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Antimicrobial effects of tea-tree oil and its major components on *Staphylococcus aureus, Staph. epidermidis* and *Propionibacterium acnes*

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A. RAMAN, U. WEIR AND S.F. BLOOMFIELD. 1995. Major components of two tea-tree oil samples were identified using thin layer and gas-liquid chromatography (TLC and GLC). Using a TLC-bioautographic technique, the tea-tree oils, terpinen-4-ol, α-terpineol and α-pinene were found to be active against *Staphylococcus aureus, Staph. epidermidis* and *Propionibacterium acnes* whereas cineole was inactive against these organisms. The MIC values of the three active compounds increased in the order α-terpineol < terpinen-4-ol < α-pinene for all three micro-organisms. MIC values of the tea-tree oils and terpinen-4-ol were lower for *P. acnes* than for the two staphylococci. This study supports the use of tea-tree oil in the treatment of acne, and demonstrates that terpinen-4-ol is not the sole active constituent of the oil.

INTRODUCTION

Tea-tree oil is obtained in Australia from various members of the *Melaleuca* genus (tea-trees). The commonest species used is *Melaleuca alternifolia*, the oil is obtained by steam-distillation of the leaves (Carson and Riley 1993). Tea-tree oil is a popular component of skin preparations, and a number of its suggested uses imply an anti-microbial effect (Drury 1991). Studies demonstrating the antimicrobial activity of the oil have been reviewed recently by Carson and Riley (1993), who point out that the contributions of the various oil components to activity have not been fully evaluated.

Tea-tree oil is recommended for the treatment of acne vulgaris. A study comparing tea-tree oil gel to benzoyl peroxide lotion demonstrated the efficacy of the oil for treating this condition (Bassett *et al.* 1990). *Propionibacterium acnes* and coagulase-negative staphylococci have been implicated in the aetiology of acne vulgaris (Shanson 1989), and it is possible that the oil works by eradicating these micro-organisms from acne lesions.

In this study, two commercial samples of tea-tree oil were compared. Thin layer chromatography (TLC), bioautography and agar dilution methods were used in order to investigate the activity of the oils and some of their components against *P. acnes* and the coagulase-negative *Staph. epidermidis* (Boyd and Hoerl 1991) which is commonly found on the skin. Since tea-tree oil has shown activity against *Staph. aureus* in a number of independent studies (Carson and Riley 1993), this organism served as a positive control in this investigation.

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MATERIALS AND METHODS

Test organisms

Propionibacterium acnes (NCTC 737), *Staph. aureus* (NCTC 9518) and *Staph. epidermidis* (NCTC 11047) were cultivated on tryptone soya agar (Oxoid Ltd, Basingstoke) slopes. For working cultures, one loopful of the slope culture was placed in tryptone soya nutrient broth (20 ml) and incubated for 24 h at 37°C (overnight culture, ONC).

Chemicals and test samples

Antiseptic tea-tree oil (ATTO) and Thursday Plantation tea-tree oil (TPTTO) were supplied by Dr G. J. Murtagh of the North Coast Agricultural Institute, Wollongbar, Australia. Terpinen-4-ol was obtained from Aldrich Chemical Co. Ltd, UK. α-Terpinene, γ-terpinene, cineole and *p*-iodonitrotetrazolium chloride (INT) were obtained from Sigma Chemicals, UK. *p*-Cymene, α-pinene and α-terpineol were available in the Pharmacognosy Laboratory.

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Chromatography

TLC was performed on silica gel G plates (0·25 mm) using toluene: ethylacetate (85 : 15) as mobile phase with double development. To visualize zones, plates were sprayed with an anisaldehyde reagent (anisaldehyde: methanol: sulphuric acid: acetic acid, 0·5 : 85 : 5 : 10) and heated for 15 min at 105°C. For bioautography, 1, 3 and 5 μl of the oil samples and terpenes were applied to each plate. GLC was performed using a 10 m × 0·3 mm Carbowax 20M capillary column with nitrogen carrier gas (30 ml min^{-1}), injection temperature 210°C, detector temperature 300°C and an oven temperature programme of 100°C for 4 min, then 100–160°C at 6° min^{-1}, then 160°C for 10 min.

Bioautography

Developed TLC plates were overlaid with molten agar inoculated with micro-organisms (2 ml of ONC in 100 ml of agar) and incubated for 24 h at 37°C. The overlaid plates were sprayed with INT solution (2 mg ml^{-1}) and incubated for a further 30 min. Areas of microbial growth stained pink, zones of inhibition appeared pale yellow.

Minimum inhibitory concentration (MIC)

Oil samples and terpenes (1 ml) were added separately to sterile molten agar (20 ml) in sealed bottles, shaken well and left overnight at 55°C to allow mixing. Eight serial 1 : 1 dilutions were performed to give concentrations of 0·02–5% v/v of each substance, with prolonged shaking before each sampling. Plates were poured from each dilution, and 10 μl of ONC of the three micro-organisms applied to different sections of the plate. The presence or absence of growth of the organisms was noted after overnight incubation at 37°C. Each test was performed in duplicate.

RESULTS

The two oil samples ATTO and TPTTO gave very similar TLC and GLC profiles (Tables 1 and 2). From the TLC results, it was confirmed that the oil contained terpinen-4-ol and 1,8-cineole. Although TPTTO and ATTO gave zones at the same R_f values as α-terpinene and cymene, these zones had different colours from the reference substances. The TLC system did not resolve α-pinene and α-terpineol, but these compounds were well separated by GLC and peaks corresponding to these were present in the oils. Thus the main compounds identified in the oils were α-pinene, α-terpineol, terpinen-4-ol and 1,8-cineole. The GLC results (normalized areas) showed that the proportions of these substances varied in the two oils.

In bioautography (Table 3), the two oils, α-pinene, α-terpineol and terpinen-4-ol were all active against the three micro-organisms in a dose-dependent manner. In similar amounts, cineole was inactive. In general, the zones of inhibition obtained with TPTTO were larger than for ATTO,

Table 1 Results of TLC analysis of antiseptic tea-tree oil (ATTO) and Thursday Plantation tea-tree oil (TPTTO) against reference terpenes

Test substance	R_f value and colour reaction of major zones observed								
ATTO	0·02 pink	0·10 purple	0·23 pink	0·41 purple	0·51 purple	0·55 purple	0·69 purple	0·77 purple	1·00 purple
TPTTO	0·02 pink	0·10 purple	0·23 pink	0·41 purple	0·51 purple	0·55 purple	0·69 purple	0·77 purple	1·00 purple
α-Terpinene				0·42 green					
α-Pinene					0·51 purple				
α-Terpineol					0·51 purple				
Cymene						0·55 pink			
Terpinen-4-ol							0·67 purple		
1,8-Cineole								0·77 purple	

Table 2 Results of GLC analysis of antiseptic tea-tree oil (ATTO) and Thursday Plantation tea-tree oil (TPTTO) against reference terpenes

Test substance	R_f values (and % area by normalization) of major peaks observed						
ATTO	1·06 (11·95)	4·86 (14·63)	6·34 (7·20)	—	14·71 (29·50)	15·25 (17·74)	17·03 (10·73)
TPTTO	1·23 (24·87)	4·82 (12·47)	6·33 (6·25)	—	14·72 (28·59)	15·25 (4·69)	17·04 (3·15)
α-Pinene	1·27						
α-Terpinene		4·77					
γ-Terpinene			6·46				
1,8-Cineole				14·12			
Terpinen-4-ol					14·77		
p-Cymene						15·12	
α-Terpineol							17·16

but this was not reflected in a difference in MIC values (Table 4). The two oils had similar MIC values, but both had lower values for *P. acnes* than for the staphylococci. The MIC values of the terpenes (Table 4) against all three organisms increased in the order α-terpineol < terpinen-4-ol < α-pinene.

DISCUSSION

The results of this study confirm the anti-microbial effects of tea-tree oil and indicate that there is not much difference in activity between the two commercial samples tested. Activity of the oil against *P. acnes* and *Staph. epidermidis* supports its use in the treatment of acne. The MIC values of between 0·31 and 0·63% v/v obtained in this study for *P. acnes* are in good agreement with values of 0·25–0·5% quoted by Carson and Riley (1994) for 32 strains of this organism. *Propionibacterium acnes* seems more sensitive than the staphylococci to the oil and its components under the conditions of this experiment.

Terpinen-4-ol is generally believed to be the active constituent of the oil (Williams *et al.* 1988), but in this experiment, α-terpineol and α-pinene were also found to be active. Despite the low content of α-terpineol (2–12%) compared to terpinen-4-ol (up to 50%; Williams *et al.* 1988), it has a lower MIC value and must therefore make some contribution to the antimicrobial effect of the oil. Cineole was found to be inactive against all three micro-organisms. The current trend is to promote low cineole content tea-tree oils as these are less irritant to the skin. This study suggests that there would be no loss of antimicrobial activity as a result of this. However, it must be noted that a recent study found that some patients

Table 3 Zones of inhibition (cm^2) produced by antiseptic tea-tree oil (ATTO), Thursday Plantation tea-tree oil (TPTTO) and some constituent terpenes using TLC-bioautography

	Zones of inhibition (cm^2)								
	Propionibacterium acnes			*Staphylococcus epidermidis*			*Staph. aureus*		
Substance	1 μl	3 μl	5 μl	1 μl	3 μl	5 μl	1 μl	3 μl	5 μl
ATTO	0·0	6·4	7·8	6·3	13·8	21·0	7·5	15·0	23·0
TPTTO	3·7	8·7	11·0	7·3	18·0	22·7	14·0	18·0	24·0
Cineole	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
Terpinen-4-ol	0·0	6·0	6·0	0·8	2·0	4·5	1·0	4·0	4·25
α-Pinene	7·8	14·0	15·0	3·0	10·0	12·0	4·0	10·5	16·0
α-Terpineol	1·4	3·5	8·80	1·4	6·5	5·8	1·5	13·0	13·0

Table 4 Summary of minimum inhibitory concentration (MIC) values (in % v/v) of antiseptic tea-tree oil (ATTO), Thursday Plantation tea-tree oil (TPTTO) and some constituent terpenes

Organism	MIC values (% v/v)				
	ATTO	TPTTO	α-Pinene	α-Terpineol	Terpinen-4-ol
Staphylococcus aureus	0·63–1·25	0·63–1·25	1·25–2·50	0·16–0·31	0·31–0·63
Staphylococcus epidermidis	0·63–1·25	0·63–1·25	>2·50	0·08–0·16	0·31–0·63
Propionibacterium acnes	0·31–0·63	0·31–0·63	>2·50	0·08–0·16	0·16–0·31

sensitive to tea-tree oil also showed an allergic reaction to terpinen-4-ol (Knight and Hansen 1994).

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