonene, α-phellandrene, γ-terpinene, linalool, pinocarveol, terpinen-4-ol, and α-terpineol. The results of the antimicrobial activity tests revealed that the essential oil of *E. globulus* has rather a strong antimicrobial activity, especially against *Streptococcus pyogenes*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. Minimum inhibitory concentration revealed the lowest activity against *Pseudomonas aeruginosa* and *Salmonela infantis* (3.13 mg/ml) while the highest activity was against *S. aureus, E. coli*, and *S. pyogenes* (0.09 mg/ml).

Keywords: *Eucalyptus globulus* Labill.; eucalyptus essential oil; chemical composition; antimicrobial activity

The use of essential oils as functional ingredients in foods, drinks, toiletries, and cosmetics is gaining *momentum*, both for the growing consumers' interest in the ingredients coming from natural sources, and also because of the increasing concern with harmful synthetic additives (Sacchetti *et al.* 2005). Due to their bioactive components, essential oils are indeed promising in view of their use as effective antibacterial, antifungal, and antioxidant agents. With the growing interest in the use of essential oils in both food and pharmaceutical industries, a systematic examination of the plant extracts has become increasingly important (Baratta *et al.* 1998).

The *Eucalyptus* genus comprises over 500 species of aromatic trees and shrubs. The previous research suggests that some species counteract influenza viruses; others are antimalarial or highly active against bacteria (Ody 1994).

Of all Australian eucalypts, *Eucalyptus globulus* Labill., Myrtaceae (Tasmanian Blue Gum) is the species most widely introduced overseas and has been established especially throughout the Mediterranean region (including Montenegro).

Supported by the Ministry of Education and Science, Montenegro, Project No. 05/1-3-3368, 2008.

Eucalyptus species extracts are now entering into common herbal use for the treatment of cold, chest pain, or cough. Eucalyptus leaf extracts have been used to treat influenza, chest problems, and skin rashes while their vapour is inhaled to fight inflammation (Musyimi & Ogur 2008).

They contain great amounts of essential oil (*Oleum eucalypti*), up to 3.5% (w/w), which is used in medicine, aromatherapy, and perfumes (Bremness 2000).

Cytotoxicity of eucalyptus essential oil was evaluated by Schnitzler *et al.* (2001) in a standard neutral red dye uptake assay. The toxicity of eucalyptus oil for RC-37 cells (monkey kidney) approached 50% (TC_{50}) at concentrations of 0.03%.

According to the classification system that allows a prediction of systemic toxicity *in vivo* from the cell culture data (Halle & Gores 1987), the expected systemic toxicity of eucalyptus essential oils can be rated as very little toxic. Clinical studies about the topical use of eucalyptus essential oil demonstrate that it is tolerated well both when inhaled and when applied onto the skin in topical formulations. However, the ingestion of a few milliliters of essential oils may cause severe symptoms of intoxication like vomiting, respiration failure, and unconsciousness and may lead to death, especially when infants are concerned (Woolf 1999).

In addition, the undiluted oils or preparations with high concentrations of essential oils should not be applied to mucous membranes or damaged skin. Riechelmann *et al.* (1997) reported a decrease of ciliary beat frequency in human ciliated respiratory cells when exposed to air concentrations of more than 5 g/m^3 of menthol, eucalyptus oil, or pine needle oil.

In order to evaluate the benefits and risks of essential oils application, it has to be taken into account that they are not primarily used as antibacterial/antiviral agents. When used at concentrations below their MIC, they may as well exert rubefacient, local anaesthetic, spasmolytic, antiphlogistic, secretolytic, or secretomotoric effects, which altogether contribute to their therapeutic efficacy (Reichling *et al.* 2009).

Much work has recently been done on the composition and antimicrobial activity of *Eucalyptus* essential oil (Kumar 1988; Chalchat *et al*. 1995; Zira & Benjilali 1996; Lawrence 1997; Chao *et al.* 2000; Delaquis *et al.* 2002; Viturro 2003; Takahashi *et al.* 2004; Trivedi & Hotchandani 2004; Cermelli *et al*. 2008).

The objectives of this study were: (*i*) the extraction of the essential oil from the leaves of *Eucalyptus globulus* Labill., Myrtaceae (Tasmanian Blue Gum) grown in Montenegro, and the quantification of the yield thereof, (*ii*) the identification and quantification of the compounds in the essential oil obtained, and (*iii*) the determination of its antimicrobial activity against the selected Gram-positive and Gram-negative bacteria and *Candida albicans* fungi by the plate diffusion and broth microdilution methods.

MATERIAL AND METHODS

Collection of herb material. Leaves of *Eucalyptus globulus* Labill., Myrtaceae (Tasmanian Blue Gum), were collected in the southern part of Montenegro (Boka Kotorska Bay), in March 2006, from a single collection site. The leaves were air-dried in the shade at the ambient temperature and stored in double-layer paper bags at the room temperature, protected from the direct light, until further analysis. The identity of the plant specimen was confirmed at the Department of Biology, Faculty of Natural Sciences and Mathematics, University of Montenegro, where the voucher specimen was deposited.

Preparation of herb material. The initial water content present in the herb leaves was found to be 12.7% (w/w) using a Dean and Stark apparatus with *n*-heptane as the reflux solvent. The herb material was milled in a coffee mill and, after sieving, a sample with a mean particle diameter size of 0.9 mm was obtained. The prepared batch was kept in an airtight resealable polypropylene bag and stored at 8°C for maximum of 3 days before use, in order to avoid the losses of the volatile compounds.

Volatile oil preparation. The herb material (20 g) was submitted to hydrodistillation in a Clevenger-type apparatus for 2 h according to Yugoslav Pharmacopoeia IV. The obtained oil was dried over anhydrous sodium sulphate, measured, poured in hermetically sealed dark-glass containers, and stored in a freezer at –4°C until analysed by GC-MS.

Gas chromatography-mass spectrometry (GC-MS). The analyses were carried out using a Shimadzu QP 5050 Gas Chromatograph-Mass Spectrometer with a MDN-5S fused silica column (30 m × 0.32 mm, film thickness 0.25 μm). The column temperature

was programmed from 60°C (2 min) to 280°C at 4°C/minute. The injection port temperature was 260°C while the interface temperature was 290°C. The samples of oil (1 µl), previously dissolved in hexane (Riedel-de Haën, Seelze, Germany), were injected by splitting and the split ratio was adjusted to 1:20. Helium was used as the carrier gas at a flow rate of 0.5 ml/min and 16.8 kPa inlet pressure. The MS conditions were: the ionisation voltage 70 eV, scanning interval 1.5 s, detector voltage 1.3 kV, and *m/z* range 40–500. The components were identified by comparing their mass spectral data with those in the WILEY229 and the NIST107 mass spectra libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature and, whenever possible, by co-injection with the authentic standards (Fluka, Greenford, UK).

Microbial strains. In order to evaluate the activity of the essential oil of *E. globulus*, the following microorganisms were used: as reference strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 10231 (Torlak, Belgrade, Serbia); further, the clinically isolated *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Streptococcus pyogenes, Morganella morganii, Providencia stuartii, Enterobacter cloacae, Acinetobacter baumannii, Citrobacter freundii, Salmonella infantis*, and *Candida albicans.*

The microorganisms were isolated from policlinically treated or hospitalised patients of the Medical Health Centre (Podgorica, Montenegro). The microorganisms were chosen so as to represent several major groups.

Antimicrobial screening. The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oil (NCCLS 1997). Briefly, the above-mentioned bacteria were cultured on the Nutrient broth (Torlak, Belgrade, Serbia) at 37 ± 0.1°C while the fungi was grown on Sabouraud dextrose broth (Merck, Darmstadt, Germany) at 20 ± 0.1°C.

Briefly, each suspension of the tested microorganism (1×10^6 CFU/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with undiluted *E. globulus* essential oil (5 µl, 10 µl, 15 µl, 20 µl, and 30 µl) and placed on the incubated plates.

The plates were kept at room temperature for 30 min and than incubated at 37°C for 20 h (for bacteria strains) and 30°C for 72 h (for fungi). The results reading was carried out by measuring the diameters of the inhibition zones, clear growth in mm. In addition, reference antibiotic discs (provided by the Institute for Serums, Vaccines and Diagnostic Preparations – Torlak, Belgrade, Serbia) such as ampicillin, ceftriaxone, oflaxacin, erythromycin, amykacine, tetracycline, and nystatin were used for comparison under the same conditions as in the essential oil experiment. All tests of the inhibitory activity were carried out in duplicates and the developing inhibition zones were compared with those of reference discs.

The essential oil was also subjected to the sterility test and was found to be free of microorganisms.

Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The broth microdilution method was used to determine MIC and MBC in according to NCCLS (1999) and Yu *et al.* (2004). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the oil were prepared in a 96-well microtiter plate ranged from 0.09 mg/ml to 25.00 mg/ml. To each well, 10 µl of resazurin indicator solution (prepared by dissolving a 270-mg tablet in 40 ml of sterile distilled water) and 30 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve a concentration of 10^4 CFU/ml. Two columns in each plate were used as controls: one column with a broad-spectrum antibiotic as a positive control (amykacine), and one column containing methanol as a negative control. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plated were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The colour change was then assessed visually. The lowest concentration at which the colour change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested oil. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the essential oil at which the incubated microorganism was completely killed.

Assay of in vitro antifungal activity. Broth microdilution assays were performed in accordance with the guidelines given in CLSI document M27-A2 (NCCLS 2000). Briefly, stock solutions were prepared in water for nystatin and in methanol for oil. The final dilution was prepared in RPMI 1640 medium, adjusted to pH 7.0 with 0.165M morpholenepropanesulfonic acid buffer, in the range from 0.09 mg/ml to 25 mg/ml and inoculum size of 10^3 CFU/ml. The growth (drug free) and sterility controls were also included. Microdilution trays were incubated in ambient air at 35°C. MICs were determined visually after 48 h of incubation, as the lowest concentration of the drug that caused no detectable growth. The average of 3 values was calculated giving the MIC and the MBC for the tested oil.

RESULTS AND DISCUSSION

Hydrodistillation of the *E. globules* leaves yielded 1.8% of essential oil (w/w, based on the fresh weight of the mature leaves) with a spicy aromatic odour. The results obtained are similar to those reported in the literature where the yield was 1.3–1.8% (w/w, based on the fresh weight of the young leaves) in Buenos Aires (Bandoni *et al.* 1993), but rather small compared to the results reported in the literature where the yield was 1.9–2.7% (w/w, based on the fresh weight of the young leaves) in Morocco (Zira & Benjilali 1996) and 2.68% (w/w, based on the fresh weight of the adult leaves) in Argentina (Viturro 2003).

The chemical composition of the hydrodistilled eucalyptus essential oil is shown in Table 1. GC-MS analyses revealed the presence of twelve compounds representing 99.77% of the total oil. The major component was 1,8-cineole (85.8%), while α-pinene (7.2%) and β-myrcene (1.5%) were minor main components. Other compounds identified in the oil obtained were β-pinene, limonene, α-phellandrene, γ-terpinene, linalool, pinocarveol, terpinen-4-ol, and α-terpineol. The eucalyptus oil consisted mostly of oxygenated monoterpenes (87.32%) and monoterpene hydrocarbons (12.45%), 1,8-cineole determines the commercial value of the oil and its importance as a raw material for different industries. Different percentages of 1,8-cineole in *E. globules* leaf oil have been reported: 64.5% in Uruguay, 77% in Cuba, 86.7% in California, 58% to 82% in Morocco, 48.7% in Africa, and 50% to 65% in Argentina (Viturro 2003).

Table 1. Chemical composition of the leaf essential oil of *Eucalyptus globulus*

Compound	(%)	RRI	Method of identification*
α-Pinene	7.16	939	1, 2, 3
Camphene	tr	953	2
β-Pinene	1.10	980	1, 2, 3
β-Myrcene	1.52	991	1, 2, 3
α-Phellandrene	0.55	1005	1, 2, 3
p-Cymene	tr	1026	2
Limonene	0.85	1031	1, 2, 3
1.8 Cineole	85.82	1033	1, 2, 3
γ-Terpinene	1.16	1062	1, 2, 3
α-Terpinene	0.11	1088	1, 2, 3
Linalool	0.43	1098	1, 2, 3
Pinocarveol	0.44	1139	2, 3
Terpinen-4-ol	0.49	1177	1, 2, 3
α-Terpineol	0.14	1189	2, 3
Grouped components (%)			
Monoterpene hydrocarbons		12.45	
Oxygenated monoterpenes		87.32	
Total identified		99.77	

*1 – co-injection with authentic compounds; 2 – MS; 3 – literature comparison; tr < 0.05

The antimicrobial activity of *E. globulus* essential oil is due to the presence of a mixture of monoterpenes and oxygenated monoterpenes (most of the antimicrobial activity in the oils has been attributed to the oxygenated monoterpenes). The identification of such compounds with a wide biological activity is critical for the mankind as it helps in the search for chemical structures that should assist in designing new drugs as therapeautic agents against human pathogens.

Antimicrobial activity

The antimicrobial plate diffusion assay for *Eucalyptus globulus* essential oil, as summarised in the Table 2 showed that different microorganisms tested had different susceptibility to the same essential oil. Eucalyptus oil was very potent against all the selected microorganisms except *Salmonella infantis* and *Pseudomonas aeruginosa* (at the lowest

Table 2. Antimicrobial activity of the *E. globulus* essential oil and some standard antibiotics against Gram-positive and Gram-negative bacteria and fungi *Candida albicans*

Microorganism	*E. globulus* oil (µl)					Standard antibiotics (µg)*						
						AMP	CTR	OFL	ER	AMYK	TE	NY
	Inhibition zone (mm)**											
	5	10	15	20	30	10	30	5	15	30	30	100
Gram-positive bacteria												
Streptococcus pyogenes	25	28	32	40	51	46	48	32	41	24	22	
S. aureus ATCC 25923	23	27	34	39	47	28	32	32	33	26	41	
Staphylococcus aureus	22	27	33	39	46	20	14	27	30	25	12	
*C. albica*ns ATCC 10231	15	18	21	41	47							19
Candida albicans	14	16	19	41	46							18
Gram-negative bacteria												
E. coli ATCC 25922	25	30	32	37	49	27	35	37	20	30	29	
Escherichia coli	23	28	32	36	47	–	36	–	16	26	–	
Klebsiella pneumoniae	25	28	33	36	44	15	35	35	15	25	26	
Acinetobacter baumannii	21	25	31	36	45	–	18	–	–	24	–	
Morganella morganii	22	27	32	36	42	–	26	34	12	28	28	
Proteus mirabilis	20	24	26	30	37	30	40	–	–	26	–	
Providencia stuartii	23	26	30	34	40	–	36	–	–	20	–	
Enterobacter cloacae	13	19	24	28	35	–	–	37	14	19	23	
Citrobacter freundii	19	29	22	25	31	26	36	35	–	30	20	
P. aeruginosa ATCC 27853	–	13	17	19	26	–	25	25	19	32	–	
Pseudomonas aeruginosa	–	12	15	17	24	–	16	25	–	22	17	
Salmonella infantis	–	12	14	16	20	39	39	32	15	31	29	

*AMP – ampicillin; CTR – ceftriaxone; ER – erythromycin; AMYK – amykacine; TE – tetracycline; NY – nystatin
**Includes diameter of well and disc (6 and 9 mm, respectively); (–) not active

oil concentrations), whether as clinically isolated strains or as ATCC strains. Since *Pseudomonas* species are known to have the ability to metabolise a wide range of organic compounds and for this fact is used extensively in bioremediation, this may explain their high level of resistance. They may simply metabolise the compounds in the oils that are inhibitory to many of the other bacteria (Chao *et al.* 2000). Because of that fact, the obtained activity of 30 µl of eucalyptus oil against *P. aeruginosa* for the clinically isolated and ATCC strains is noteworthy (24 mm and 26 mm, respectively).

The essential oil activity against the tested microorganisms was increased in both cases (for ATCC strains and clinically isolated strains) with increased amounts of the essential oil investigated.

Outof the tested ATCC strains, *E. globulus* essential oil showed the strongest antimicrobial activity against *S. aureus* ATCC 25923, followed by *E. coli* ATCC 25922 and fungi *C. albicans* ATCC 10231.

Regarding the clinically isolated bacterial strains, the highest inhibition zone values were observed against the medically important pathogens S*treptococcus pyogenes*, *Staphylococcus aureus*, and *Escherchia coli*, ranging from 25 mm to 51 mm, from 22 mm to 48 mm, and from 23 mm to 47 mm, respectively. For comparison the diameters of the growth inhibition zones ranged with the stan-

dard antibiotics used from 18 mm to 24 mm (for *S. pyogenes*), from 12 mm to 30 mm (for *S. aureus*), and from 16 mm to 36 mm (for *E. coli*). A very high antibacterial activity of eucalyptus essential oil was shown against *Acinetobacter baumanii* and *Citrobacter freundii* which were significantly susceptible to the essential oil at concentrations of 20 µl and 30 µl, with significant diameters of the growth inhibition zones (36 mm and 45 mm) and (25 mm and 31 mm), respectively. Also, very susceptible strains were *Enterobacter cloacae* (13 mm to 35 mm), *Providencia stuartii* (23–40 mm), and *Morganella morganii* (22–42 mm), with the measured diameters of the growth inhibition zones of *E. globulus* essential oil larger as compared to those of the tested conventional antibiotics (14–23 mm), (20–36 mm), and (12–28 mm), respectively. *Eucalyptus* oil showed an activity similar to those of the conventional antibiotics against *Klebsiella pneumoniae* and *Proteus mirabilis*: 14–29 mm compared to 25–44 mm, and 20–37 mm compared to 26–40 mm, respectively. Eucalyptus oil showed a noteworthy activity against *Salmonella infantis* only at the concentration of 30 µl, with the diameter of the growth inhibition zone of 20 mm (with the used antibiotics, the diameters of the growth inhibition zones vary from 15 mm to 39 mm).

The oil exhibited a very strong activity against the fungus *Candida albicans* in all concentrations, which may be significant since *C. albicans* invades different parts of the human body causing cutaneous, mucutaneous, and opportunistic infections. In our experiment, the diameters of the growth inhibition zones ranged from 14 mm to 46 mm, giving two times the effect of nystatin. It is probably due to the high amount of 1,8-cineole in the eucalyptus essential oil (over 85%), already known as the component that inhibits the growth of fungi, especially *C. albicans* (Tabanca *et al*. 2001).

The results of the antimicrobial broth microdilution assay are summarised in Table 3. MIC values did not exhibit substantial variations when compared to the trend of inhibition shown with the plate diffusion method. Generally, larger inhibition zone values correlated with lower MIC. Eucalyptus essential oil showed a high activity against a majority of the selected microorganisms. MIC ranged between 0.09 mg/ml and 3.13 mg/ml, with the lowest activity for *P. aeruginosa* and *S. infantis* (3.13 mg/ml) while the highest activity was observed against *S. aureus, E. coli*, and *S. pyogenes* (0.09 mg/ml). The lowest MBC was 0.09 mg/ml for *E. coli* and *S. pyogenes* while *K. pneumoniae* had the highest MBC of 6.25 mg/ml.

Table 3. Antimicrobial activity of *E. globulus* essential oil expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Microorganism	Eucalyptus oil (mg/ml)	
	MIC	MBC
E. coli ATCC 25922	0.09	0.09
Streptococcus pyogenes	0.09	0.09
Escherichia coli	0.09	0.18
Staphylococcus aureus	0.09	0.18
S. aureus ATCC 25923	0.09	0.18
Proteus mirabilis	0.36	1.57
C. albicans ATCC 10231	0.36	0.36
Citrobacter freundii	0.36	0.36
Candida albicans	0.36	0.72
Morganella morganii	0.36	0.72
Providencia stuartii	0.72	0.72
Enterobacter cloacae	0.72	0.72
Acinetobacter baumannii	1.57	1.57
P. aeruginosa ATCC 27853	1.57	3.13
Pseudomonas aeruginosa	3.13	3.13
Salmonella infantis	3.13	3.13
Klebsiella pneumoniae	1.57	6.25

Most of the antimicrobial activity of the essential oils has been attributed to the oxygenated monoterpenes (Knoblach *et al.* 1989; Aggarwal *et al.* 2002). The antimicrobial activity of eucalyptus essential oil could be associated with the presence of 1,8-cineole, linalool, and pinocarveol. 1,8-Cineole as well as linalool are well-known substances with pronounced antimicrobial properties (Tzakou *et al.* 2001; Mourey & Canillac 2002; Viljoen *et al.* 2003).

Also, a number of researchers have shown that limonene is especially effective in inhibiting the proliferation of a variety of microorganisms that cause food spoilage (Aggarwal *et al.* 2002). In addition, the components present in lower amounts in eucalyptus essential oil, such as α-terpineol and terpinen-4-ol, could also contribute to the antimicrobial activity of the oil. It was found that terpinen-4-ol is responsible for the bacteriostatic activity against several microorganisms, especially

against the bacteria that infect the urinary tract (Barel *et al.* 1991). α-Terpineol had been also reported to possess antibacterial activity (Cosentino *et al.* 1999). In fact, it is also possible that the components which are present in lower amounts might be involved in some type of synergism with the other active compounds.

Although the antibacterial activities of the essential oils from many herb species have been extensively surveyed (Rios & Recio 2005), their antimicrobial mechanisms have not been reported in great details. Since the active antimicrobial compounds of essential oils are terpenes and phenolics in nature, it seems reasonable to suppose that their modes of action might be similar to those of other phenolic compounds (Shunying *et al.* 2005). Any individual essential oil contains complex mixtures of such compounds, however, little is known about the effect of the interaction between the individual constituents on the antimicrobial activity. Interactions between the constituents may lead to additive, synergistic, or antagonistic effects (Delaquis *et al.* 2002).

This study has shown that essential oil of *Eucalyptus globulus* Labill., Myrtaceae (Tasmanian Blue Gum) grown in Montenegro possesses rather a significant activity against different microorganisms, including human pathogens, food poisoning and spoilage bacteria, and blastomycete opportunistic fungus *C. albicans*. These results confirm the potential use of *E. globulus* essential oil in the food and pharmaceutical industries, which may be useful as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious diseases. When used at concentrations below its MIC, it may as well exert rubefacient, secretolytic or secretomotoric effects, which altogether contribute to its therapeutic efficacy.

References

Aggarwal K.K., Khanuja S.P.S., Ahmad A., Kumar T.R.S., Gupta V.K., Kumar S. (2002): Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicita* and *Anethum sowa*. Flavour and Fragrance Journal, **17**: 59–63.

Bandoni A., Juarez M., Mizrahi I. (1993): Estudio deesencias »tipo eucaliptol« obtenidas de algunes especies de Eucalyptus de la provincia de Buenos Aires. Anales de S.A.L.P.A., **11**: 171–181.

Baratta M.T., Dorman H.J., Deans S.G., Figueiredo A.C., Barroso J.G., Ruperto G. (1998): Antimicrobial and antioxidant properties of some commercial essential oils. Flavour and Fragrance Journal, **13**: 235–244.

Barel S., Segal R., Yashphe J. (1991): The antimicrobial activity of the essential oil from *Achillea fragrantissima*. Journal of Ethnopharmacology, **33**: 187–191.

Bremness L. (2000): Herbs. Dorling Kindersley, London: 54.

Cosentino S., Tuberoso C.I.G., Pisano B., Satta M., Mascia V., Arzedi E., Palmas F. (1999): *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Letters in Applied Microbiology, **29**: 130–135.

Cermelli C., Fabio A., Fabio G., Quaglio P. (2008): Effect of eucalyptus oil on respiratory bacteria and viruses. Current Microbiology, **56**: 89–92.

Chalchat J.C., Cabard J.L., Gorunović M.S., Đermanović V., Bulatović V. (1995): Chemical composition of *Eucalyptus globulus* oils from the Montenegro coast and east coast of Spain. Journal of Essential Oil Research, **7**: 147–152.

Chao S.C., Young D.G., Oberg C.J. (2000): Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. Journal of Essential Oil Research, **12**: 639–649.

Delaquis P.J., Stanich K., Girard B., Mazza G. (2002): Antimicrobial activity of individual and mixed fractions of dill, cilanto, coriander and eucalyptus essential oils. International Journal of Food Microbiology**, 74**: 101–109.

Halle W., Gores E. (1987): Prediction of LD_{50} values by cell culture. Pharmazie, **42**: 245–248.

Knoblach K., Pauli A., Iberi B., Wegand H., Weis N. (1989): Antibacterial and antifungal properties of essential oil components. Journal of Essentisal Oil Research, **1**: 119–128.

Kumar A. (1988): Antibacterial properties of some eucalyptus oils. Fitoterapia, **59**: 113–123.

Lawrence B.M. (1997): Progress in essential oils. Perfumer and Flavorist, **22**: 49–56.

Mourey A., Canillac N. (2002): Anti-*Lysteria monocytogenes* activity of essential oils components of conifers. Food Control, **13**: 289–292.

Musyimi D.M., Ogur J.A. (2008): Comparative assessment of antifungal activity of extracts from *Eucalyptus globulus* and *Eucalyptus citriodora*. Research Journal of Phytochemistry, **2**: 35–43.

NCCLS (1997): Performance standards for antimicrobial disk susceptibility test. 6th Ed. Approved Standard, M2-A6. National Committee for Clinical Laboratory Standards. Wayne.

NCCLS (1999): Performance standards for antimicrobial susceptibility testing. In: Proceedings 9th International Supplement. M100-S9. National Committee for Clinical Laboratory Standards, Wayne.

NCCLS (2002): Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved Standard M27-A2. 2nd Ed. National Committee for Clinical Laboratory Standards, Wayne.

Ody P. (1994): The Complete Medicinal Herbal. Dorling Kindersley, London: 61.

Riechelmann H., Brommer C., Hinni M., Maartin C. (1997): Response of human ciliated respiratory cells to a mixture of menthol, eucalyptus oil and pine needle oil. Arzneimittelforschung, **47**: 1035–1039.

Reichling J., Schnitzler P., Suschke U., Saller R. (2009): Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties – an overview. Forschende Komplementärmedizin, **16**: 79–90.

Rios J.L., Recio M.C. (2005): Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, **100**: 80–84.

Sacchetti G., Maietti S., Muzzoli M., Scaglianti M., Manfredini S., Radice M., Bruni R. (2005): Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chemistry, **91**: 621–632.

Schnitzler P., Schön K., Reichling J. (2001): Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. Pharmazie, **56**: 343–347.

Shunying Z., Yang Y., Huaidong Y., Yue Y., Guolin Z. (2005): Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. Journal of Ethnopharmacology, **96**: 151–158.

Tabanca N., Kirimer N., Demirci B., Demirci F., Baser K.H.C. (2001): Composition and antimicrobial activity of the essential oils of *Micromeria cristata* subsp. *phyrgia* and the enantiomeric distribution of borneol. Journal of Agricultural and Food Chemistry, **49**: 4300–4303.

Takahashi T., Kokubo R., Sakaino M. (2004): Antimicrobial activities of eucalyptus leaf extracts and falovonoids from eucalyptus from *Eucalyptus maculate*. Letters in Applied Microbiology, **39**: 60–64.

Trivedi N.A., Hotchandani S.C. (2004): A study of the antimicrobial activity of oil of *Eucalyptus*. Indian Journal of Pharmacology, **32**: 93–95.

Tzakou O., Pitarokili D., Chinou I.B., Harvala C. (2001): Composition and antimicrobial activity of the essential oil of *Salvia ringens*. Planta Medica, **67**: 81–83.

Viljoen A., Vuuren S.V., Ernst E., Klepser M., Demirci B., Baser H., Wyk B.E.V. (2003): *Osmitopsis asteriscoides* (Asteraceae) – the antimicrobial and essential oil composition of a Cape-Dutch remedy. Journal of Ethnopharmacology, **88**: 137–143.

Viturro C.I. (2003): Volatile components of *Eucalyptus globulus* Labill. ssp. *bicostata* from Jujuy, Argentina. Journal of Essential Oil Research, **15**: 206–208.

Woolf A. (1999): Essential oil poisoning. Journal of Toxicology - Clinical Toxicology, **37**: 721–727.

Yu J.Q., Lei J.C., Yu H., Cai X., Zou G.L. (2004): Chemical composition and antimicrobial activity of the essential oil of Scutellaria barbata. Phytochemistry, **65**: 881–884.

Zira S.S., Benjilali B.B. (1996): Seasonal changes in the volatile oil and cineole contents of five *Eucalyptus* species growing in Morocco. Journal of Essential Oil Research, **8**: 19–24.

Received for publication May 28, 2009
Accepted after corrections September 10, 2010

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