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Comparative study on the *in vitro* antibacterial activity of Australian tea tree oil, cajuput oil, niaouli oil, manuka oil, kanuka oil, and eucalyptus oil

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To compare the antibacterial activity of the Australian tea tree oil (TTO) with various other medicinally and commercially important essential myrtaceous oils (cajuput oil, niaouli oil, kanuka oil, manuka oil, and eucalyptus oil) the essential oils were first analysed by GC-MS and then tested against various bacteria using a broth microdilution method. The highest activity was obtained by TTO, with MIC values of 0.25% for *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella choleraesuis, Shigella flexneri, Bacillus subtilis, Listeria monocytogenes, Staphylococcus aureus, S. saprophyticus,* and *S. xylosus*. It is noteworthy that manuka oil exhibited a higher activity than TTO against gram-positive bacteria, with MIC values of 0.12%. Both TTO and manuka oil also demonstrated a very good antimicrobial efficacy against various antibiotic-resistant *Staphylococcus* species. *Pseudomonas aeruginosa* was resistant to all essential oils tested, even at the highest concentration of 4%.

1. Introduction

The common name tea tree is not only typical for *Melaleuca alternifolia* but it has also been given to various other members of the genera *Melaleuca, Leptospermum* and *Kunzea*, all belonging to the family Myrtaceae. The name tea tree came into being because the aromatic foliage of several species of these genera were sometimes used by the early settlers in Australia as a substitute for tea. Leaves and terminal branchlets of the different tea trees are steam distilled to give essential Australian tea tree oil (plant species: *Melaleuca alternifolia*), cajuput oil (plant species: *Melaleuca cajuputi*), niaouli oil (plant species: *Melaleuca viridiflora* and *M. quinquenervia*, respectively), kunzea oil (plant species: *Kunzea ericoides*), and manuka oil (plant species: *Leptospermum scoparium*). The purpose of this study was to evaluate the antimicrobial activity of these essential myrtaceous oils, including eucalyptus oil, as they are used worldwide in various health care products, cosmetics and household disinfectants [1–10].

2. Investigations, results and discussion

2.1. Chemical characterization of the essential myrtaceous oils

Since the chemical composition of the essential oils affects microbial growth, each commercial oil was characterized chemically before using it in the bioassay. The results are listed in Table 1. The major components of each essential oil were identified by comparing their mass spectral data with those of authentic terpene standards, literature data, and those mass spectral data stored on the spectrometer database as well as by coinjection with authentic substances.

The Australian tea tree oil (TTO) consisted mainly of terpinene-4-ol (39.3%), γ-terpinene (20.67%), and α-terpinene (10.5%) whereas cajuput oil, eucalyptus oil, and niaouli oil exhibited a very high 1,8-cineol content (61.0%, 80.12%, and 52.04%, respectively) in addition to smaller amounts of other terpenes. The manuka oil belonged to the group with a high triketone chemotype, containing 14.36% leptospermone and 3.94% isoleptospermone. In addition to already known substances [11] we were able to identify several other terpenes, such as γ-terpinene, terpinen-4-ol (monoterpenes), β-caryophyllene, aromadendrene, allo-aromadendrene, viridiflorene, δ-cadinene, α-humulene, α-cubebene, α-copaene, α-gurjunene, calamene, and selinene (sesquiterpenes). The kanuka oil represented an α-pinene rich essential oil (70.63%). Besides the compounds already known [12] we additionally were able to identify the following mono- and sesquiterpenes: myrcene, limonene, terpinolene, terpenen-4-ol (monoterpenes), aromadendrene, viridiflorene, δ-cadinene, α-cubebene, α-copaene, and α-gurjunene (sesquiterpenes).

2.2. Antibacterial activity of the essential myrtaceous oils

The difficulty in testing essential oils in standard test media is that the pure oil is not water soluble. The lipophilic oil separates out and floats on the top of the test medium. Therefore, the insolubility of the essential oil in water rendered the agent unsuitable for antibacterial assay by dircet dilution in the watery culture medium. Attempts to overcome this problem have included the use of an emulsifier, such as Tween 80. It could be shown by several investigators that the presence of Tween 80 at a final concentration of 0.5% (v/v) in the watery media did not inhibit the growth of any bacteria [5–8, 13]. Our tests with Tween 80 confirmed these findings. The detergent was used successfully to enhance the solubility of essential oils in the test medium. Moreover, Tween 80 neither affected the visual determination of MICs nor had any effect on the growth of test organisms.

The MIC and MBC results are given in Tables 2 and 3, respectively. The most susceptible microorganism was *Listeria monocytogenes*, with MIC values of 0.12% to 0.25% for all essential oils. In contrast, *Pseudomonas aeruginosa* was not affected by any of the essential myrtaceous oils tested, even at the highest concentration of 4%.

TTO was the most potent of the six essential myrtaceous oils with a broad spectrum of antibacterial activity. Except for *Pseudomonas aeruginosa* all the test strains were inhibited. The MIC values ranged from 0.25 to 1.0% TTO. In this study the MIC values of TTO often corresponded to the MBC values, and this may be indicative of a bactericidal effect.

In contrast to TTO, all other myrtaceous oils showed a more differentiated spectrum of antimicrobial effects. Ca-

Table 1: Composition of various essential myrtaceous oils

Identification	Compounds	Tea tree oil	Cajuput oil	Niaouli oil	Eucalyptus oil	Manuka oil	Kanuka oil
a/b	α-thujene	0.90	0.30				0.88
a/b	α-pinene	2.30	9.05	8.80	3.26	1.14	70.63
a/b	camphene			0.23			
a/b	β-pinene	1.10	3.82	2.58	0.59	0.11	0.59
a/b	myrcene	0.77	0.88	0.35	0.55	0.42	0.17
a/b	α-phellandrene	0.48	0.18		0.52		
a/b	α-terpinene	10.50	0.63	0.50			
a/b	p-cymene	2.00	2.00	1.51	2.17	0.15	5.09
a/b	1,8-cineol	2.30	61.00	52.04	80.12	0.29	3.48
a/b	limonene	0.88	5.99	14.07	6.81		1.28
a/b	γ-terpinene	21.67	3.10	0.66	2.19	0.73	3.94
a/b	linalool						1.15
a/b	terpinolene	3.59	1.25	0.57	0.26		0.76
a/b	borneol			0.49			
a/b	terpinene-4-ol	39.30	1.25	1.48	1.22	1.76	1.09
a/b	α-terpineol	2.62	3.50	8.79	0.76		0.29
a/b	bornylacetat			0.83			
a/b	β-caryophyllene		1.46	0.20		2.79	
a/b	aromadendrene	0.63				2.11	0.48
a/b	alloaromadendrene					0.79	
a/b	viridiflorene	1.10	0.34			4.40	1.05
b	δ-cadinene	1.10				6.02	0.30
a/b	α-humulene		0.56			0.30	
b	α-cubebene					4.38	0.17
b	α-copaene					6.58	0.23
b	α-gurjunene					1.05	0.20
b	calamanene					15.93	1.04
b	selinene					6.10	
b	leptospermone					14.36	
b	isoleptospermone					3.94	

Data are % (v/v) of total essential oil. a: Identification by authentic substances. b: Identification by GC/MS.

juput oil, eucalyptus oil, and niaouli oil exhibited a very similar antimicrobial activity against gram-negative and gram-positive bacteria. Besides *Pseudomonas aeruginosa*, which was generally resistant, *Eschericha coli* was also insensitive to cajuput oil and eucalyptus oil while *Proteus mirabilis* was only resistant to cajuput oil. Both manuka oil and kanuka oil were less active against gram-negative bacteria than against gram-positive bacteria. The most effective one was manuka oil, with MIC values of 0.12% for all the gram-positive bacteria.

2.3. Susceptibility of antibiotic-resistant Staphylococcus species to TTO and manuka oil

From a more practical point of view, it seems particularly interesting that all the *Staphylococcus* species tested were very sensitive to low concentrations of TTO and manuka oil. Staphylococci are important nosocomial pathogens causing localized purulent and generalized septic infections. Methicillin-resistent *Staphylococcus aureus* (MRSA) are worldwide of major concern due to its concurrent resistance against a wide spectrum of antibiotics. MRSA usually colonizes the anterior nares of patients and healthy individuals, and from those carriers the bacteria are disseminated to others causing epidemics in hospitals. For eradication of MRSA from mucosal surfaces the topical application of mupirocin is recommended. However, its widespread use inheres the risk of development of resistance. Therefore, alternative substances with anti-MRSA activity are urgently needed. To this end, we investigated the susceptibility of MRSA strains as well as different strains of multiresistant *Staphylococcus capitis, S. epider-*

Table 2: Antimicrobial activity of various essential myrtaceous oils against gram-negative bacteria

Bacteria	Tea tree oil		Cajuput oil		Niaouli oil		Eucalyptus oil		Manuka oil		Kanuka oil	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Citrobacter freundii	0.50	1.0	1.0	1.0	1.0	1.0	1.0	1.0	>2.0	>2.0	>2.0	>2.0
Enterobacter aerogenes	0.25	0.50	0.50	1.0	0.50	0.50	2.0	>2.0	>2.0	>2.0	>2.0	>2.0
Escherichia coli	0.25	0.25	>4.0	>4.0	0.50	1.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0
Klebsiella pneumoniae	0.25	0.25	0.50	0.50	0.50	0.50	0.50	0.50	>2.0	>2.0	>2.0	>2.0
Proteus mirabilis	0.25	0.50	>4.0	>4.0	1.0	1.0	2.0	2.0	2.0	4.0	>4.0	>4.0
Pseudomonas aeruginosa	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0	>4.0
Salmonella choleraesuis	0.25	0.50	1.0	2.0	1.0	2.0	2.0	>2.0	>2.0	>2.0	>2.0	>2.0
Shigella flexneri	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.50	0.50	>2.0	>2.0

Data are concentration of essential oil in % (v/v)

Table 3: Antimicrobial activity of various essential myrtaceous oils against gram-positive bacteria

Bacteria	Tea tree oil		Cajuput oil		Niaouli oil		Eucalyptus oil		Manuka oil		Kanuka oil	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus subtilis	0.25	0.25	0.50	0.50	0.25	0.25	1.0	2.0	0.12	0.25	0.50	0.50
Corynebacterium pseudodiphtheriae	0.50	1.0	1.0	1.0	0.50	1.0	2.0	>2.0	0.12	0.12	1.0	1.0
Enterococcus durans	1.0	1.0	2.0	>2.0	2.0	>2.0	2.0	>2.0	0.12	0.12	2.0	>2.0
Enterococcus faecalis	1.0	1.0	2.0	>2.0	2.0	>2.0	2.0	>2.0	0.12	0.12	2.0	>2.0
Enterococcus faecium	1.0	1.0	2.0	>2.0	2.0	>2.0	2.0	>2.0	0.12	0.12	2.0	>2.0
Listeria monocytogenes	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.12	0.25	0.12	0.12
Staphylococcus aureus	0.25	0.50	1.0	4.0	0.50	1.0	2.0	2.0	0.12	0.25	0.50	1.0
Staphylococcus epidermidis	0.25	0.25	0.50	0.50	0.50	0.50	0.50	1.0	0.12	0.12	0.50	0.50
Staphylococcus saprophyticus	0.25	0.25	0.50	0.50	0.50	1.0	0.50	0.50	0.12	0.12	0.50	0.50
Staphylococcus xylosus	0.25	0.50	0.50	1.0	0.50	0.50	0.50	0.50	0.12	0.25	0.50	0.50

Data are concentration of essential oil in % (v/v)

Table 4: Antimicrobial activity of tea tree oil and manuka oil against several methicillin-resistant *Staphylococcus aureus* strains and other strains of multiresistant *Staphylococcus* species

Multiresistant *Staphylococcus* species	Tea tree oil		Manuka oil	
	MIC	MBC	MIC	MBC
Staphylococcus aureus (n = 3)	0.25	0.25	0.06–0.12	0.12
Staphylococcus capitis (n = 3)	0.12–0.25	0.25	0.06–0.12	0.12
Staphylococcus epidermitis (n = 3)	0.25–0.5	0.25–1.0	0.06–0.12	0.12
Staphylococcus haemolyticus (n = 4)	0.25–0.5	0.25–0.5	0.06–0.12	0.12
Staphylococcus hominis (n = 1)	0.12	0.25	0.06	0.12
Staphylococcus saprophyticus (n = 2)	0.25–0.50	0.25–0.5	0.12–0.25	0.12–0.5
Staphylococcus xylosus (n = 1)	0.25	0.50	0.12	0.12

Data are concentration of essential oil in % (v/v). n: number of strains

midis, S. haemolyticus, S. hominis, S. saprophyticus, and *S. xylosus* to TTO and manuka oil. The antimicrobial activities of both oils are shown in Table 4. Manuka oil was more active than TTO, with MIC and MBC values of 0.06–0.25% and 0.12–0.50%, respectively. The activity of TTO was one or two dilution steps lower than those of manuka oil. Our results on the antimicrobial activity of TTO against MRSA were identical to those reported previously by Carson et al. [14].

2.4. Antibacterial active principles of the essential myrtaceous oils

An essential oil always represents a complex mixture of different chemical substances. For that reason it is very difficult to reduce the antimicrobial effect of a total essential oil to one or a few active principles. In general, it cannot be excluded that, in addition to the main compounds, other minor compounds are making a significant contribution to the oils' activity. For TTO terpinen-4-ol, α-terpineol and linalool exhibited the best antimicrobial activity [7]. Obviously, 1,8-cineol was found to be less active against several microorganisms. The antimicrobial activity of TTO was much greater than that of cajuput oil (61% 1,8-cineol), eucalyptus oil (about 80% 1,8-cineol), and niaouli oil (about 52% 1,8-cineol), which suggests that essential oils with high 1,8-cineol levels are less active.

The very strong antimicrobial effect of manuka oil against the gram-positive bacteria tested is difficult to explain. As of yet, it is unclear whether calamene (about 16%) or leptospermone (about 14%) or the two compounds in combination are responsible for the antimicrobial activity against gram-positive bacteria.

2.5. Conclusion

In summary, our data show that essential myrtaceous oils exhibit remarkable antimicrobial activity against a wide spectrum of bacteria important in human medicine. Out of all oils tested TTO was the most active substance.
Pseudomonas aeruginosa, however, was uniformely resistant against all test substances. The good *in vitro* activities of TTO and manuka oil against different multiresistant *Staphylococcus* species suggest that these agents may have potential for topical use, and clinical trials designed to assess their efficacies *in vivo* are warranted. Based on its broad activity spectrum, TTO is also a possible candidate for topical antiseptic application in cosmetic products.

3. Experimental

3.1. Apparatus and chemicals

3.1.1. Essential oils

The essential oils tested were commercial products: Australian tea tree oil, manuka oil [ALVA, Wallenhorst, Germany], cajuput oil [Spinnrad, Gelsen-

kirchen, Germany], Oleum Eucalypti Ph. Eur. (eucalyptus oil), Oleum Niaouli (niaouli oil) [Caesar & Loretz, Hilden, Germany], and kanuka oil [Colimex, Köln, Germany].

3.1.2. Terpene standards

α-Thujene, α-pinene, borneol, bornylacetate, camphene, β-caryophyllene, 1,8-cineole, p-cymene, α-humulene, limonene, β-pinene, myrcene, α-terpinene, γ-terpinene, terpinen-4-ol, α-terpineol, terpinolene [Roth, Karlsruhe], allo-aromadendrene, aromadendrene, linalool, α-phellandrene, viridiflorene [Fluka, Buchs, Switzerland].

3.1.3. GC method

The essential oils were analysed as 1% solution in n-hexane containing tridecane as the internal standard. GC was performed using a Carlo Erba GC 6000 chromatograph equipped with a Spectra Physics Integrator SP 4290. The GC column was a 15 m × 0.25 mm (i.d.) fused silica capillary column coated with OV 1 (phase thickness 0.25 μm) and with He as the carrier gas (flow rate: 2 mL/min.); split: 1 : 5. Temperature program: The initial column temperature was 40 °C for 4 min. Subsequently, the temperature rate was programmed from 40 °C to 300 °C in two steps, first 4 °C/min. up to 120 °C and then 10 °C/min. up to 300 °C. Injector temperature: 250 °C; detector temperature: 300 °C; injection volume: 1 μl.

3.1.4. GC-MS method

A gas chromatograph Carlo Erba HRGC 4160 was coupled via an open, split interface to a Finnigan MAT 4500 mass spectrometer. GC column: 30 m × 0.25 mm (i.d.) fused silica capillary column coated with OV 1 (phase thickness: 0.25 μm). Split: 1 : 20. Temperature program: 46 °C for 4 min; 3 °C/min. up to 76 °C, then 4 °C/min. up to 136 °C and 6 °C/min. up to 300 °C. EI ionizing voltage 70 eV.

3.2. Microbiology

3.2.1. Bacteria

All the bacteria used for susceptibility testing were test strains derived from Type Culture Collection (ATCC, U.S.A.; NCTC, UK; DSM, Germany) and from blood culture samples (BC) isolated in the routine laboratory of the Institute of Hygiene, University Hospital Heidelberg, Germany.
Gram-negative bacteria: *Citrobacter freundii* DSM 30039, *Enterobacter aerogenes* DSM 30053, *Escherichia coli* ATCC 11229, *Klebsiella pneumoniae* DSM 30104, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella choleraesuis* DSM 554, *Shigella flexneri* DSM 4782.
Gram-positive bacteria: *Bacillus subtilis* DSM 347, *Corynebacterium pseudodiphtheriae* ATCC 10700, *Enterococcus durans* ATCC 19433, *Enterococcus faecalis* ATCC 19433, *Enterococcus faecium* ATCC, *Listeria monocytogenes* DSM 20600, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus saprophyticus* ATCC 15305, and *Staphylococcus xylosus* ATCC 29971. Methicillinresistant *Staphylococcus aureus* BC 52, BC 54, NCTC 10442, multiresistant *Staphylococcus capitis* BC 2812, BC 3559, BC 3476, *Staphylococcus epidermidis* BC 3334, BC 3389, BC 3396, *Staphylococcus haemolyticus* BC 3468, BC 3516, BC 2794, BC 2887, *Staphylococcus hominis* BC 3427, *Staphylococcus saprophyticus* BC 3368, BC 3418, and *Staphylococcus xylosus* BC 3456. All Strains were cultured over night on blood agar and incubated aerobically at 37 °C.

3.2.2. Broth microdilution method

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the different essential oils against the test organisms [6]. All tests were performed in Iso-Sensitest broth (ISB; Oxoid) supplemented with Tween 80 detergent (final conc. of 0.5% (v/v)). The final inoculum of the test strains was adjustd to 5×10^5 cfu/ml, which was controlled by the spiral plater counting method (Spiral Systems, Cincinnati, OH, USA).
Geometric dilutions ranging from 0.06 to 4.0% essential oil were prepared in a 96-well microtiter plate, including one growth control (ISB + Tween 80) and one sterility control (ISB + Tween 80 + test oil) in each plate. Plates were incubated under normal atmospheric conditions at 37 °C for 20 to 24 hours. Each test was performed in duplicate and repeated twice. To confirm MICs and to determine MBCs, 100 μl broth was removed from each well, transferred to 10 ml ISB, and incubated for 72 h at 37 °C.
The MIC is defined as the lowest concentration of the essential oil at which the microorganism being tested does not demonstrate visible growth. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom. The MBC is defined as the lowest concentration of the essential oil at which 99.9% of the bacteria have been killed.

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