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RESEARCH ARTICLE

Antibacterial activity of essential oils from *Eucalyptus* and of selected components against multidrug-resistant bacterial pathogens

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Abstract

Context: *Eucalyptus globulus* Labill (Myrtaceae) is the principal source of eucalyptus oil in the world and has been used as an antiseptic and for relieving symptoms of cough, cold, sore throat, and other infections. The oil, well known as 'eucalyptus oil' commercially, has been produced from the leaves. Biological properties of the essential oil of fruits from *E. globulus* have not been investigated much.

Objective: The present study was performed to examine the antimicrobial activity of the fruit oil of *E. globulus* (EGF) and the leaf oils of *E. globulus* (EGL), *E. radiata* Sieber ex DC (ERL) and *E. citriodora* Hook (ECL) against multidrug-resistant (MDR) bacteria. Furthermore, this study was attempted to characterize the oils as well as to establish a relationship between the chemical composition and the corresponding antimicrobial properties.

Materials and methods: The chemical composition of the oils was analyzed by GLC-MS. The oils and isolated major components of the oils were tested against MDR bacteria using the broth microdilution method.

Results: EGF exerted the most pronounced activity against methicillin-resistant *Staphylococcus aureus* (MIC ~ 250 µg/ml). EGF mainly consisted of aromadendrene (31.17%), whereas ECL had citronellal (90.07%) and citronellol (4.32%) as the major compounds. 1,8-cineole was most abundant in EGL (86.51%) and ERL (82.66%).

Discussion and conclusion: The activity of the oils can be ranked as EGF > ECL > ERL ~ EGL. However, all the oils and the components were hardly active against MDR Gram-negative bacteria. Aromadendrene was found to be the most active, followed by citronellol, citronellal and 1,8-cineole.

Keywords: Eucalyptus, essential oil, antimicrobial activity, aromadendrene, 1,8-cineole, multidrug-resistant bacteria

Introduction

Increasing resistance of pathogens toward antibiotics presents a major threat to public health because it reduces the effectiveness of antibiotic treatment, which could lead to an increase in morbidity and mortality. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) are the most important resistant pathogens among Gram-positive bacteria concerning nosocomial infections. Emergence of resistance in Gram-negative bacteria (*Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa*, and *Acinetobacter*) has also been documented. Many antibiotics that have frequently been used in the past have become less effective against these pathogens (Jones, 2001). Hence, there is an urgent need to find alternative antimicrobial agents for the treatment of resistant pathogenic microorganisms.

Many essential oils including those from *Eucalyptus* have been used in folk medicine throughout the world, and their medicinal properties have been investigated (Coppen, 2002). Essential oils from *Eucalyptus* exhibit antibacterial, antifungal, analgesic and anti-inflammatory properties and have also been widely

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used in pharmaceutical, food, and cosmetics products (Ramezani et al., 2002; Silva et al., 2003; Sartorelli et al., 2007).

Eucalyptus globulus Labill (Myrtaceae) is the principal source of eucalyptus oil in the world and has been used as an antiseptic and for relieving symptoms of cough, cold, sore throat and other infections (Van Wyk and Wink, 2004; Kumar et al., 2007). The oil, well known as 'eucalyptus oil' commercially, has been produced from the leaves (Lis-Balchin et al., 1998). Furthermore, biological properties of the essential oil of fruits from *E. globulus* have not been much investigated, whereas the chemical composition of the fruit oil has been studied (Cimanga et al., 2002; Pereira et al., 2005). Instead of *E. globulus,* essential oils from *E. radiata* Sieber ex DC (Myrtaceae) and *E. citriodora* Hook (Myrtaceae) are widely used for aromatherapy. *Eucalyptus radiata* oil has also been shown to be useful for treating disorders of the respiratory system (Coppen, 2002). *Eucalyptus citriodora* is another well-known eucalypt with antibacterial, antifungal, analgesic and anti-inflammatory properties (Lis-Balchin et al., 1998; Silva et al., 2003). However, no report is available on its activity against multidrug-resistant bacteria.

In this investigation, we have examined the antimicrobial activity of the fruit oil of *E. globulus* and the leaf oils of *E. globulus*, *E. radiata* and *E. citriodora* against multidrug-resistant Gram-positive and Gram-negative bacteria. The major components of the oils (aromadendrene, 1,8-cineole, citronellal, and citronellol) were also evaluated. Furthermore, the chemical composition of the oils was reinvestigated by GLC-MS to characterize the oils as well as to establish a relationship between the chemical composition and the corresponding antimicrobial properties.

Materials and methods

Plant material

The fruits of *E. globulus* were kindly provided by Prof. Thomas Efferth. The identity of the plant has been authenticated by Dr. Wahyono at Department of Pharmacognosy, Gadjah Mada University, Indonesia, and the voucher specimen (P6868) was deposited at the Department of Biology, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany.

Essential oils and monosubstances

The dried powder of fruits from *E. globulus* was subjected to hydrodistillation for 6 h using a Clevenger-type apparatus. After separation, the essential oil was kept in separate sealed vials at 4°C for further analysis. Essential oils of the leaves from *E. globulus* (Bergland-Pharma, Heimertingen, Germany), *E. radiata* (Primavera Life, Sulzberg, Germany) and *E. citriodora* (Primavera Life, Sulzberg, Germany) were obtained commercially. (+)-Aromadendrene (≥97% purity), (±)-citronellal (≥80% purity), (±)-citronellol (90–95% purity) were purchased from Fluka, Switzerland, and 1,8-cineole (99% purity) from Sigma-Aldrich (St. Louis, MO).

Microbial strains

The essential oils and their components were tested against multidrug-resistant Gram-positive and Gram-negative bacteria. Gram-positive bacteria: reference strain of MRSA (NCTC 10442), clinical isolates of MRSA (MR134/93, MR131/98, MR 2387/00, MR1150/93, MR 1000/93, MR635/93, MR1678/96, BL7127/98, and USA300), reference strain of VRE (ATCC 51299), and clinical isolates of VRE (VRE902247, VRE902251, VRE902316, and VRE 902267). Gram-negative bacteria: *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC BAA 747. All microorganisms were obtained from the Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg University, Germany. The strains were sub-cultured on Columbia 5% sheep blood agar (Becton Dickinson, Germany) 24 h prior to any antimicrobial test.

Minimal inhibitory concentration and minimal bactericidal concentration determination

The minimal inhibitory concentration (MIC) of the samples was determined by broth microdilution methods (Mulyaningsih et al., 2010a). Briefly, the samples were pipetted into 96-well microtiter plates in Mueller Hinton broth (Fluka, Switzerland) followed by a twofold serial dilution. An inoculum suspension was added to give a final concentration of 5×10^5 cfu/ml. After incubation at 37°C for 24 h, MIC was determined as the lowest concentration without bacterial growth (no color change in MTT assay). The minimal bactericidal concentration (MBC) was determined by subculturing 3 µl from each well without apparent microbial growth on Columbia 5% sheep blood agar and incubated at 36°C for 24 h. The lowest concentration without apparent microbial growth was taken as the MBC. The experiments were performed in duplicate and repeated twice.

Gas liquid chromatography/flame ionization detector

High resolution capillary gas liquid chromatography (GLC) was carried out on a Varian 3400 equipped with flame ionization detector (FID) detector and DB-5 column (30 m × 0.25 mm × 0.25 µm) (Ohio Valley, Marietta, GA). The operating conditions were as follows: carrier gas: helium with a flow rate of 2 ml/min, split ratio 1:20. The oven temperature was programmed with an initial temperature 40°C, 2 min isothermal, 300°C, 4°C/min, then 10 min isothermal. Injector and detector temperatures were set at 250°C and 300°C, respectively. PeakSimple® 2000 chromatography data system (SRI Instruments, Torrance, CA) was used for recording and integration of the chromatograms.

Gas liquid chromatography-mass spectrometry (GLC-MS) analysis

GLC-MS was carried out on a Hewlett-Packard gas chromatograph (GC 5890 II) equipped the same column as GLC (see above). Samples (2 µl) were injected in a split mode (split ratio, 1:15) with the carrier gas helium at a

flow rate of 2 ml/min. The capillary column was coupled to a quadrupole mass spectrometer (SSQ 7000, ThermoFinnigan, Bremen, Germany). The injector temperature was 250°C. Helium carrier gas flow rate was 2 ml/min. All the mass spectra were recorded with the following conditions: electron energy, 70 eV; ion source, 175°C. The oil components were identified by their retention time, retention indices relative to C8-C28 *n*-alkanes, computer matching with the Wiley Registry of Mass Spectral Data 8th edition, NIST Mass Spectral Library (December 2005) and by comparing their mass spectra with data already available in the literature and in our own database (Adams, 2004; Mulyaningsih et al., 2010a).

Results and discussion

Chemical composition of the essential oils

The chemical composition of the fruit oil of *E. globulus* (EGF) was determined by GLC-MS (Table 1), in comparison to the leaf oils from *E. globulus* (EGL), *E. radiata* (ERL), and *E. citriodora* (ECL). About 65 compounds were unambiguously identified, representing 97.02–99.89% of the total oils. Monoterpenoids were predominantly found in the three leaf oils (EGL, ERL, and ECL), whereas sesquiterpenoids were more abundant in the fruit oil (EGF).

EGF contained mainly aromadendrene (31.17%) followed by 1,8-cineole (14.55%), globulol (10.69%), and ledene (7.13%) (Mulyaningsih et al., 2010b). This result was in agreement with a previous study on EGF grown in Portugal (Pereira et al., 2005). However, another report on the EGF had found 1,8-cineole as the main compound (Basias and Saxena, 1984). Some differences can occur in composition of oils from the same plant species probably due to genetic variation and different environmental factors (climate, harvesting seasons, geographical location) (Nishimura and Calvin, 1979; Pereira et al., 2005).

In contrast to EGF, 1,8-cineole (86.51%), α-pinene (4.74%), γ-terpinene (2.57%) and α-phellandrene (1.40%) were the main compounds in EGL, and aromadendrene was only present as a minor compound. EGL is well known to be a 1,8-Cineole-rich oil. The monoterpene 1,8-cineole has been used for medicinal, flavor and fragrance purposes. 1,8-cineole exhibits mosquito repellency (Klocke, 1987), antitumor properties in rats, and anti-inflammatory activity (Santos et al., 2004).

1,8-Cineole (82.66%) occurs as a major compound of ERL (Table 1) followed by α-terpineol (7.03%), and α-pinene (3.68%). *Eucalyptus radiata* oil is sometimes preferred by aromatherapists, because its fragrance is more pleasant than EGL. This oil appears to be useful for treating disorders of the respiratory tract and is known with a high 1,8-cineole content of about 80% (Lis-Balchin et al., 1998). For medicinal purpose, British Pharmacopoeia, European Pharmacopoeia and Chinese Pharmacopoeia specify that eucalyptus oil must contain 1,8-cineole by not less than 70%. In the case of *E. citriodora* oil, it should contain at least 65% citronellal (Coppen, 2002).

Neither aromadendrene nor 1,8-cineole was detected in ECL, but citronellal (90.07%) and citronellol (4.32%) were found to be major constituents. The high abundance of citronellal, the characteristic monoterpene of EGL (lemon-scented *Eucalyptus*), has been reported by previous authors (Silva et al., 2003; Batish et al., 2006). It was noteworthy that linalool, terpinen-4-ol, α-terpineol, piperiton, viridoflorol, and globulol were commonly found 1,8-cineole-rich oils, whereas the monoterpene α-pinene and γ-terpinene were present in all the oils.

Antimicrobial activity of the essential oils and the major components of the oils

The antimicrobial activities of the four essential oils and their major components (aromadendrene, 1,8-cineole, citronellal and citronellol) against multidrug-resistant bacteria are summarized in Table 2 and 3. EGF exerted a powerful activity against MRSA strains with MIC values between 250 and 1000 μg/ml. All VRE strains were inhibited by EGF with MIC values of 500–1000 μg/ml. Against MRSA and VRE, the antimicrobial activity of aromadendrene was stronger than that of 1,8-cineole. It seems that the antimicrobial activity of EGF can be attributed to aromadendrene. This compound has a reactive exocyclic methylene group and a cyclopropane ring which can alkylate proteins and thereby disturb the conformation of proteins. Additionally, since the compound is highly lipophilic, it can disrupt the fluidity and permeability of biomembranes (Sikkema et al., 1995; Wink, 2008).

In contrast to aromadendrene, the tested microorganisms were not inhibited by 1,8-cineole up to concentration 8000 μg/ml (Mulyaningsih et al., 2010b). This result was in agreement with previous studies (Inouye et al., 2001; Aridogan et al., 2002). The leaf oils with high 1,8-cineole contents (EGL and ERL) showed moderate activities against MRSA (MIC value of EGL: 2000 to >4000 μg/ml and of ERL: ≥4000 μg/ml). However, 1,8-cineole has been used as a flavor and fragrance purpose and for medicinal purposes. 1,8-Cineole works as a mosquito repellency (Klocke, 1987) and shows antitumor activity in rats and anti-inflammatory properties (Santos et al., 2004).

ECL which contains predominantly citronellal and citronellol exhibited anti-MRSA activity with MIC values ranging from 1000 to >4000 μg/ml. It was surprising that citronellal had lower activity than citronellol although it is more reactive because of its aldehyde group. Secondary metabolites with aldehyde groups normally possess good antibacterial activity through alkylation of amino group of proteins and DNA (Wink, 2008). Citronellol which bears a polar hydroxyl group may disturb protein conformation and cell membrane fluidity (Pelczar et al., 1988). The lower activity of citronellal might be due to its relatively high evaporation rate which would decrease the amount of citronellal in the assay (Lertsatitthanakorn et al., 2008). A number of studies had confirmed that citronellal was a weakly active monoterpene, whereas citronellol was evaluated to be more active than citronellal as an antifungal agent. A previous study reported a synergistic effect in

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Table 1. Composition of the essential oil of fruits from *Eucalyptus globulus* and from *Eucalyptus* leaves.

No	Constituents	RI (DB-5)	Relative abundance (%)			
			EGF[§]	EGL	ECL	ERL
1	α-Thujene	903	—	—	0.46	—
2	α-Pinene	925	1.53	4.74	0.38	3.68
3	Sabinene	968	—	—	0.58	1.40
4	β-Pinene	970	—	0.88	0.91	—
5	β-Myrcene	989	—	0.88	0.15	—
6	α-Phellandrene	1002	2.61	1.40	—	—
7	*p*-Cymene	1024	0.49	—	—	—
8	Limonene	1027	—	—	0.5	—
9	**1,8-Cineole**	1030	14.55	86.51	—	82.66
10	γ-Terpinene	1057	0.18	2.57	0.25	Tr
11	*p*-Menth-3,8-diene	1069	—	—	0.13	—
12	Terpinolene	1085	—	0.77	0.1	Tr
13	τ-Terpinene	1087	0.18	—	—	—
14	Isoterpinolene	1098	0.27	—	—	—
15	Linalool	1111	0.12	Tr	—	Tr
16	**Citronellal**	1160	—	Tr	90.07	—
17	Isopulegol	1177	—	Tr	tr	—
18	Carvenone	1164	0.07	—	—	—
19	Borneol	1166	0.41	0.09	—	—
20	Terpinen-4-ol	1176	1.87	0.69	—	1.53
21	α-Terpineol	1189	0.85	0.50	—	7.03
22	Sabinol	1199	1.14	—	—	—
23	Citronellol	1227	—	0.16	4.32	Tr
24	*p*-Ment-1(7)-en-2-one	1231	0.62	—	—	—
25	Piperiton	1249	0.31	Tr	—	Tr
26	Geraniol	1252	Tr	—	—	—
27	Thymol	1302	Tr	—	—	—
28	Exo-2-hydroxycineole acetate	1338	0.14	—	—	—
29	α-Terpinyl acetate	1348	1.27	—	—	Tr
30	Citronellyl acetate	1351	—	—	Tr	—
31	Geranyl acetate	1373	0.20	—	—	—
32	Isoledene	1378	0.81	—	—	—
33	Tricyclo(6.3.0.0(2.4))undec-8-ene-3,3,7,11-tetramethyl	1396	0.18	—	—	—
34	α-Gurjunene	1412	5.10	0.11	—	—
35	β-Caryophyllene	1418	—	—	1.46	—
36	*Aromadendrene*	1446	31.17	0.41	—	Tr
37	α-Caryophyllene	1452	—	—	Tr	0.04
38	*allo*-aromadendrene	1466	3.68	0.16	—	Tr
39	γ-Gurjunen	1476	0.70	—	—	—
40	α-Selinene	1490	0.84	—	—	—
41	Longifolene	1493	1.75	—	—	—
42	Ledene	1504	7.13	Tr	—	—
43	γ-Cadinene	1518	0.24	—	—	—
44	Dehydroaromadendrene	1526	0.75	—	—	—
45	δ-Cadinene	1543	0.64	—	Tr	0.55
46	α-Calacorene	1555	0.16	—	—	—
47	Epiglobulol	1566	5.17	Tr	—	—
48	Spathulenol	1567	—	Tr	—	Tr
49	Caryophyllen oxide	1572	—	—	—	—
50	Palustrol	1581	0.22	—	—	—
51	Viridiflorol	1593	0.24	Tr	—	Tr
52	Globulol	1595	10.69	Tr	—	Tr
53	τ-Eudesmol	1600	1.24	—	—	—

Table 1. Continued on next page

Table 1. Continued.

No	Constituents	RI (DB-5)	Relative abundance (%) EGF[§]	EGL	ECL	ERL
54	Guaiol	1607	0.79	—	—	—
55	β-Eudesmol	1611	0.31	—	—	—
56	Cubenol	1616	0.11	—	—	—
57	Sesquiterpene alcohol	1627	0.55	—	—	—
58	τ-Cadinol	1631	0.17	—	—	—
59	α-Eudesmol	1657	0.18	—	—	—
	Monoterpene hydrocarbons		4.77	11.24	3.46	5.10
	Oxygenated monoterpenes		21.58	87.96	94.41	91.26
	Sesquiterpene hydrocarbons		52.15	0.68	1.49	0.62
	Oxygenated sesquiterpenes		19.67	0.01	0.01	0.04
	Total identified		98.17	99.89	99.37	97.02

Relative abundance of the components are given as % (total peak area = 100%).
[§]Previously reported (Mulyaningsih et al., 2010b).
Tr: trace amount, less than 0.05%; —: not detected.
EGF, *E. globulus* fruits; EGL, *E. globulus* leaves; ECL, *E. citriodora* leaves; ERL, *E. radiata* leaves; RI, retention index.

Table 2. The antimicrobial activity of the essential oils of *Eucalyptus* against multidrug-resistant bacteria.

Microorganisms	ECL MIC	ECL MBC	ERL MIC	ERL MBC	EGL MIC	EGL MBC	EGF[§] MIC	EGF[§] MBC
Gram-positive								
MRSA								
MRSA NCTC 10442*	2	4	4	>4	4	>4	0.25	0.06
USA300	2	4	>4	NT	>4	NT	0.5	1
1678/98	4	>4	>4	NT	>4	NT	0.125	0.5
635/93	2	4	>4	NT	4	>4	0.25	0.25
2387/00	2	4	4	>4	4	>4	0.12	0.12
1000/93	1	2	4	>4	2	4	0.12	0.25
BL7127/98	4	>4	>4	NT	4	>4	0.5	0.5
MR131/98	4	>4	>4	NT	>4	NT	0.5	0.5
MR134/93	>4	NT	>4	NT	4	>4	0.5	2
MR1150/93	>4	NT	>4	NT	>4	NT	1	2
VRE								
VRE *E. faecalis* ATCC 51299*	>4	NT	>4	NT	>4	NT	1	2
VR902291	>4	NT	>4	NT	>4	NT	0.5	0.5
VR902316	>4	NT	>4	NT	>4	NT	0.5	0.5
VR902247	>4	NT	>4	NT	>4	NT	1	2
VR902267	>4	NT	>4	NT	>4	NT	1	2
Gram-negative								
*Escherichia coli**	>4	NT	>4	NT	>4	NT	8	NA
*Pseudomonas aeruginosa**	>4	NT	>4	NT	>4	NT	>8	NT
*Klebsiella pneumonia**	>4	NT	>4	NT	>4	NT	>8	NT
*Acinetobacter baumannii**	2	2	1	1	2	2	1	1

[§]Previously reported (Mulyaningsih et al., 2010b).
*Reference strain.
Concentrations are given in mg/ml; NT: not tested.
EGF, *Eucalyptus globulus* fruits; EGL, *E. globulus* leaves; ECL, *E. citriodora* leaves; ERL, *E. radiata* leaves; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration.

a combination of citronellol and citronellal which would make the oil more active (Low et al., 1974).

The four essential oils of *Eucalyptus* were hardly active against multidrug-resistant Gram-negative bacteria, except *Acinetobacter baumannii* (MIC of EGF, EGL, ERL, ECL was 1000, 2000, 1000, 2000 μg/ml, respectively). The low susceptibility of Gram-negative bacteria is probably due to the presence of an outer lipopolysaccharide membrane which acts a physical barrier to lipophilic compounds including essential oils (Vaara, 1992). Moreover, Tegos et al. reported that the low activity of lipophilic plant compounds might be due to MDR efflux through by ABC transporter (Tegos et al., 2002). *Pseudomonas aeruginosa* was reported to have a MexAB-OprM pump, whereas

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Table 3. The antimicrobial activity of the major components of the *Eucalyptus* oils against multidrug-resistant bacteria.

	Aromadendrene[§]		1,8-Cineole[§]		Citronellal		Citronellol	
Microorganisms	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive								
MRSA								
MRSA NCTC 10442*	0.5	0.5	>8	NT	2	2	1	2
USA300	1	4	>8	NT	8	>8	2	2
1678/98	0.25	0.5	>8	NT	1–2	2	0.125	0.125
635/93	0.25	0.25	>8	NT	>8	>8	8	8
2387/00	0.25	0.5	>8	NT	8	8–>8	2	4
1000/93	0.25	0.25	>8	NT	>8	>8	4	4
BL7127/98	1	1	>8	NT	8	>8	1–2	4
MR131/98	0.5	1	>8	NT	0.5–1	1–2	0.125	0.5
MR134/93	1	4	>8	NT	8	>8	4	8
MR1150/93	1	2	>8	NT	8	>8	4	8
VRE								
VRE *E. faecalis* ATCC 51299*	1	2	>8	NT	>8	>8	2	4
VR902291	1	2	>8	NT	8–>8	>8	8	4
VR902316	1	2	>8	NT	>8	>8	8	8
VR902247	1	4	>8	NT	>8	>8	8	8
VR902267	1	4	>8	NT	8–>8	>8	8	8
Gram-negative								
Escherichia coli	>8	NT	>8	NT	>8	>8	4	8
Pseudomonas aeruginosa	>8	NT	>8	NT	>8	>8	>8	>8
Klebsiella pneumonia	>8	NT	>8	NT	>8	>8	>8	>8
Acinetobacter baumannii	2	2	8	8	2–4	4	0.125–0.25	0.25–0.5

[§]Previously reported (Mulyaningsih et al., 2010b).
*Reference strain.
Concentrations are given in mg/ml; NT: not tested.
EGF, *Eucalyptus globulus* fruits; EGL, *E. globulus* leaves; ECL, *E. citriodora* leaves; ERL, *E. radiata* leaves; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration.

E. coli posses an AcrAB multidrug efflux pump (Ma et al., 1993). Multidrug efflux pumps were also reported from Enterobacteriaceae including efflux pumps that remove antibiotics as well as other lipophilic noxious substances from cells (Schweizer, 2003). *Acinetobacter baumannii* is an important cause of nosocomial infections mainly affecting immunocompromised patients and shows an outstanding ability to rapidly evolve resistance to new antibiotics (Joly-Guillou, 2005). *Acinetobacter baumannii* was more susceptible to the essential oils than the other tested bacteria probably due to the difference of its outer membrane from Enterobacteriaceae (Scott et al., 1976). Our results indicate that the *Eucalyptus* oils can be a good source of antibacterial agents particularly against *A. baumannii*.

Among the four oils tested, the antimicrobial activity of the oils can be ranked as EGF > ECL > ERL > EGL. Previous studies showed that *E. globulus* oil (91% 1,8-cineole) was less antibacterial than *E. radiata* (84% 1,8-cineole), whereas *E. citriodora* oil exhibited higher antifungal activity (Lis-Balchin et al., 1998; Ramezani et al., 2002). Regarding the activity of the major component of the oils, aromadendrene has shown the most active substance followed by citronellol, citronellal and 1,8-Cineole.

Our previous investigation demonstrated synergistic properties of combinations of aromadendrene and 1,8-cineole from the essential oil of EGF (Mulyaningsih et al., 2010b). For future work, it might be interesting to use the essential oils alone or in combinations with other antibacterial drugs to treat patients with nosocomial infections.

Conclusion

Our results indicate that the EGF oils are promising to be an antibacterial agent. The chemical composition of oils apparently determines the antimicrobial property. The major components will certainly contribute to the antimicrobial activity; however, minor components should also be considered because they can produce a synergistic, additive or even antagonistic interaction.

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Declaration of interest

The authors report no declarations of interest.

References

Adams RP (2004). Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Carol Stream: Allured Pub Corp.

Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E. (2002). Antimicrobial activity and chemical composition of some essential oils. *Arch Pharm Res*, 25, 860–864.

Basias R, Saxena S. (1984). Chemical examination of essential oil from the fruits of *Eucalyptus globulus* Labill. *Herba Hungarica*, 23, 21–23.

Batish DR, Singh HP, Setia N, Kaur S, Kohli RK. (2006). Chemical composition and inhibitory activity of essential oil from decaying leaves of *Eucalyptus citriodora*. *Z Naturforsch, C, J Biosci*, 61, 52–56.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totté J, Pieters L, Vlietinck AJ. (2002). Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol*, 79, 213–220.

Coppen JJW. (2002). *Eucalyptus, The Genus Eucalyptus*. London: Taylor and Francis.

Inouye S, Takizawa T, Yamaguchi H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *J Antimicrob Chemother*, 47, 565–573.

Joly-Guillou ML. (2005). Clinical impact and pathogenicity of Acinetobacter. *Clin Microbiol Infect*, 11, 868–873.

Jones RN. (2001). Resistance patterns among nosocomial pathogens: trends over the past few years. *Chest*, 119, 397S–404S.

Klocke JA. (1987). 1,8-cineole (Eucalyptol), a mosquito feeding and ovipositional repellent from volatile oil of *Hemizonia fitchii* (Asteraceae). *J Chem Ecol*, 13, 2131–2141.

Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. (2007). Ethnopharmacological approaches to wound healing–exploring medicinal plants of India. *J Ethnopharmacol*, 114, 103–113.

Lertsatitthanakorn P, Taweechisupapong S, Aromdee C, Khunkitti W. (2008). Antibacterial activity of citronella oil solid lipid particles in oleogel against Propionibacterium acnes and its chemical stability. *Int J Essent Oil Ther*, 2, 167–171.

Lis-Balchin M, Deans SG, Eaglesham E. (1998). Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour Frag J*, 13, 98–104.

Low D, Rawal BD, Griffin WJ. (1974). Antibacterial action of the essential oils of some *Australian Myrtaceae* with special references to the activity of chromatographic fractions of oil of *Eucalyptus citriodora*. *Planta Med*, 26, 184–185.

Ma D, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. (1993). Molecular cloning and characterization of acrA and acrE genes of *Escherichia coli*. *J Bacteriol*, 175, 6299–6313.

Mulyaningsih S, Youns M, El-Readi MZ, Ashour ML, Nibret E, Sporer F, Herrmann F, Reichling J, Wink M. (2010a). Biological activity of the essential oil of *Kadsura longipedunculata* (Schisandraceae) and its major components. *J Pharm Pharmacol*, 62, 1037–1044.

Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. (2010b). Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine*, 17, 1061–1066.

Nishimura H, Calvin M. (1979). Essential oil of *Eucalyptus globulus* in California. *J Agric Food Chem*, 27, 432–435.

Pelczar MJ, Chan ECS, Krieg NR. (1988). Control of microorganisms by physical agents. In *Microbiology*. New York: McGraw-Hill International, 469–509.

Pereira SI, Freire CSR, Neto CP, Silvestre AJD, Silva AMS. (2005). Chemical composition of the essential oil distilled from the fruits of *Eucalyptus globulus* grown in Portugal. *Flavour Frag J*, 20, 407–409.

Ramezani H, Singh HP, Batish DR, Kohli RK. (2002). Antifungal activity of the volatile oil of *Eucalyptus citriodora*. *Fitoterapia*, 73, 261–262.

Santos FA, Silva RM, Campos AR, De Araújo RP, Lima Júnior RC, Rao VS. (2004). 1,8-cineole (eucalyptol), a monoterpene oxide attenuates the colonic damage in rats on acute TNBS-colitis. *Food Chem Toxicol*, 42, 579–584.

Sartorelli P, Marquioreto AD, Amaral-Baroli A, Lima ME, Moreno PR. (2007). Chemical composition and antimicrobial activity of the essential oils from two species of Eucalyptus. *Phytother Res*, 21, 231–233.

Schweizer HP. (2003). Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. *Genet Mol Res*, 2, 48–62.

Scott CCL, Makula RA, Finnerty WR. (1976). Isolation and characterization of membranes from a hydrocarbon-oxidizing Acinetobacter sp. *J Bacteriol*, 127l, 469–480.

Sikkema J, de Bont JA, Poolman B. (1995). Mechanisms of membrane toxicity of hydrocarbons. *Microbiol Rev*, 59, 201–222.

Silva J, Abebe W, Sousa SM, Duarte VG, Machado MI, Matos FJ. (2003). Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *J Ethnopharmacol*, 89, 277–283.

Tegos G, Stermitz FR, Lomovskaya O, Lewis K. (2002). Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. *Antimicrob Agents Chemother*, 46, 3133–3141.

Vaara M. (1992). Agents that increase the permeability of the outer membrane. *Microbiol Rev*, 56, 395–411.

Van Wyk E, Wink M. (2004). *Medicinal Plants of the World*. Portland: Timber Press, Inc.

Wink M. (2008). Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. *Curr Drug Metab*, 9, 996–1009.

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