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Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics

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Keywords
bacteriostatic and bactericidal activity, *Cinnamomum verum* bark, essential oil, nosocomial strains.

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Abstract

Aims: To compare the bacteriostatic and bactericidal activity of 13 chemotyped essential oils (EO) on 65 bacteria with varying sensitivity to antibiotics.
Methods and Results: Fifty-five bacterial strains were tested with two methods used for evaluation of antimicrobial activity (CLSI recommendations): the agar dilution method and the time-killing curve method. EO containing aldehydes (*Cinnamomum verum* bark and *Cymbopogon citratus*), phenols (*Origanum compactum*, *Trachyspermum ammi*, *Thymus satureioides*, *Eugenia caryophyllus* and *Cinnamomum verum* leaf) showed the highest antimicrobial activity with minimum inhibitory concentration (MIC) <2% (v/v) against all strains except *Pseudomonas aeruginosa*. Alcohol-based EO (*Melaleuca alternifolia*, *Cymbopogon martinii* and *Lavandula angustifolia*) exhibited varying degrees of activity depending on Gram status. EO containing 1·8-cineole and hydrocarbons (*Eucalyptus globulus*, *Melaleuca cajeputii* and *Citrus sinensis*) had $MIC_{90\%} \geq 10\%$ (v/v). Against *P. aeruginosa*, only *C. verum* bark and *O. compactum* presented MIC ≤2% (v/v). *Cinnamomum verum* bark, *O. compactum*, *T. satureioides*, *C. verum* leaf and *M. alternifolia* were bactericidal against *Staphylococcus aureus* and *Escherichia coli* at concentrations ranging from to 0·31% to 10% (v/v) after 1 h of contact. *Cinnamomum verum* bark and *O. compactum* were bactericidal against *P. aeruginosa* within 5 min at concentrations <2% (v/v).
Conclusions: *Cinnamomum verum* bark had the highest antimicrobial activity, particularly against resistant strains.
Significance and Impact of the Study: Bacteriostatic and bactericidal activity of EO on nosocomial antibiotic-resistant strains.

Introduction

Antibiotic-resistant bacteria continue to be a major health concern worldwide, in particular methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas aeruginosa*, *Acinetobacter* and enterobacteria producing extended-spectrum ß-lactamase (ESBL). Several studies have documented the antimicrobial activity of essential oils (EO), in particular on susceptible bacteria, by determining their minimum inhibitory concentration (MIC). Agar dilution methods are widely used to determine the MIC of EO, but the ability to compare data from different studies is limited owing to differences in test methodologies, in particular concerning the use of solubilization agent (Gustafson *et al.* 1998; Mann and Markham 1998; Hammer *et al.* 1999, 2003; Banes-Marshall *et al.* 2001; Prabuseenivasan *et al.* 2006). The aim of this study was to evaluate the bacteriostatic and bactericidal activity of 13 EO, respectively, using the agar dilution method and the time-killing curve method, on 65 bacterial strains having varying sensitivity to antibiotics.

Materials and methods

Bacteria

Sixty-five bacterial strains, both ATCC (26) and clinical (39), were examined: 34 strains with low-level resistance to antibiotics [two *Escherichia coli*, one *Salmonella typhimurium*, one *Salmonella enteritidis*, one *Citrobacter koseri*, one *Klebsiella pneumoniae*, one *Hafnia alvei*, one *Vibrio cholerae*, two *Pasteurella multocida*, one *Bordetella bronchisepta*, one *Branhamella catarrhalis*, two methicillin-susceptible *S. aureus*, five methicillin-susceptible coagulase-negative *Staphylococcus*, 11 *Streptococci* (of which two *Streptococci pyogenes*), one *Enterococcus faecalis*, one *Listeria monocytogenes*, two *Corynebacterium* spp.] and 31 strains with marked resistance to antibiotics (seven penicillinase-producing enterobacteria, ESBL or AmpC, or overproducing OXY-1 β-lactamase: two *E. coli*, two *Enterobacter cloacae*, two *Citrobacter* spp. and one *Klebsiella oxytoca*, one *Vibrio cholerae* with carbenicillinase 6, four *Acinetobacter baumanii* with imipenemase or VEB1, two *Stenotrophomonas maltophilia*, two *Aeromonas hydrophila*, two *P. aeruginosa*, including one with ESBL, four MRSA, two glycopeptide-intermediate *S. aureus* (GISA and hetero-GISA), one methicillin-resistant *Staphylococcus epidermidis*, one penicillin-resistant *Streptococcus pneumoniae*, five VRE: three *Enterococcus faecium van-A* and *van-B*, one *Enterococcus gallinarum* and 1 *Enterococcus casseliflavus*). Identification of clinical strains was made using the API® system (bioMerieux, Marcy l'Etoile, France).

EO

A total of 13 EO, supplied by Pranarôm (International S.A., Ghislenghien, Belgium) were tested. The EO were extracted by low-pressure hydrodistillation without chemical descalers. The EO analyses were carried out by gas chromatography–mass spectrometry (GC–MS) using a Hewlett-Packard GCD system. An HP-Innowax capillary column (60 m × 0·25 mm, 0·5-μm film thickness) was used with helium as the carrier gas at a flow rate of 22–25 psi. The GC oven temperature was held at 50°C for 6 min, and programmed to increase by 2°C per min till 250°C and then held at this temperature for 20 min. The injector and detector temperatures were 250 and 280°C, respectively; injection was in split mode and the injected volume comprised 1 μl of a 5/100 solution of the oil in hexane. Automatic calibration of masses by auto-tuning was used in MS. The mass range was from *m/z* 30 to 350. A library search was carried out on a combination of the NBS library and 75 000 spectra provided with software for spectral interpretation from the NIST Scientific and Technical database and a personal aromatic library. Table 1 lists only the major components of each of the oils obtained by gas chromatography.

Bacteriostatic activity

The EO were diluted in sterile 0·15% agar solution (Difco™ Agar Granulated; Becton Dickinson) to ensure dissolution in an aqueous medium. The powdered agar was solubilized in heated distilled water. MIC were determined, in duplicate, using an agar-dilution method based on the CLSI guidelines (Anon. 2006). Twofold serial dilutions of each EO ranging from 50% to 0·1% (v/v) were prepared in agar solution 0·15% (v/v). Dilute and pure EO (4 ml) were added to sterile molten MH (Mueller–Hinton; Becton Dickinson®), with or without sheep blood (36 ml), giving final concentrations in the range of 0·01–10%. Inoculation was performed by means of spots containing 10^4 CFU per spot using a multi-inoculator device. MH broth with 0·15% agar solution was used as a positive growth control. The inoculated plates were incubated at 37°C for 48 h. MIC were taken as the lowest concentrations of oil inhibiting visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded.

Bactericidal activity

Killing curves for cinnamon bark, oregano, thyme, cinnamon leaf and tea tree oils were prepared at 37°C on clinical strains of MRSA ATCC 43300, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027 and *S. pyogenes*. Killing curves were prepared in duplicate using the standardized method described by Courvalin *et al.* (2006). One millilitre of EO dilution was added to 9 ml of broth, MH or Todd Hewitt broth, for *S. pyogenes* (Becton Dickinson®), containing an inoculum of around 10^7 CFU ml^{-1} of bacteria. A growth control in 1 ml of agar solution was studied simultaneously. The surviving colonies were counted at fixed times (5, 15, 30 min, 1, 2, 4, 6, 24 and 24 h). In order to eliminate carry-over of EO, 10-fold dilution series were prepared for each broth in sterile distilled water, and 100 μl of these dilutions and the initial broth were then removed and placed in MH agar (Becton Dickenson®) for *E. coli*, *S. aureus* and *P. aeruginosa*, and in blood agar (Becton Dickinson®) for *S. pyogenes*. A bactericidal effect was defined as a 5_{log} reduction for the initial inoculum within 1 h, as recommended by the French Standards Association (AFNOR) (Anon. 1989).

Results

Bacteriostatic activity

The mean MIC (% v/v) ± SD of the 13 EO against the 65 bacteria strains are given in Table 2. EO containing

Table 1 Principal components (%) of the 13 essential oils tested

Botanical name →	*Cinnamomum verum, bark*	*Cinnamomum verum, leaf*	*Cymbopogon citratus*	*Cymbopogon martinii*	*Eugenia caryophyllus*	*Eucalyptus globulus*	*Lavendula angustifolia*	*Melaleuca alternifolia*	*Melaleuca cajeputii*	*Origanum compactum*	*Thymus satureioides*	*Citrus sinensis*	*Tachyspermum ammi*
Usual name	Cinnamomum bark	Cinnamomum leaves	Lemon grass	Palmarosa	Clove	Eucalyptus	Lavender	Tea tree	Cajeput	Origano	Thyme	Orange	Ajowan
Batch number → Principal components↓ (%)	CVECPR0304	CVFEIN0205	CCPAIL0405	CMPAIL0905	ECBFGR0505	EGFEYD1105	LASFPR0505	MAFEDA0505	MCFEIN0105	OCSFPH0305	TSSFPH0405	CSZEIL0405	TAFRIL0205
Limonene	0·19	0·42	2·57	0·14		**7·47**	0·23	1·13	**9·19**	0·48		**91·84**	1·03
Cinnamaldehyde	**68·79**												
Eugenol	6·96	**65·28**	0·24		**75·52**								
Geranyl acetate			3·56	**12·13**			0·75						
α, β and γ-Terpinene	0·13	0·16				3·71	0·08	**25·41**	3·56	20·19	4·10		**40·75**
Linalyl acetate							**37·68**						
Eugenyl acetate					**12·25**								
Benzyl benzoate	0·45	5·63											
Neral			**28·87**	0·19				0·15		0·18		0·11	0·13
Geranial			**40·04**	0·46						0·04		0·19	
Geraniol			4·52	**77·98**		0·05	0·36						
Linalol	0·80	2·87	1·31	2·80		0·08	**26·75**	0·05	4·63	2·22	5·39	0·56	
β-Caryophyllene	1·16	4·99		1·77	8·73		4·15			2·57	10·93		
1·8-Cineole		0·15				**80·43**	0·65	4·39	**65·05**	0·20			
Terpinene-4-ol	0·06	0·13	0·31			0·30	1·79	**35·51**	0·97	0·19			0·36
p-Cymene		0·81				2·47	0·20		1·21	11·68	3·65		14·75
Thymol								2·28		**22·07**	13·54		**33·98**
Carvacrol										**29·69**	2·21	0·11	0·70
α-Terpineol		0·40	0·29	0·04		1·07	0·64	3·87	3·62	0·50	6·69		0·28
Borneol		0·05	0·40				1·54			0·35	**27·31**		

Figures in bold indicate the principal components of each oil.

Table 2 Antimicrobial activity [mean minimum inhibitory concentration (MIC % v/v)] of 13 essential oils for 65 bacteria (in decreasing order of efficacy from left to right)

Strains (number tested)	Cinnamon bark	Origano	Ajowan	Thyme	Clove	Cinnamon leaf	Lemon grass	Tea tree	Palmarosa	Eucalyptus	Cajeput	Lavender	Orange
Escherichia coli (4)	0·07 ± 0·03	0·24 ± 0·08	0·37 ± 0·15	0·35 ± 0·1	0·43 ± 0·15	0·5 ± 0·15	0·94 ± 0·32	0·62 ± 0·27	0·62 ± 0·27	2·25 ± 2·17	5·63 ± 1·65	6·88 ± 2·42	>10 ± 0
Enterobacter cloacae (2)	0·1 ± 0·04	0·27 ± 0·07	0·2 ± 0·07	0·39 ± 0·13	0·31 ± 0	0·47 ± 0·16	1·09 ± 0·27	0·47 ± 0·16	>10 ± 0	7·5 ± 2·5	7·5 ± 2·5	>10 ± 0	>10 ± 0
Salmonella (2)	0·08 ± 0	0·24 ± 0·08	0·31 ± 0	0·39 ± 0·13	0·47 ± 0·16	0·47 ± 0·16	0·94 ± 0·32	0·62 ± 0	>10 ± 0	8·75 ± 2·17	7·5 ± 2·5	>10 ± 0	>10 ± 0
Citrobacter (3)	0·01 ± 0·04	0·26 ± 0·07	0·26 ± 0·07	0·35 ± 0·1	0·52 ± 0·16	0·47 ± 0·16	1·04 ± 0·3	0·67 ± 0·28	>10 ± 0	8·33 ± 2·36	7·5 ± 2·5	>10 ± 0	>10 ± 0
Hafnia alvei	0·08 ± 0	0·24 ± 0·08	0·24 ± 0·08	0·31 ± 0	0·47 ± 0·16	0·47 ± 0·16	0·62 ± 0	0·94 ± 0·32	0·62 ± 0	5 ± 0	5 ± 0	>10 ± 0	>10 ± 0
Vibrio cholerae (2)	0·06 ± 0·02	0·14 ± 0·04	0·16 ± 0	0·16 ± 0	0·39 ± 0·13	0·47 ± 0·16	0·24 ± 0·08	0·62 ± 0	0·24 ± 0·08	3·75 ± 1·25	3·13 ± 1·08	1·25 ± 0	0·62 ± 0
Klebsiella (2)	0·1 ± 0·04	0·2 ± 0·07	0·31 ± 0	0·47 ± 0·16	0·62 ± 0	0·47 ± 0·16	1·25 ± 0	0·78 ± 0·27	>10 ± 0	10 ± 0	10 ± 0	>10 ± 0	>10 ± 0
Pasteurella multocida (2)	0·04 ± 0·01	0·2 ± 0·07	0·27 ± 0·07	0·27 ± 0·08	0·47 ± 0·16	0·31 ± 0	0·12 ± 0·04	0·47 ± 0·16	0·13 ± 0·04	1·25 ± 0	1·88 ± 0·63	0·27 ± 0·06	4·38 ± 1·08
Bordetella bronchisepta	0·08 ± 0	0·24 ± 0·08	0·47 ± 0·16	0·31 ± 0	0·62 ± 0	0·47 ± 0·16	0·16 ± 0	0·47 ± 0·16	0·47 ± 0·16	2·5 ± 0	2·75 ± 1·25	0·31 ± 0	5 ± 0
Acinetobacter baumanii (4)	0·08 ± 0	0·16 ± 0	0·27 ± 0·15	0·49 ± 0·18	0·25 ± 0·07	0·58 ± 0·1	0·5 ± 0·15	0·62 ± 0·27	0·35 ± 0·1	4·06 ± 1·21	2·75 ± 1·25	1·88 ± 0·63	>10 ± 0
Stenotrophomonas maltophilia (2)	0·07 ± 0·02	0·18 ± 0·08	0·16 ± 0	0·16 ± 0	0·31 ± 0	0·47 ± 0·16	0·47 ± 0·16	0·62 ± 0	0·27 ± 0·06	3·13 ± 1·08	2·75 ± 1·25	8·75 ± 2·17	2·75 ± 1·25
Aeromonas hydrophila (2)	0·03 ± 0·01	0·12 ± 0·04	0·14 ± 0·04	0·14 ± 0·03	0·27 ± 0·06	0·35 ± 0·17	0·31 ± 0	0·39 ± 0·13	0·27 ± 0·06	2·5 ± 0	1·25 ± 0	0·94 ± 0·32	0·62 ± 0
Branhamella catarrhalis	0·03 ± 0·01	ND	ND	ND	0·47 ± 0·16	1·25 ± 0	0·31 ± 0	ND	ND	ND	ND	1·25 ± 0	ND
Pseudomonas aeruginosa (2)	0·24 ± 0·08	2·19 ± 0·54	7·4 ± 2·5	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0	>10 ± 0
Staphylococcus aureus (8)	0·1 ± 0·03	0·24 ± 0·08	0·24 ± 0·08	0·29 ± 0·05	0·6 ± 0·08	0·48 ± 0·15	0·38 ± 0·1	1·05 ± 0·29	0·31 ± 0	7·19 ± 2·48	5·16 ± 1·39	2·58 ± 1·29	10 ± 0
Coagulase – staphylococci (6)	0·08 ± 0·03	0·24 ± 0·08	0·24 ± 0·07	0·38 ± 0·14	0·62 ± 0	0·7 ± 0·25	0·34 ± 0·13	1·41 ± 0·52	0·29 ± 0·06	7·29 2·79	4·79 ± 1·90	6·45 ± 2·6	7·71 ± 3·41
Streptococci (12)	0·06 ± 0·04	0·2 ± 0·08	0·31 ± 0·14	0·34 ± 0·18	0·27 ± 0·1	0·58 ± 0·24	0·26 ± 0·13	0·56 ± 0·35	0·22 ± 0·07	2·78 ± 2·08	2·92 ± 2·24	0·4 ± 0·31	5·14 ± 3·33
Enterococci (6)	0·16 ± 0	0·34 ± 0·08	0·47 ± 0·15	0·94 ± 0·3	0·52 ± 0·14	1·15 ± 0·23	0·54 ± 0·13	1·87 ± 0·63	0·31 ± 0	5·83 ± 1·86	8·75 ± 2·17	2·91 ± 0·93	>10 ± 0
Listeria monocytogenes	0·08 ± 0	0·31 ± 0	0·47 ± 0·15	0·62 ± 0	0·47 ± 0·16	1·25 ± 0	0·24 ± 0·08	1·87 ± 0·63	0·24 ± 0·08	5 ± 0	7·5 ± 2·75	1·25 ± 0	10 ± 0
Corynebacterium (2)	0·09 ± 0·04	0·2 ± 0·07	0·27 ± 0·06	0·47 ± 0·16	0·35 ± 0·17	0·78 ± 0·27	0·18 ± 0·08	0·47 ± 0·16	0·18 ± 0·08	3·75 ± 1·25	3·75 ± 1·25	0·38 ± 0·13	7·5 ± 2·5

ND, not determined.

Values are mean MIC (% v/v) ± SD of strains. Each strain was tested in duplicate.

phenols (oregano, ajowan, thyme, clove and cinnamon leaf) and aldehydes (cinnamon bark and lemon grass) exhibited marked activity against all strains except *P. aeruginosa*, with MIC < 2% (v/v). Alcohol-based EO (tea tree, palmarosa and lavender) exhibited varying degrees of activity depending on whether the strains were Gram-positive or Gram-negative. Tea tree oil was the most efficient alcohol-based EO against all Gram-negative bacteria [MIC ≤ 1·25% (v/v)] except *P. aeruginosa*. For palmarosa and lavender, $MIC_{90\%}$ was >2% (v/v), and the activity of these two oils was better against Gram-positive bacteria than against Gram-negative bacteria. EO containing 1·8-cineole and hydrocarbons (eucalyptus, cajuput and orange) exhibited little activity, with $MIC_{90} \geq 10\%$ (v/v). Against *P. aeruginosa*, only cinnamon bark and oregano presented a low MIC ≤ 2·5% (v/v), with the MIC of the other EO ranging from 5% to >10% (v/v). The MIC for antibiotic-resistant strains were identical to those for susceptible strains.

Bactericidal activity

Killing curves were performed for EO with MIC < 2% (v/v) having different principal components: aldehyde (cinnamaldehyde) for cinnamon bark, phenol for oregano (carvacrol and thymol), cinnamon leaf (eugenol) and a monoterpenol group for thyme (borneol) and tea tree (terpinen-4-ol). The results obtained for the time-killing curves are summarized in Table 3. For *E. coli*, all EO were bactericidal within 5 min at concentrations <2% (v/v). In contrast, with *S. aureus*, EO formed three distinct groups. The first group, oregano and cinnamon leaf oils, comprising mainly phenols, acted within 5 min at concentrations <2% (v/v). The second group of EO (cinnamon bark, thyme) were bactericidal at concentrations <2% (v/v) at 30 min and exhibited time-dependent bactericidal kinetics, as may be seen in Fig. 1 for the time-killing curve of cinnamon bark EO on *S. aureus*. The third group consisted of tea tree oil, which is bactericidal only at concentrations >2% (v/v), even after 24 h. For *S. pyogenes*, only cinnamon bark and oregano were tested, with bactericidal activity being rapid for oregano and slower for cinnamon bark. The only EO bacteriostatic against *P. aeruginosa*, cinnamon bark and oregano exhibited good bactericidal activity within 5 min at concentrations <2% (v/v).

Table 3 Minimum bactericidal concentration* (% v/v) at 37°C of cinnamon bark, oregano, thyme, cinnamon leaf and tea tree oil at 5, 15, 30 min, 1 and 24 h

Bacteria tested		Cinnamon bark	Oregano	Thyme	Cinnamon leaf	Tea tree
E. coli	5 min	0·62	0·31	0·31	1·25	1·25
	15 min	0·62	0·31	0·31	1·25	1·25
	30 min	0·31	0·31	0·31	1·25	1·25
	60 min	0·31	0·31	0·31	1·25	1·25
	24 h	0·08	0·31	0·31	1·25	1·25
S. aureus	5 min	>10	0·62	>10	1·25	>10
	15 min	2·5	0·62	2·5	1·25	>10
	30 min	1·25	0·62	0·62	1·25	>10
	60 min	0·62	0·62	0·62	1·25	10
	24 h	0·16	0·62	0·62	1·25	2·5
P. aeruginosa	5 min	0·62	1·25	>10	ND	>10
	15 min	0·62	1·25	>10	ND	>10
	30 min	0·62	1·25	>10	5	>10
	60 min	0·62	1·25	>10	5	>10
	24 h	0·31	ND	>10	ND	ND
Str. pyogenes	5 min	>10	1·25	ND	ND	ND
	15 min	>10	1·25			
	30 min	>10	0·62			
	60 min	2·5	0·62			
	24 h	0·31	0·62			

E., Escherichia; S., Staphylococcus; P., Pseudomonas; *Str., Streptococci*; ND, not determined.

*Minimum bactericidal concentration needed to achieve a reduction in numbers of 5 log CFU ml^{-1}.

Killing curves were prepared in duplicate and the results were identical to one dilution.

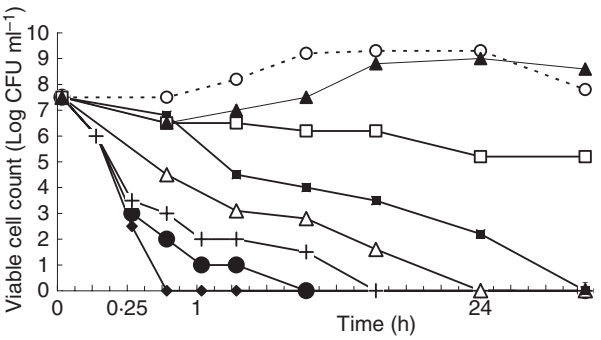

Figure 1 Time-killing curve for cinnamon bark essential oil on methicillin-resistant *Staphylococcus aureus* ATCC 43300 [minimum inhibitory concentration (MIC) 0·12% v/v]. A viable bacteria count was performed at different concentrations after 5, 15, 30 min, 1, 2, 4, 6 and 24 h. Killing curve was prepared at 37°C in duplicate and the results were identical to within one dilution. Results: (○) growth control; (▲) 0·04% (v/v); (□) 0·08% (v/v); (■) 0·16% (v/v); (Δ) 0·31% (v/v); (+) 0·62% (v/v); (●) 1·25% (v/v); (◆) 2·5% (v/v).

Discussion

The highly standardized antibiotic techniques that have gained wide acceptance over the last 25 years for the evaluation of antibiotics constitute excellent tools for assessing EO, namely the agar dilution method to determine MIC and the bactericidal kinetic method to determine minimum bactericidal concentration (MBC). Our results show good reproducibility and results for all 13 EO in terms of MIC and MBC.

The presence of certain predominant chemical groups determines the bacteriostatic activity of an EO, although synergistic action between the various biochemical molecules is also extremely important (Edwards-Jones *et al.* 2004). Our determination of the bacteriostatic activity of 13 EO against 65 bacterial strains allowed the following classification of EO by efficacy based on chemical composition: phenols, aldehydes and monoterpenols followed by oxides and hydrocarbons. This classification is in general agreement with previously reported studies (Inouye *et al.* 2001; Edwards-Jones *et al.* 2004; Penalver *et al.* 2005). EO are generally more effective against Gram-positive bacteria, as demonstrated by other authors (Smith-Palmer *et al.* 1998; Hammer *et al.* 1999; Prabuseenivasan *et al.* 2006). In addition, we showed that the activity of EO determined for a number of strains highly resistant to antibiotics was independent of the level of resistance to antibiotics. These results were described for several bacteria, such as *S. aureus*, *Helicobacter pylori, Salmonella* spp. and *S. pneumoniae* but not for bacteria involved in nosocomial infections, i.e. GISA, VRE, ESBL-producing enterobacteria, *Acinetobacter* spp., *St. maltophilia* (Mann and Markham 1998; Ohno *et al.* 2003; Shin 2005; Carson *et al.* 2006).

Pseudomonas aeruginosa is notorious for its involvement in nosocomial infections and frequent resistance to antibiotics. This was the bacteria most highly resistant to EO, regardless of the level of resistance to antibiotics. According to Longbottom *et al.* (2004), this resistance of *P. aeruginosa* appears to be the result of an external membrane particularly impermeable to EO molecules and the presence of efflux mechanisms and porine-dependent inhibition, protecting the bacteria against the action of EO. The two strains of *P. aeruginosa* tested, the wild phenotype and that having acquired ESBL, exhibited identical MIC. They were sensitive to only two of the 13 EO tested: cinnamon bark and oregano. Prabuseenivasan *et al.* (2006) also demonstrated significant inhibitory effects of cinnamon oil against one strain of *P. aerugionsa* susceptible to antibiotics. MIC of *P. aeruginosa* was 10% (v/v) for tea tree oil, whereas Papadopoulos *et al.* (2006) found $MIC_{90\%}$ values of 4% using Tween as the solubilization agent. However, in our study (data not shown), Tween exhibited the antimicrobial activity previously noted by Remmal *et al.* (1993) resulting in our decision to solubilize the EO in agar (Remmal *et al.* 1993; Mann and Markham 1998).

As bacteriostatic effects in themselves are insufficient to confer antiseptic and disinfectant qualities on a substance, we examined the bactericidal activity of several chemotyped EO. To our knowledge, tea tree oil alone has been assessed for bactericidal activity comparatively with known antiseptics, such as mupirocin and alcohol derivatives (Elsom and Hide 1999; Carson *et al.* 2002; Messager *et al.* 2005). We demonstrated that the bactericidal activity of each EO varied according to its chemotype. With chiefly phenolic EO, a bactericidal effect appeared quickly within 5 min. For other EO (cinnamon bark, thyme and tea tree), bactericidal effects against Gram-positive bacteria (*S. aureus* and *S. pyogenes*) were slower than those seen against Gram-negative bacteria. Carson *et al.* (2002); Cox *et al.* (2000) and Marino *et al.* (2001) also showed the bactericidal activity of tea tree oil to be slower against *S. aureus* than against *E. coli*. Cinnamon bark and oregano appeared the most active EO as they had the greatest bactericidal effect. Cinnamon bark was the only EO to exhibit pronounced bactericidal activity against *P. aeruginosa*. However, against Gram-positive organisms, it exhibited longer killing times than the chiefly phenolic oregano oil, acting on *S. aureus* within 15 min at 2·5% (v/v), while oregano took 5 min to induce a 5_{log} reduction in the *inoculum* at a concentration of 0·62% (v/v). Further studies to investigate the bactericidal synergy of these two oils would be useful.

Thanks to the use of two methods long employed in the evaluation of antibiotics, because of which we were able to classify 13 EO in terms of their bacteriostatic and bactericidal activities on bacteria with varying sensitivity to antibiotics. The agar dilution MIC method appeared highly reproducible and the results were confirmed using the time-killing curve method. Because of its rapid bactericidal effects, cinnamon bark seems to be a promising antiseptic and disinfectant agent, above all in hospital units, where antibiotic-resistant strains are common.

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