

Melaleuca Oil  
- A Natural Healthy Alternative

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## 1. Introduction

Australian Tea Tree Oil (TTO) has been used by the indigenous aboriginal people of Australia for thousands of years for a variety of ailments. After being acclaimed as a near perfect antiseptic in the 1930's its use declined with the discovery of the sulphonamides and antibiotics. It has now been rediscovered as a safe and effective antiseptic without many of the problems associated with synthetic antiseptics. It is proving to be a most versatile ingredient in human and veterinary pharmaceuticals, personal care products and household sanitation applications. The many features of Tea Tree Oil include a natural product from a renewable resource, broad spectrum of activity, a mild local anaesthetic effect and environmentally safe. Tea Tree Oil is an excellent solvent, penetrates skin and mucous membranes, is non staining, has a low incidence of skin irritation and a long history of use.

## 2. Chemistry

In common with other essential oils, Australian TTO is a complex mixture of individual components. These are mainly monoterpenes, sesquiterpenes and terpene alcohols. Over 90 components have been isolated from the oil.

Analysis of a typical oil is given in Fig 1. These compounds analysed account for around 95% of the components of the oil. Also shown is the composition of the oil (taken from a large commercial production batch of 6000L) used in our studies.

Component	ISO 4730 Standard	Sample Oil
1,8 cineole	max 15%	4.6%
a-terpinene	5.0 to 13.0%	7.8%
g-terpinine	10.0 to 28.0%	17.1%
p-cymene	0.5 to 12.0%	2.0%
terpinen-4-ol	min 30%	41.6%
a-terpineol	1.5 to 8.0%	3.4%
a-pinene	1.0 to 6.0%	1.8%
terpinolene	1.5 to 5.0%	3.2%
limonene	0.5 to 4.0%	1.0%
aromadendrene	trace to 7%	
cadinene	trace to 8%	
globulol	trace to 3%	
viridiflorol	trace to 1.5%	
sabinene	trace to 3.5%	

**Fig 1 ISO Standard and Analysis of Melaleuca alternifolia oil terpinene -4-ol type**

There is an inverse relationship between content of 1,8 - cineole and terpinene-4-ol. As the content of 1,8 - cineole increases the content of terpinene-4-ol decreases (3). In some oils, the higher cineole content produces an odour profile much closer to Eucalyptus Oil. These oils are less acceptable for personal care products, but may still comply with the ISO standard.

## 3.Characterisation of the Oil Used in our Studies

Because the cineole component has been associated with skin irritation, it is important to formulate with high quality oil which far exceeds the minimum ISO requirement. We do not work with oil with a cineole level above 5%, and most oils produced in Australia do not exceed

this 5% content. The Melaleuca Oil used in our studies was produced by Main Camp in their plantation on the N.S.W. north coast and complies with the ISO standard.

#### 4. Degradation pathways of Tea Tee Oil Components

Tea tree oil may undergo two major chemical degradation routes. In one direction, oxidation of the alpha-terpene, gamma-terpinene, alpha-terpinolene and related components leads to an increase in the p-cymene content of the oil. In the other direction, hydrolysis of these same compounds will lead to an increase in terpinene-4-ol content. Thus, in theory, hydrolysis of the oil may lead to an increase in antimicrobial activity. Figure 2 shows the degradation pathways of the major Tea Tee Oil components.

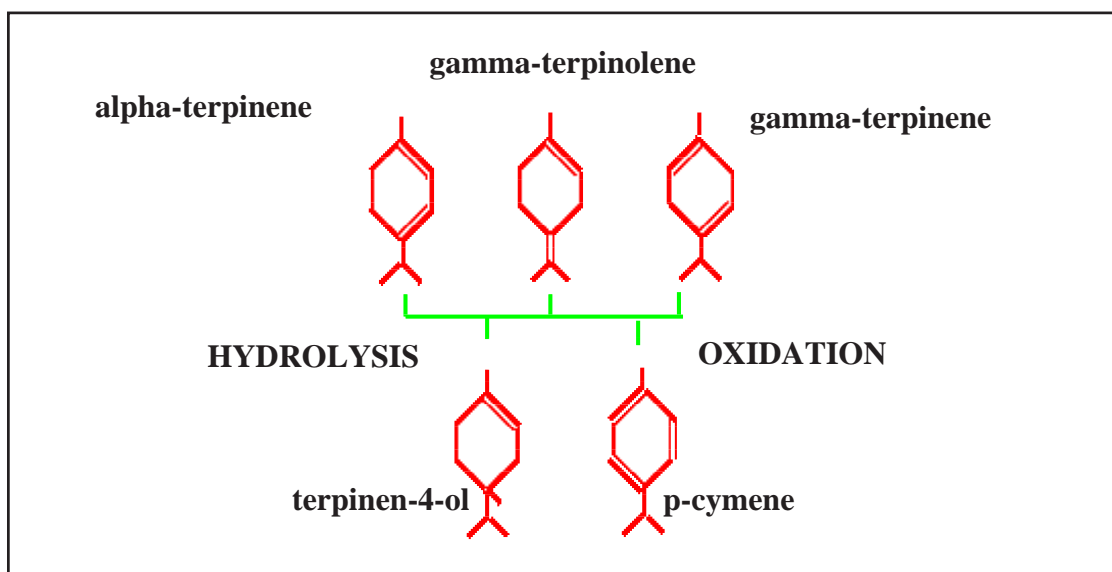


Figure 2: Degradation Pathways of Tea Tree Oil Components

#### 5. Formulation considerations in tea tree oil products

##### 5.1 Solubilisation of Tea Tree Oil

Tea Tree oil may be solubilised by a variety of surfactants. It was found necessary to utilise 4 parts of polysorbate 20 to one part Tea Tree Oil to achieve a clear solution on subsequent dilution with an infinite amount of water. However only 1.5 parts of the surfactant, polyoxyl 35 castor oil was required to effect the same result. The method of preparation is important and involves allowing the oil and surfactant to be mixed well and allowed to stand for a few minutes. Water is subsequently added gradually with constant stirring until a gelatinous mass results due to hydration of the oil. Further addition of water results in a lowering of the viscosity and a clear liquid. Should the initial addition of water be done too quickly or the TTO not adequately mixed then at best an opalescent solution results.

The use of polyhydroxyalcohols such as glycerol or propylene glycol can effect solubilisation without having to go through the initial gelling phase. This can be achieved by triturating the glycerol with the mixture of the surfactant and Tea Tree Oil, prior to the addition of water. The addition of alcohol to the formulation enhances solubilisation producing a clear solution. Typically a range of 5 to 10% alcohol is employed.

##### 5.2. Effect of Concentration and Type of Surfactant

The effects of various surfactants and solubilising agents on the antimicrobial activity of a range of antiseptic and disinfectant products has been well documented. The present investigation aimed to determine the effect of varying the concentration of surfactants on the antimicrobial activity of TTO. The concentration of TTO remained constant at 0.5% and the surfactants were varied to

achieve a ratio of Oil:Surfactant of between 1:1 to 1 : 5 on a weight for weight basis. Simple aqueous solutions of TTO were prepared and subjected to a combined USP/BP preservative efficacy test. The two surfactants investigated were polyoxyl 35 castor oil and polysorbate 20 and the results were as follows:

- a. Polyoxyl 35 castor oil had no effect on TTO activity when used as the solubilising agent in the range of concentration studied (Up to 2.5% surfactant with 0.5% oil). All formulations passed the USP test criteria and all passed the BP criteria with the exception of the mould *A.niger*.
- b. Increasing the polysorbate 20 : TTO ratio decreased the activity of the TTO as the ratio of surfactant to oil increased from between 1 : 1 and 1 : 5 (oil to surfactant). Higher levels of this surfactant resulted in failure of the BP/USP test for both some bacteria and yeast and mould.

These findings are in simple aqueous solution. In formulated products it can be anticipated that the situation will be even more complex.

### 5.3 Effect of other Excipients

The effect of a variety of commonly employed excipients on the activity of TTO was examined in a simple 5% aqueous solution of TTO solubilised with 10% polyoxyl 35 castor oil. Figure 3 shows that the addition of some excipients cause the simple formulation to fail the BP requirements for preservative efficacy. However all formulations with added excipients do pass the less stringent USP test for preservative efficacy.

Excipient		B.P.	U.S.P	
Sodium lauryl sulphate 2%		√	√	
Sodium laureth sulphate 2%		x	√	
Cocoamidobetaine 5%		√	√	
Polysorbate 20 5%		x	√	
Sodium Edetate 0.2%		√	√	
Capric/Caprylic triglycerides 2%		√	√	
Cetomacrogol Emulsifying Wax 5%		√	√	
Propylene Glycol 5%		x	√	
Ethanol 2%			x	√
Propylene Glycol 5%		x		√
Carboxy Polymer 0.5%		x		√
Sepigel 2%			x	√
Hydroxyethyl cellulose 0.2%	x			√

**Fig 3 : Effect of Excipients on Tea Tree Activity.**

### 5.4 Effect of pH

The pH of the final product can be critical to the activity of TTO formulations. TTO aqueous solutions (5%), solubilised with polyoxyl 35 castor oil were adjusted to varying pH values with organic and inorganic acid and base. Either lactic acid/ sodium lactate or hydrochloric acid/ potassium hydroxide was used for this purpose. It was observed that when the pH is adjusted with inorganic acid or base then extreme pH values are preferred for maximum activity of the oil. Utilising organic pH adjustments neutral pH values seem to be preferable. The basis for these findings are still to be determined.

### 5.6 Phase Partitioning

It is well recognised that the antimicrobial activity of multi-phase systems often resides in the

aqueous phase. To estimate the likely concentration of oil in the aqueous phase of a simple two component system, samples were prepared containing equal parts of Capric/Caprylic triglycerides and water. To this 0.5% TTO was added, either with or without prior addition of polyoxyl 35 castor oil.

The samples were vortex mixed and allowed to equilibrate for 3 days, with occasional hand shaking. The ratio of TTO in each phase was determined by gas chromatography assay of terpinene-4-ol after centrifugation. The results show that in the preparation without surfactant, almost all of the TTO had migrated into the oil phase, whilst in the preparation containing surfactant, 80% of the TTO remained in the aqueous phase. These results were consistent for ratios of oil to surfactant from 1:1 to 1:3. This has important implications in the formulation of products when TTO is to be used as a preservative.

## 6. Microbiological Activity

TTO is a broad spectrum antibacterial agent. This has been evidenced by a number of Minimum Inhibitory Concentration (MIC) studies (Fig 4). MIC values against commonly encountered gram positive and gram negative bacteria are commonly in the range of 0.5 to 1.0%. Figure 5 also shows our own results for MIC's against these organisms in solubilised and free oil form as a gum suspension. There has been considerable debate as to the major contributor to the activity of the oil, with terpinene-4-ol being noted as the major active component. However, it is apparent that this component alone does not account for the high activity of the oil. A study by Southwell et al in 1993 (1) showed that a content of cineole between 1.5% and 28.5% did not influence the MIC performance of the oil against *E. coli*, *S. aureus*, or *C. albicans*, provided the terpinene-4-ol content was maintained above 30%.

We have utilised a suspension test as a measure of antimicrobial activity in some studies on formulated products. This involves challenging the product with the test organism, with subsequent sampling at various time intervals and noting the rate of reduction in surviving organisms compared to a saline control. Effectively this method enables the rates of kill to be compared over a short time period. We do not know at this point how this correlates to the MIC test, but we can see that this is a far more demanding challenge for the oil, which still produces spectacular results against some species.

Organism	Previously Reported	Solubilised Oil	Gum Susp. of free oil
<i>Staph. aureus</i>	0.2	0.1	<0.05
<i>Staph. epidermidis</i>	0.5	0.2	<0.05
<i>Strept. mutans</i>	0.4	0.4	0.4
<i>Strept. pyogenes</i>	1	0.5	0.5
<i>P. aeruginosa</i>	1-2	2.0	2.0
<i>P. cepacia</i>	?	0.5	0.5
<i>S. marcescens</i>	0.2-0.3	0.4	0.1
<i>C. albicans</i>	0.2	0.1	0.1
<i>A. niger</i>	0.3-0.4	0.4	0.4
<i>T. rubrum</i>	1.0	1.0	1.0

**Fig 4 Typical Minimum Inhibitory Concentrations of Tea Tree Oil**

## 7. Synergisation of Tea Tree Oil

Topical nicotinamide has previously been employed in cell-mediated tissue injury (2). Recently it has been successfully used the treatment of acne because of its anti-inflammatory effect (3). Nicotinamide has not been previously reported to have antimicrobial properties and initial

studies sought to combine the antibacterial activity of TTO with the anti-inflammatory effect of nicotinamide in the treatment of acne vulgaris. However microbial challenge tests using the Suspension Test Method as described above showed an unexpected synergistic combination between TTO and nicotinamide against the organism Propionobacter acnes, the principal organism associated with acne. Figure 5 shows the synergistic effect of nicotinamide and TTO in a typical cream formulation using a Suspension Test with P.acnes as the test organism compared to a saline control in which the concentration of both nicotinamide and TTO were both 5%. It was observed that when nicotinamide was present a greatly enhanced kill rate results. \*\*

Actives	5 min	15 min	30 min	45 min	60 min
TTO 5%	$2.0 \times 10^6$		$4.8 \times 10^5$	$9.5 \times 10^4$	
TTO 5% Nic 5%	$1.4 \times 10^4$	$6.1 \times 10^3$	$2.5 \times 10^2$	<10	<10
Saline Control	<10	$6.5 \times 10^6$		$7.0 \times 10^6$	

- Suspension Test using P. acnes ATCC 6919 cfu/mL against time. Inoculum Density  $6.5 \times 10^6$

**Fig 5 : Effect of TTO Nicotinamide Combination on a Typical Cream**

Further studies as illustrated in Figure 6 show similar findings and confirm that nicotinamide alone exhibits no antibacterial effect. The efficacy of two commercially available preparations are also shown for comparison. The mechanism for the synergistic combination and its clinical significance has not been established.

Contact Time	5 min	15 min	30 min	45 min	60 min
Control		$8.3 \times 10^6$			$1.5 \times 10^7$
Base alone		$>2.5 \times 10^6$	$>2.5 \times 10^6$	$>2.5 \times 10^6$	$>2.5 \times 10^6$
Base/TTO		$2.7 \times 10^6$	$2.5 \times 10^6$	$2.2 \times 10^6$	$8.2 \times 10^5$
Base/Nic		$4.7 \times 10^6$	$2.8 \times 10^6$	$1.4 \times 10^6$	$2.0 \times 10^5$
Base/TTO/Nic		$4.3 \times 10^6$	$3.4 \times 10^6$	$3.5 \times 10^6$	$3.1 \times 10^6$
BenzacW10		$1.8 \times 10^6$	$6.8 \times 10^4$	$2.6 \times 10^3$	$1.1 \times 10^2$
Ultra Clearasil		$2.2 \times 10^6$	$2.7 \times 10^3$	$5.0 \times 10^1$	<10
Control					
Base alone					
Base/TTO					
Base/Nic					
Base/TTO/Nic					
BenzacW10					
Ultra Clearasil					

**Fig 6 : Effect of Nicotinamide / TTO Combination on P.acnes**

## 8. Preservation

As well as the now established antibacterial uses for the oil, there has recently been a great deal of interest in the area of personal care product preservation and TTO is an ideal candidate, because of its natural origin. Priest (4) has shown that it is possible to formulate clear solutions with preservative levels of TTO at around 0.5%, but the more demanding requirements of the preservative efficacy test of the British Pharmacopoeia over that of the United States Pharmacopoeia are not always met and present a challenge to the formulator.

The present investigation sought to complement the antimicrobial action of TTO with a variety of aromatic alcohols with the view to producing the ideal combination product which would be suitable as a preservative for a range of pharmaceutical and personal care products. The alcohols chosen for the study were benzyl alcohol, phenoxyethanol and phenyl ethyl alcohol. These were chosen because benzyl alcohol while failing the BP preservative efficacy test at a level of 0.5% in simple aqueous solution is active against *A.niger*, but has weaker activity against bacteria, particularly *S.aureus*. On the other hand, TTO while having relatively poor activity against *A.niger* has excellent activity against the gram positive and negative bacteria. It was thought that a combination of a suitable aromatic alcohol and TTO might be an ideal combination for a preservative mix.

A range of combinations of the various alcohols and TTO were prepared. The concentrations ranged from 0.2% to 1.0% of the actives which were solubilised using 1.0% polyoxyl 35 castor oil and diluted to volume with water. The results of combined BP/USP preservative efficacy tests are shown in Figure 7. These studies indicate that at a level of 0.5%, neither the alcohols nor TTO will pass both the BP and USP criteria. However, if used in combination, at a level of 0.5% alcohol, a level of 0.3% TTO is sufficient to enable the combination to satisfy both pharmacopoeial requirements.

Combination			Monograph		U.S.P.	
TTO	BA	PHE	PEA	B.P.		
0.1					X	√
0.3					X	√
0.5					X	√
1.0					X	√
	0.5				X	X
0.5	0.2				X	X
0.5	0.5				√	√
0.3	0.2				X	X
0.3	0.5				√	√
		0.5			X	√
		1.0			√	√
0.5		0.2			√	√
0.5		0.5			√	√
0.3		0.2			X	√
0.3		0.5			√	√
			0.5		X	X
			1.0		√	√
0.5			0.2		X	X
.5			0.5		√	√
0.3			0.2		X	X
0.3			0.5		√	√

BA = Benzyl alcohol PHE = Phenoxyethanol PEA = Phenylethylalcohol

### **Figure 7: Preservative Combinations of Tea Tree Oil. ( Solubilised)**

Most of the failures at lower concentrations of the alcohols are due to poor activity of the combination against *A.niger*, whilst low concentrations of TTO result in poorer activity against the gram positive and negative bacteria.

### **9. Packaging Considerations**

The pure oil has high plasticiser and solvent properties which need to be taken into account in packaging design. It is also volatile and can permeate many plastics. The main components which present difficulties are polystyrene and lower density polyethylene.

As cosmeceuticals would typically contain 0.25 - 2% oil, these incompatibilities are not of as much importance and aluminium tubes and glass do not appear to present any problem. Newer materials such as P.E.T., surface flourinated polypropylene, multi-laminate tubes and co-extruded bottles are all generally suitable. Naturally, it is always necessary to perform stability testing to verify this. For pharmaceutical products with higher levels of oil content, packaging presents a major consideration.

### **10. Summary**

1. Australian Tea Tree Oil from *Melaleuca terpinene-4-ol* type is a versatile material for use in a variety of pharmaceutical and personal care products.
2. Tea Tree oil, by virtue of its natural origins, has great attraction as a renewable resource, non-animal derived material.
3. TTO and nicotinamide act synergistically against *P.acnes*, the main organism associated with acne when tested by a Suspension Test.
3. The oil can be used successfully to produce "preservative free" formulations with internationally recognised preservative efficacy.

### **11. Acknowledgements**

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\*\* Subject of current Patents by International Treaty

### **12. References**

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