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Pharmacological and Antimicrobial Studies on Different Tea-tree Oils *(Melaleuca alternifolia, Leptospermum scoparium* or Manuka and *Kunzea ericoides* or Kanuka), Originating in Australia and New Zealand

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Three different species of Myrtaceae growing in Australia and New Zealand are known as 'Tea-tree': the Australian Tea tree (*Melaleuca alternifolia*), the New Zealand Manuka (*Leptospermum scoparium*) and Kanuka (*Kunzea ericoides*). All three essential oils are used by aromatherapists, although only *Melaleuca* has been tested for toxicity, and its antimicrobial effects studied. The pharmacology and antimicrobial activity of the three 'tea-tree' oils was determined using guinea-pig ileum, skeletal muscle (chick biventer muscle and the rat phrenic nerve diaphragm) and also rat uterus *in vitro*. Differences were shown between the three essential oils in their action on smooth muscle: Manuka had a spasmolytic action, while Kanuka and *Melaleuca* had an initial spasmogenic action. Using the diaphragm, Manuka and *Melaleuca* decreased the tension and caused a delayed contracture; Kanuka had no activity at the same concentration. The action on chick biventer muscle was, however, similar for all three oils, as was the action on the uterus, where they caused a decrease in the force of the spontaneous contractions. The latter action suggests caution in the use of these essential oils during childbirth, as cessation of contractions could put the baby, and mother, at risk. The comparative antimicrobial activity showed greater differences between different samples of Manuka and Kanuka than *Melaleuca* samples. The antifungal activity of Kanuka was inversely proportional to its strong antibacterial activity, whilst Manuka displayed a stronger antifungal effect, though not as potent as *Melaleuca*. The antioxidant activity of Manuka samples was more consistent than that of Kanuka, while *Melaleuca* showed no activity. The variability in the Manuka and Kanuka essential oils suggests caution in their usage, as does the fact that the oils have not been tested for toxicity.

Keywords: pharmacology; antimicrobial action; tea tree oils; *Melaleuca alternifolia*; Manuka; *Leptospermum scoparium*; Kanuka; *Kunzea ericoides*.

INTRODUCTION

Both Australia and New Zealand have indigenous 'tea-trees' in the family Myrtaceae which were reputedly used for brewing tea by Captain Cook. There is, however, no resemblance between real tea, *Camellia sinensis*, Camelliaceae, and the taste or odour of these species.

The Australian tea tree oil from *Melaleuca alternifolia* and other *Melaleuca* species has strong antimicrobial potential (Carson and Riley, 1993, 1994, 1995; Lis-Balchin *et al.*, 1999). The whole plant extract had been used originally by aboriginals and the essential oils themselves had been used during the Second World War as a general antimicrobial agent and insect repellant, and provided in the first aid kits of serving Australian soldiers. The essential oil is nowadays used as a strong antimicrobial and antifungal agent in creams, soaps, toothpastes and other preparations and has been used both externally and internally by both herbalists and aromatherapists for some years (Blackwell, 1991; Lis-Balchin, 1997).

There is scant evidence that Manuka (*Leptospermum scoparium*) and Kanuka (*Kunzea ericoides*) has such potential (Bloor, 1992; Cooke and Cooke, 1994; Lis-Balchin *et al.*, 1996a; Lis-Balchin *et al.*, 1998b), but as the essential oils are said to have remarkable powers of healing, based on folk medicinal usage (Brooker *et al.*, 1987), these oils are being used by some aromatherapists, although there have been no safety/toxicological evaluations performed on them.

Many of the folk-medicinal uses of the New Zealand 'tea-tree' oils are related to both species, e.g. the leaves of Manuka and Kanuka were used as vapour baths for colds; an infusion was very astringent and various uses were found for concoctions including urinary complaints and

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as a febrifuge (Brooker *et al.*, 1987). Kanuka was applied to scalds and burns, used to stop coughing and as a sedative; it was also used against dysentery. The decoction of boiled leaves and bark was used to treat stiff backs etc. Seed capsules were boiled to give a decoction for external application to treat inflammation or drank for diarrhoea; the capsules or leaves were also chewed for dysentery. The water from boiled bark was used for treating inflamed breasts and also to treat mouth, throat and eye problems.

The antibacterial effect of honeys derived from Kanuka and Manuka blossom against *Staphylococcus aureus* was shown by Allen *et al.*, (1991), and more recently Manuka honey was shown to be active against *Helicobacter pylori* (Somal *et al.*, 1994). *L. scoparium* contains leptospermone, which has antihelminthic properties and is closely related to compounds having similar properties in male ferns; leptospermone has insecticidal properties, and is similar in structure to the insecticide valone (Brooker *et al.*, 1987). There are virtually no studies on the pharmacological effects of any of these 'tea-tree' oils, except for Lis-Balchin *et al.* (1996a), Lis-Balchin and Hart (1998); therefore the present studies were initiated to study the pharmacological basis of their 'aromatherapeutic' action (if any) and to compare the mode of action of the different 'tea-tree' oils in different tissues *in vitro*. Due to their use in body massage, the action was studied in skeletal muscle, using chick biventer muscle and rat phrenic nerve diaphragm preparations, as well as in smooth muscle (guinea-pig ileum), due to the possible absorption of some components (Bronough *et al.*, 1990). The pharmacological action was also studied in the uterus (using rat uterine preparations), as aromatherapists use various essential oils during pregnancy and childbirth (Lis-Balchin, 1997), without knowing their possible effects or side-effects (Burns and Blamey, 1994). Some of the major components of the 'tea-tree' oils were also assessed, in order to ascertain which components were responsible for the particular pharmacological activity.

The comparative antimicrobial activity was also investigated *in vitro* for the tea-tree oils using 25 different bacterial species, 20 different varieties of *Listeria monocytogenes* and three species of fungi. The antioxidant activity was also compared. Some of the major components of the oils were also investigated.

MATERIALS AND METHODS

Essential oils. These were donated by Butterbur and Sage, Reading, UK; The components were purchased from Sigma, UK.

Pharmacological studies. Guinea-pig ileum was used as the smooth muscle and 2 cm lengths were mounted in a 25 mL organ bath, with field stimulation applied using two parallel platinum electrodes placed either side of the tissue and attached to a stimulator (0.5 ms, 0.1 Hz, 70 V). Changes in tension of the muscle were recorded with an isometric transducer attached to a pen recorder. Essential oils and components, diluted in methanol, were introduced into the bath at 5×10^{-5} v/v in a maximum volume of 0.2 mL, at which volume there was no solvent interference.

Studies on skeletal muscle were performed *in vitro* using male albino rat diaphragm preparations (Bulbring, 1946) with the muscle stimulated directly or via the phrenic nerve. The diaphragm was suspended in a 100 mL organ bath in Krebs buffer at 34 °C, gassed with 95% oxygen in carbon dioxide. Stimulation was at 0.21 Hz (0.5 ms, 40 V). The essential oils and components were presented at 2.5×10^{-3} v/v.

Preparations of chick biventer muscle were also used as a skeletal muscle, with the essential oils and components presented at 1×10^{-3} v/v.

All experiments were repeated at least five times on different tissues: results, where appropriate, were expressed as the mean percentage inhibition (or change in the electrically induced or spontaneous contractions) $\pm$ SEM.

The chemical composition of the essential oils was determined by gas chromatography using a Shimadzu Model GC 8A with an OV1 column, 0.32 mm $\times$ 58 m; temperature programme from 100 °–230 °C at 4 °C min.

Microorganisms used. The 25 different species of bacteria included both food spoilage organisms and pathogenic bacteria, with 9 Gram-positive and 16 Gram-negative (Deans and Ritchie, 1987); the 20 strains of *Listeria monocytogenes* used were obtained from various airline foods (Lis-Balchin *et al.*, 1997).

Microbiological techniques. The antibacterial studies were conducted as previously described (Lis-Balchin *et al.*, 1996c) using 10 μL of essential oils pipetted into wells made in agar previously inoculated with a single bacterium. Zones of inhibition were measured after 48 h incubation in the dark, at the appropriate temperature for each bacterium. Antifungal activity was assessed against *Aspergillus niger*, *A. ochraceus* and *Fusarium culmorum* (Lis-Balchin *et al.*, 1996b). 1 μL and 10 μL doses of essential oil were added to test tubes containing 10 μL of YES broth, then seeded with each single fungus. After incubation in the dark for 10 days at 25 °C the solution was filtered and the dry fungi weighed. The % Inhibition was calculated from:

$$\frac{\text{Test weight (g)} - \text{control weight (g)}}{\text{Control weight (g)}} \times 100.$$

RESULTS

The pharmacological action on guinea-pig ileum *in vitro* showed a strong spasmolytic activity for Manuka in contrast to an initial spasmogenic action for Kanuka and *Melaleuca*, which was followed by a spasmolytic action (Table 1). α-Terpineol and terpinen-4-ol produced a substantial spasmolytic action, while α-terpinene, β-pinene and α(−)pinene produced an initial contraction followed by spasmolysis at the same concentration; the (+) α-pinene enantiomer produced only a spasmogenic response. l,8-cineole produced a rise in tone of the electrically induced contractions.

The action of the three 'tea-tree' oils on the phrenic nerve diaphragm muscle illustrated a different response for *Melaleuca* and Manuka oils (showing both a decrease in tension and a delayed increase in resting tone, or

Table 1. Pharmacological studies on tea tree oils and their major components

Essential oil/ component	Pharmacological action	
Smooth Muscle Guinea-pig ileum Conc. 5×10^{-5} v/v	Spasmogenesis %	Spasmolysis %
Melaleuca oil	15 (3.5) initially	46.8 (4.6)
Manuka		38.1 (1.4)
Kanuka	28.6 (3.1) initially	59.7 (4.6)
Components		
1, 8-Cineole	25 (4.5) rise in tone	
α-Pinene (+)	7 (4.6)	
β-Pinene (−)	98 (4.7)	23.5 (3.5)
γ-Terpineol		86 (2.5)
α-Terpinene	78 (3.2)	87 (2.5)
γ-Terpinene	3.8 (0.5)	73 (2.2)
Terpinen-4-ol		93 (3.5)

contracture) compared with Kanuka, which showed no response at that concentration (Table 2). The two enantiomers of α-pinene showed a similar effect to that of *Melaleuca* and Manuka, but the response was lower in the (−) isomer (Table 2). The response was almost identical if the diaphragm muscle was stimulated directly.

Using the chick biventer muscle, all three 'tea-tree' oils produced a decrease in tension, in contrast to the increase in tone or contracture shown by α- and β-pinene and also p-cymene (Table 2).

The effect on the uterine muscle by all the 'tea-tree' oils and components was invariably a decrease in force, which in the case of α-terpinene was also accompanied by

Table 2. Pharmacological activity of *Melaleuca* oil and components in skeletal muscle

	Increase in resting tone	Decrease in tension
Rat phrenic nerve diaphragm at 2.5×10^{-3}		
Melaleuca		87.5 (4.8)
	+delayed increase to 100%	
Manuka		82.5 (2.5)
	+delayed increase to 100%	
Kanuka	No effect	
α-Pinene (+)		85 (4.0)
	+delayed increase to 56%	
β-Pinene (−)		18 (2.4)
Rat diaphragm direct at 2.5×10^{-3}		
Melaleuca		66.7 (3.8)
	+delayed increase to 100%	
Manuka		65.0 (8.8)
	+delayed increase to 100%	
Kanuka	No effect	
Chick biventer muscle at 1×10^{-3}		
Melaleuca		71.0 (5.8)
Manuka		27.8 (4.7)
Kanuka		24.0 (2.5)
α-Pinene	15% rise in resting tone	
β-Pinene	10% rise in resting tone	
ρ-Cymene	20% increased twitch, +14% increase in resting tone	

Numbers indicate mean (SEM) %

Table 3. Pharmacological activity of *Melaleuca* oil, Manuka and Kanuka and some of their components in uterine muscle

	Pharmacological effect
Rat uterus at 1×10^{-5}	
Melaleuca	26.0 (1.5) decrease in force[a]
Manuka (at 1×10^{-6})	100% decrease in force, quick recovery
Kanuka (at 1×10^{-6})	100% decrease in force, quick recovery
1,8-Cineole	51.0 (2.5) decrease in force
α-Pinene	21.0 (1.7) decrease in force
β-Pinene	25.0 (2.5) decrease in force
α-Terpineol	100% decrease in force and loss of contractions
α-Terpinene	100% decrease in force and frequency, quick recovery
ρ-Cymene	25 (0.5) decrease in force
Terpinen-4-ol	100% decrease in force

[a] Decrease in force of spontaneous contractions.

a decrease in the frequency of the spontaneous contractions (Table 3).

The antibacterial activity against 25 different bacterial species and 20 *Listeria monocytogenes* varieties was strongest for *Melaleuca* oils compared with Manuka and Kanuka oils from different sites in New Zealand (Table 4); different samples of the New Zealand Manuka and Kanuka oils showed considerable variation, whilst *Melaleuca* oils from different sites remained more constant.

The actual activity of two samples of *Melaleuca alternifolia* oil and some of their components against individual bacteria (Table 5a,b) showed some variation, with γ-terpinene exhibiting very low activities on all twenty five of 25 bacteria and α-terpinene showing only one high antibacterial activity. The individual activities against *Listeria monocytogenes* strains are shown in Table 6. The activities were generally low for all the components, with γ-terpinene exhibiting no activity at all.

The antifungal activity of the *Melaleuca* and Manuka

Table 4. Summary of the activity of the different samples of three 'tea-tree' oils against 25 different bacterial species and 20 different *Listeria monocytogenes* strains

	25 bacteria species	20 *Listeria* strains
Melaleuca alternifolia Austr.	24	20
Melaleuca alternifolia NZ	24	15
Manuka NI	15	20
Manuka SI	11	0
Kanuka NI	12	19
Kanuka SI	16	20
Kanuka/Manuka NI	18	18
1,8-Cineole	16	0
ρ-Cymene	6	0
Terpinene-4-ol	24	20
α-Terpinene	14	8
γ-Terpinene	5	0
α-Pinene	23	9

Table 5a. Antibacterial activity of 'tea-tree oils' from *Melaleuca alternifolia, Leptospermum scoparium* (Manuka) and *Kunzea ericoides* (Kanuka) and some of their main components against 25 different species of bacteria

	mm diameter					
Organism	a	b	c	d	e	f
1	9.1	5.4	6.0	6.3	6.8	0
2	12.9	5.8	6.4	7.8	7.6	5.9
3	9.4	5.7	0	11.6	9.8	5.6
4	10.7	0	14.3	7.3	6.3	6.3
5	11.6	6.5	5.4	4.9	9.3	6.3
6	11.4	6.5	10.6	8.7	8.0	7.7
7	8.5	6.3	19.9	10.7	7.6	10.6
8	11.0	0	0	0	0	0
9	16.0	12.2	26.0	31.5	9.3	22.0
10	8.3	0	0	0	0	0
11	11.8	0	10.6	5.6	0	0
12	10.4	5.5	6.3	12.8	6.3	0
13	5.8	0	5.9	7.0	0	0
14	5.9	0	0	0	0	0
15	8.3	5.7	4.7	5.4	5.9	5.1
16	6.9	0	16.7	5.7	7.0	5.7
17	10.4	0	24.2	6.5	6.0	5.0
18	9.5	0	0	0	0	0
19	12.2	0	0	0	5.5	0
20	9.1	0	0	0	0	0
21	9.8	0	0	7.4	0	0
22	8.0	0	0	6.5	0	0
23	7.6	8.9	11.7	12.6	12.3	10.8
24	6.2	0	8.0	14.4	6.0	0
25	9.3	0	0	0	5.3	0
No. +ve	9	5	9	8	9	8
No. −ve	14	6	6	10	7	4
Total	25	11	15	18	16	12

a, *Melaleuca* oil, Australian; b, Manuka from South Island; c, Manuka from North Island; d, Manuka/Kanuka mix; e, Kanuka from South Island; f, Kanuka from North Island.

Table 5b. Antibacterial activity of tea tree *Melaleuca alternifolia* Australian and New Zealand oil and some of its main components against 25 different species of bacteria

	(*Melaleuca* oils and components) mm diameter							
Organism	a	b	c	d	e	f	g	h
1	12.6	8.4	0	7.9	15.6	7.5	8.0	9.1
2	9.1	8.6	0	8.3	7.5	5.8	8.7	12.9
3	13.1	11.5	0	9.0	10.8	0	26.9	9.4
4	11.5	9.6	0	8.4	10.3	5.3	6.2	10.7
5	7.7	8.9	0	10.5	7.2	8.3	14.9	11.6
6	11.6	0	6.2	8.5	14.3	7.5	17.9	11.4
7	11.7	6.9	7.7	14.1	16.9	8.8	13.0	8.5
8	8.2	0	0	10.4	10.8	0	15.6	11.0
9	36.1	31.5	0	8.6	19.5	37.2	6.9	16.0
10	8.7	7.3	0	9.2	6.0	5.2	5.9	8.3
11	12.2	7.4	0	7.6	9.5	0	19.2	11.8
12	6.5	0	7.0	9.6	6.2	6.5	21.4	10.4
13	10.7	8.9	0	8.5	12.9	5.0	18.3	5.8
14	9.4	0	0	6.0	8.1	0	6.0	5.9
15	9.4	8.2	0	9.8	10.4	6.0	7.5	8.3
16	34.1	0	8.4	0	20.7	7.9	15.7	6.9
17	8.4	0	9.1	8.0	9.7	9.5	6.6	10.4
18	9.4	0	0	8.9	11.6	0	5.3	9.5
19	9.6	8.3	0	7.5	10.9	0	16.1	12.2
20	0	0	0	6.4	0	0	0	9.1
21	9.7	0	0	8.8	10.9	0	22.2	9.8
22	0	0	0	8.6	6.0	0	7.8	8.0
23	15.6	9.0	0	8.8	7.0	6.4	11.9	7.6
24	11.6	0	0	5.8	6.0	5.3	6.2	6.2
25	8.6	9.6	0	8.0	9.2	4.9	12.0	9.3
Total	23	14	5	24	24	16	24	25

a, α-pinene; b, β-terpinene; c, γ-terpinene; d, α-terpineol; e, terpinen-4-ol; f, 1,8-cineole; g, *Melaleuca* oil, New Zealand; h, *Melaleuca* oil, Australian.

and Kanuka oils from different sites (Table 7) again show greater differences in activity between the two Manuka and Kanuka samples than the *Melaleuca* samples. This difference is also reflected in the antioxidant activity (Table 8). Only γ-terpinene and terpinen-4-ol out of the components studied exhibited antioxidant activity.

DISCUSSION AND CONCLUSIONS

The possible therapeutic application of *Melaleuca*, Manuka and Kanuka was initially investigated by studying their mode of action in smooth muscle (using guinea-pig ileum), due to the possible absorption of at least some of its components, if not all (Bronough *et al.*, 1990). The pharmacological action was different between the 'tea-tree' oils, showing a strong spasmolytic activity for Manuka in contrast to an initial spasmogenic action for Kanuka and *Melaleuca*, which was followed by a spasmolytic action: this was a reflection of the difference in the chemical composition. Kanuka and *Melaleuca* oils contained a large proportion of monoterpenes, which have been shown to be related to the spasmogenic activity (Lis-Balchin *et al*, 1996b) whilst Manuka contained mostly sesquiterpenes. The differences in the activity of the individual components was pronounced: α-terpineol and terpinen-4-ol producing simply a strong spasmolysis, while α- and γ-terpinenes produced both an initial spasmogenic action followed by a spasmolytic action. 1,8-cineole gave a distinctive rise in tone, rather than a contraction, unlike any of the other components studied (Table 1). This is difficult to explain, as *Eucalyptus globulus*, containing about 90% of this component showed only a spasmolytic effect in the same tissue (Lis-Balchin *et al.*, l996b). The difference in activity between the enantiomers of α-pinene had previously been reported (Lis-Balchin *et al* 1999) and this could make a substantial difference to the pharmacological activity of different essential oils, depending on their enantiomeric proportions. *Melaleuca alternifolia* oils were found to contain many different enantiomeric components with different proportions of each enantiomer (Leach *et al.*, 1993); nowadays the commercial 'tea tree' oil is produced from many different species of *Melaleuca* in Australia and there are also possible enantiomeric differences in the oils produced from plants growing in other parts of the world giving rise to different grades of oil of different pharmacological action. Some of the so-called 'therapeutic grade tea-tree oils' now produced in Australia, contain double the proportion of terpinen-4-ol compared with normal grade oils and this could dramatically alter the pharmacological action.

Differences in the mode of action in guinea-pig ileum had previously been shown between Manuka and Kanuka

Table 6. Antibacterial activity of tea tree *Melaleuca alternifolia* Australian and New Zealand oil and some of its main components against *Listeria monocytogenes*

	Melaleuca oils and components mm diameter							
No. Bact.	a	b	c	d	e	f	g	h
1	0	5.9	0	7.3	5.4	0	5.8	8.7
2	7.0	6.6	0	6.0	6.4	0	5.9	8.0
3	0	0	0	6.8	5.0	0	5.6	6.9
4	6.5	0	0	7.4	6.5	0	5.4	8.6
5	6.0	5.6	0	6.6	5.1	0	0	9.2
6	0	0	0	5.4	6.3	0	5.1	7.7
7	0	0	0	5.9	7.0	0	0	7.8
8	0	0	0	7.5	6.1	0	0	9.0
9	9.1	0	0	7.2	5.6	0	8.9	8.5
10	0	0	0	6.0	6.4	0	5.6	7.7
11	6.4	6.0	0	6.0	5.5	0	5.9	6.7
12	0	6.6	0	7.0	5.2	0	6.2	6.5
13	0	0	0	6.6	5.5	0	8.3	8.8
14	0	5.1	0	7.5	6.2	0	0	9.1
15	5.1	0	0	7.9	5.4	0	6.0	7.1
16	7.0	0	0	7.9	5.4	0	6.0	7.1
17	7.0	0	0	7.0	5.5	0	6.0	8.0
18	11.9	0	0	5.9	6.2	0	0	7.6
19	0	6.6	0	7.2	5.5	0	5.9	7.3
20	0	6.6	0	6.0	5.7	0	7.2	7.3
Total no.	9	8	0	20	20	0	15	20

a, α-pinene; b, α-terpinene; c, γ-terpinene; d, α-terpineol; e, terpinen-4-ol; f, 1,8-cineole; g, *Melaleuca* oil; New Zealand; h, *Melaleuca* oil, Australian.

Table 8. Antioxidant activity of *Melaleuca alternifolia* oil against Manuka and Kanuka samples

Essential oil	Zone (mm)	Intensity
Melaleuca Australian	0	
Man 1	11.1	+
Man 2	8.2	+
Kan/Man	0	
Kan 1	17.7	
Kan 2	0	
α-Pinene	0	
α-Terpinene	7.1	+
α-Terpinene	0	
Terpinene-4-ol	9.1	+
α-Terpineol	0	

+ indicates a faint intensity.
Mel, *Melaleuca*; Man 1, Manuka from North Island; Man 2, Manuka from South Island; Man/Kan, Manuka/Kanuka mix; Kan 1, Kanuka from North Island ; Kan 2, Kanuka from South Island.

(Lis-Balchin and Hart, 1998) where there was some evidence for the involvement of cyclic AMP in the mechanism of action of Manuka, but not Kanuka; neither used cyclic GMP, and they did not seem to behave like calcium channel blockers of potassium channel openers. The activity of *Melaleuca* oils resembles Kanuka to some extent as the oil has an initial spasmogenic action followed by a potent spasmolytic action; the mechanism of the latter action was apparently neither via cAMP, nor cGMP or acting as potassium channel openers at low concentrations, but some evidence was obtained for action as calcium channel blockers at higher concentrations (Lis-Balchin and Hart, 2000).

Due to their use in body massage in aromatherapy, the action of the 'tea-tree' oils was studied in skeletal muscle, using chick biventer muscle and rat phrenic nerve diaphragm preparations. The action in skeletal muscle was also studied to ascertain whether their activity was similar to that in smooth muscle and secondly whether it was similar for all the 'tea-tree' oils. The action of Manuka was almost identical to that of *Melaleuca*, showing both a decrease in tension (through an inhibition of the twitch response to nerve stimulation) and a delayed but very profound increase in resting tone which signifies contracture whether the muscle was stimulated directly or via the phrenic nerve. The same actions were shown by clary sage, dill, fennel, frankinsence and nutmeg, all of which, except clary sage, are very strongly spasmogenic on smooth muscle (Lis-Balchin and Hart, 1997). Kanuka, at the same concentration, did not show any effect on either the nerve-stimulated or solely muscle-stimulated diaphragm, similar to that of angelica root oil (Lis-Balchin and Hart, 1997). The (+) enantiomer of α-pinene exhibited a similar reaction to Manuka and *Melaleuca*, but the (−) enantiomer showed just a decrease in tension at the same concentration: this again illustrated the difference in the enantiomeric pharmacological effect. The effect of Manuka showed a difference in the two muscle tissues and indicated a rather less relaxing effect on skeletal muscle than that obviously wanted through aromatherapeutic massage: the effect of Manuka on muscle fatigue or muscle cramp could thus be very detrimental.

The activity of the three 'tea-tree' oils on the chick biventer muscle was very similar showing a decrease in tension: *Melaleuca* produced more than double the effect

Table 7. Antifungal activity of *Melaleuca alternifolia* oil against Manuka and Kanuka samples

	Antifungal activity						
	NZ Mel	Austr Mel	Man1	Man2	M/K	Kanl	Kan2
Aspergillus niger	94	85	64	68	30	1	0
Aspergillus ochraceus	89	91	49	87	13	10	27
Fusarium culmorum	78	76	25	57	0	0	16

NZ Mel, New Zealand *Melaleuca*; Austr Med, Australian *Melaleuca*; Man 1, Manuka from North Island; Man Z, Manuka from South Island; M/K, Manuka/L6 Kanuka mix, Kaul, Kanuka from North Island; Kan Z, Kanuka from South Island.
Note: Numbers approaching 100 indicate most potent antifungal activity.

of the other oils. The pinenes and ρ-cymene showed an opposing effect of contracture.

The pharmacological action on uterine muscle *in vitro* indicated a similarity for all the 'tea-tree' oils and components alike and resembled previous studies with over 30 essential oils and mixtures of different essential oils (Lis-Balchin and Hart, 1997) where a reduction in the size of the spontaneous contractions always occurred. This was often accompanied by a reduction in the frequency of contractions for many essential oils. There is therefore a possible caution for the use of essential oils during childbirth, as they could stop contractions and thereby put the baby (as well as the mother) at risk.

There is a wide range of effects of different essential oils on the same skeletal muscle *in vitro* (Lis-Balchin and Hart, 1997), and the present results show differences also in the actual skeletal tissue studied. There is therefore difficulty in understanding the aromatherapists' belief (without any scientific evidence) that a given essential oil is 'relaxing', especially as they seem to imply it achieves this through its activity when applied via massage. Perhaps, it is the massage alone which is relaxing, as there is no clinical study as yet, to prove that essential oils actually have any further benefit (Lis-Balchin, 1997).

The present results support the work of Buchbauer *et al.* (1993) in showing that essential oils have selective actions on biological tissues and that their bioactivity is not due to non-specific toxic actions on cell membranes (Buchbauer *et al.*, 1993).

The antibacterial and antifungal activities was strongest for *Melaleuca* oils compared with Manuka and Kanuka oils (Table 4); different samples of the New Zealand Manuka and Kanuka oils showed considerable variation, whilst *Melaleuca* oils remained more constant. However, the production of more potent *Melaleuca* oils (labelled as therapeutic) by some Australian companies, which contain a higher percentage of terpinen-4-ol and lower 1,8-cineole indicates that there is an expected difference in antimicrobial activity: these oils were not included in the present study.

The data presented in Table 5 show how two different *Melaleuca* oils and their main components act against individual bacteria: α-terpineol has very low antibacterial activity against only 5 of 25 different species, whilst α-terpinene has only one potent activity and again shows low activity against the rest of the 14 of 25 bacteria. Terpinen-4-ol shows the highest activity against 24 bacteria, which is comparable to that of the whole oil, whilst 1,8-cineole shows reduced antibacterial activity against most of the species. The results are in agreement with studies by Carson and Riley (1994) who studied the antibacterial effect of eight *Melaleuca* components against 10 different bacteria. The results against 20 *Listeria monocytogenes* varieties show that γ-terpinene and 1,8-cineole have no effect, whilst α-pinene has a low effect against only eight varieties (Table 6) ; α-terpineol is slightly more effective than terpinen-4-ol against most of the *Listeria* varieties, which shows that generalizations cannot be made as to the effectiveness of individual essential oils or components against all bacteria. There was a marked difference in the activity between the two *Melaleuca* samples.

The antifungal activity of the different 'tea-tree' oils showed marked variations for the Manuka and Kanuka samples but not for the *Melaleuca* samples. The possibility of mistaking the two latter species can be demonstrated in the sample marked M/K (Manuka/Kanuka), which was very little different from the Kanuka samples in the antibacterial studies, but showed profound differences against the fungi. There was a very strong negative correlation between the pure Kanuka samples and the antifungal activity, compared with the more effective antifungal activity of Manuka; the mixture of Kanuka/Manuka showed an intermediate antifungal activity.

There is a vogue for many scientifically non-qualified aromatherapists to practise 'clinical aromatherapy', where they prescribe the internal usage of essential oils. Internal prescribing involves oral, rectal and vaginal intake: however, the use of tampons soaked in various potentially toxic essential oils, like the various 'tea-tree' oils with variable biological potential could have a possible harmful effect on the delicate internal mucosal membranes. The possibility of misdiagnosis of a urinogenital condition by medically unqualified aromatherapists or by the patients themselves could also result in serious consequences.

The antioxidant activity of the 'tea-tree' oils was very variable in the present studies, the Manuka samples showing more effectiveness than one of the Kanuka samples and the Kanuka/Manuka mixture. Only γ-terpinene and terpinen-4-ol showed antioxidant potential. The latter are also the most potent antibacterial agents and possibly exert some of this activity through their antioxidant action.

The problem with many Myrtaceae is that of their genetic variation or the production of spontaneous chemotypes, giving many different essential oil compositions with differing bioactivities. The quality of commercial Australian Tea tree has now been generally stabilized, mainly by the selection of clones and the blending of different essential oils from different species of *Melaleuca* to conform with the Australian Standards, but the problem of diversity remains in other parts of the world where *Melaleuca* species grow, e.g. Zimbabwe, New Zealand, Indonesia etc. The diversity of Manuka and Kanuka oils is even more pronounced and the use of these New Zealand oils, especially in aromatherapy may therefore be premature, unless their quality can be assured and also toxicological studies (which are available for all essential oils used in the Food and Cosmetics Industry) are undertaken.

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