Melaleuca alternifolia (Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties

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INTRODUCTION

Many complementary and alternative medicines have enjoyed increased popularity in recent decades. Efforts to validate their use have seen their putative therapeutic properties come under increasing scrutiny in vitro and, in some cases, in vivo. One such product is tea tree oil (TTO), the volatile essential oil derived mainly from the Australian native plant *Melaleuca alternifolia*. Employed largely for its antimicrobial properties, TTO is incorporated as the active ingredient in many topical formulations used to treat cutaneous infections. It is widely available over the counter in Australia, Europe, and North America and is marketed as a remedy for various ailments.

COMPOSITION AND CHEMISTRY

TTO is composed of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. Terpenes are volatile, aromatic hydrocarbons and may be considered polymers of isoprene, which has the formula C_5H_8. Early reports on the composition of TTO described 12 (65), 21 (3),

and 48 (142) components. The seminal paper by Brophy and colleagues (25) examined over 800 TTO samples by gas chromatography and gas chromatography-mass spectrometry and reported approximately 100 components and their ranges of concentrations (Table 1).

TTO has a relative density of 0.885 to 0.906 (89), is only sparingly soluble in water, and is miscible with nonpolar solvents. Some of the chemical and physical properties of TTO components are shown in Table 2.

Given the scope for batch-to-batch variation, it is fortunate that the composition of oil sold as TTO is regulated by an international standard for "Oil of *Melaleuca*—terpinen-4-ol type," which sets maxima and/or minima for 14 components of the oil (89) (Table 1). Notably, the standard does not stipulate the species of *Melaleuca* from which the TTO must be sourced. Instead, it sets out physical and chemical criteria for the desired chemotype. Six varieties, or chemotypes, of *M. alternifolia* have been described, each producing oil with a distinct chemical composition. These include a terpinen-4-ol chemotype, a terpinolene chemotype, and four 1,8-cineole chemotypes (83). The terpinen-4-ol chemotype typically contains levels of terpinen-4-ol of between 30 to 40% (83) and is the chemotype used in commercial TTO production. Despite the inherent variability of commercial TTO, no obvious differences in its bioactivity either in vitro or in vivo have been noted so far. The suggestion that oil from a particular *M. alternifolia* clone possesses en-

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TABLE 1. Composition of *M. alternifolia* (tea tree) oil

Component	Composition (%)	
	ISO 4730 range[a]	Typical composition[b]
Terpinen-4-ol	≥30[c]	40.1
γ-Terpinene	10–28	23.0
α-Terpinene	5–13	10.4
1,8-Cineole	≤15[d]	5.1
Terpinolene	1.5–5	3.1
ρ-Cymene	0.5–12	2.9
α-Pinene	1–6	2.6
α-Terpineol	1.5–8	2.4
Aromadendrene	Trace–7	1.5
δ-Cadinene	Trace–8	1.3
Limonene	0.5–4	1.0
Sabinene	Trace–3.5	0.2
Globulol	Trace–3	0.2
Viridiflorol	Trace–1.5	0.1

[a] IOS 4730, International Organization for Standardization standard no. 4730 (from reference 89).
[b] From reference 25.
[c] No upper limit is set, although 48% has been proposed.
[d] No lower limit is set.

hanced microbicidal activity has been made (106), but the evidence is not compelling.

The components specified by the international standard were selected for a variety of reasons, including provenance verification and biological activity. For example, with provenance, the inclusion of the minor components sabinene, globulol, and viridiflorol is potentially helpful, since it may render the formulation of artificial oil from individual components difficult or economically untenable. With biological activity, the antimicrobial activity of TTO is attributed mainly to terpinen-4-ol, a major component of the oil. Consequently, to optimize antimicrobial activity, a lower limit of 30% and no upper limit were set for terpinen-4-ol content. Conversely, an upper limit of 15% and no lower limit were set for 1,8-cineole, although the rationale for this may not have been entirely sound. For many years cineole was erroneously considered to be a skin

TABLE 2. Properties of TTO components

Component	Type of compound	Chemical formula	Solubility (ppm)[a]	Log K_{OW}[b]
Terpinen-4-ol	Monocyclic terpene alcohol	$C_{10}H_{18}O$	1,491	3.26
γ-Terpinene	Monocyclic terpene	$C_{10}H_{16}$	1.0	4.36
α-Terpinene	Monocyclic terpene	$C_{10}H_{16}$	8.2	4.25
1,8-Cineole	Monocyclic terpene alcohol	$C_{10}H_{18}O$	907	2.84
α-Terpinolene	Monocyclic terpene	$C_{10}H_{16}$	4.3	4.24
ρ-Cymene	Monocyclic terpene	$C_{10}H_{14}$	6.2	
(+)-α-Pinene	Dicyclic terpene	$C_{10}H_{16}$	0.57	4.44
α-Terpineol	Monocyclic terpene alcohol	$C_{10}H_{18}O$	1,827	3.28
Aromadendrene	Sesquiterpene	$C_{15}H_{24}$		
δ-Cadinene	Sesquiterpene	$C_{15}H_{24}$		
(+)-Limonene	Monocyclic terpene	$C_{10}H_{16}$	1.0	4.38
Sabinene	Dicyclic monoterpene	$C_{10}H_{16}$		
Globulol	Sesquiterpene alcohol	$C_{15}H_{26}O$		

[a] From reference 63.
[b] K_{ow}, octanol-water partition coefficient, from reference 62.

and mucous membrane irritant, fuelling efforts to minimize its level in TTO. This reputation was based on historical anecdotal evidence and uncorroborated statements (20, 55, 98, 126, 153, 156–158), and repetition of this suggestion appears to have consolidated the myth. Recent data, as discussed later in this review, do not indicate that 1,8-cineole is an irritant. Although minimization of 1,8-cineole content on the basis of reducing adverse reactions is not warranted, it remains an important consideration since 1,8-cineole levels are usually inversely proportional to the levels of terpinen-4-ol (25), one of the main antimicrobial components of TTO (36, 48, 71, 126).

The composition of TTO may change considerably during storage, with ρ-cymene levels increasing and α- and γ-terpinene levels declining (25). Light, heat, exposure to air, and moisture all affect oil stability, and TTO should be stored in dark, cool, dry conditions, preferably in a vessel that contains little air.

PROVENANCE AND NOMENCLATURE

The provenance of TTO is not always clear from its common name or those of its sources. It is known by a number of synonyms, including "melaleuca oil" and "ti tree oil," the latter being a Maori and Samoan common name for plants in the genus *Cordyline* (155). Even the term "melaleuca oil" is potentially ambiguous, since several chemically distinct oils are distilled from other *Melaleuca* species, such as cajuput oil (also cajeput or cajaput) from *M. cajuputi* and niaouli oil from *M. quinquenervia* (often misidentified as *M. viridiflora*) (51, 98). However, the term has been adopted by the Australian Therapeutic Goods Administration as the official name for TTO. The use of common plant names further confounds the issue. In Australia, "tea trees" are also known as "paperbark trees," and collectively these terms may refer to species in the *Melaleuca* or *Leptospermum* genera, of which there are several hundred. For instance, common names for *M. cajuputi* include "swamp tea tree" and "paperbark tea tree," while those for *M. quinquenervia* include "broad-leaved tea tree" and "broad-leaved paperbark" (98). Many *Leptospermum* species are cultivated as ornamental plants and are often mistakenly identified as the source of TTO. In addition, the essential oils kanuka and manuka, derived from the New Zealand plants *Kunzea ericoides* and *Leptospermum scoparium*, respectively, are referred to as New Zealand TTOs (42) although they are very different in composition from Australian TTO (125). In this review article, the term TTO will refer only to the oil of *M. alternifolia*.

As explained above, the international standard for TTO does not specify which *Melaleuca* species must be used to produce oil. Rather it sets out the requirements for an oil chemotype. Oils that meet the requirements of the standard have been distilled from *Melaleuca* species other than *M. alternifolia*, including *M. dissitiflora*, *M. linariifolia*, and *M. uncinata* (113). However, in practice, commercial TTO is produced from *M. alternifolia* (Maiden and Betche) Cheel. The *Melaleuca* genus belongs to the Myrtaceae family and contains approximately 230 species, almost all of which are native to Australia (51). When left to grow naturally, *M. alternifolia* grows to a tree reaching heights of approximately 5 to 8 meters (45). Trees older than 3 years typically flower in October and November (12, 98), and flowers are produced in loose, white to

creamy colored terminal spikes, which can give trees a "fluffy" appearance (155).

COMMERCIAL PRODUCTION

The commercial TTO industry was born after the medicinal properties of the oil were first reported by Penfold in the 1920s (121–124) as part of a larger survey into Australian essential oils with economic potential. During that nascent stage, TTO was produced from natural bush stands of plants, ostensibly *M. alternifolia*, that produced oil with the appropriate chemotype. The native habitat of *M. alternifolia* is low-lying, swampy, subtropical, coastal ground around the Clarence and Richmond Rivers in northeastern New South Wales and southern Queensland (142), and, unlike several other *Melaleuca* species, it does not occur naturally outside Australia. The plant material was hand cut and often distilled on the spot in makeshift, mobile, wood-fired bush stills. The industry continued in this fashion for several decades. Legend has it that the oil was considered so important for its medicinal uses that Australian soldiers were supplied oil as part of their military kits during World War II and that bush cutters were exempt from national service (35). However, no evidence to corroborate these accounts could be found (A.-M. Conde and M. Pollard [Australian War Memorial, Canberra, Australia], personal communication). Production ebbed after World War II as demand for the oil declined, presumably due to the development of effective antibiotics and the waning image of natural products. Interest in the oil was rekindled in the 1970s as part of the general renaissance of interest in natural products. Commercial plantations were established in the 1970s and 1980s, allowing the industry to mechanize and produce large quantities of a consistent product (25, 93). Today there are plantations in Western Australia, Queensland, and New South Wales, although the majority are in New South Wales around the Lismore region. Typically, plantations are established from seedlings sowed and raised in greenhouses prior to being planted out in the field at high density. The time to first harvest varies from 1 to 3 years, depending on the climate and rate of plant growth. Harvesting is by a coppicing process in which the whole plant is cut off close to ground level and chipped into smaller fragments prior to oil extraction.

Oil Extraction

TTO is produced by steam distillation of the leaves and terminal branches of *M. alternifolia*. Once condensed, the clear to pale yellow oil is separated from the aqueous distillate. The yield of oil is typically 1 to 2% of wet plant material weight. Alternative extraction methods such as the use of microwave technology have been considered, but none has been utilized on a commercial scale.

ANTIMICROBIAL ACTIVITY IN VITRO

Of all of the properties claimed for TTO, its antimicrobial activity has received the most attention. The earliest reported use of the *M. alternifolia* plant that presumably exploited this property was the traditional use by the Bundjalung Aborigines of northern New South Wales. Crushed leaves of "tea trees"

were inhaled to treat coughs and colds or were sprinkled on wounds, after which a poultice was applied (135). In addition, tea tree leaves were soaked to make an infusion to treat sore throats or skin ailments (101, 135). The oral history of Australian Aborigines also tells of healing lakes, which were lagoons into which *M. alternifolia* leaves had fallen and decayed over time (3). Use of the oil itself, as opposed to the unextracted plant material, did not become common practice until Penfold published the first reports of its antimicrobial activity in a series of papers in the 1920s and 1930s. In evaluating the antimicrobial activity of *M. alternifolia* oil and other oils, he made comparisons with the disinfectant carbolic acid or phenol, the gold standard of the day, in a test known as the Rideal-Walker (RW) coefficient. The activity of TTO was compared directly with that of phenol and rated as 11 times more active (121). The RW coefficients of several TTO components were also reported, including 3.5 for cineole and 8 for cymene (122), 13 for linalool (123), and 13.5 for terpinen-4-ol and 16 for terpineol (121). As a result, TTO was promoted as a therapeutic agent (5–7). These publications, as well as several others (60, 70, 84, 102, 120, 124, 152), describe a range of medicinal uses for TTO. However, in terms of the evidence they provide for the medicinal properties of TTO, they are of limited value, since by the standards of today the data they provide would be considered mostly anecdotal.

In contrast, contemporary data clearly show that the broad-spectrum activity of TTO includes antibacterial, antifungal, antiviral, and antiprotozoal activities. Not all of the activity has been characterized well in vitro, and in the few cases where clinical work has been done, data are promising but thus far inadequate.

Evaluation of the antimicrobial activity of TTO has been impeded by its physical properties; TTO and its components are only sparingly soluble in water (Table 2), and this limits their miscibility in test media. Different strategies have been used to counteract this problem, with the addition of surfactants to broth and agar test media being used most widely (11, 13, 15, 31, 32, 61). Dispersion of TTO in liquid media usually results in a turbid suspension that makes determination of end points in susceptibility tests difficult. Occasionally dyes have been used as visual indicators of the MIC, with mixed success (31, 32, 40, 104).

Antibacterial Activity

The few reports of the antibacterial activity of TTO appearing in the literature from the 1940s to the 1980s (11, 15, 100, 153) have been reviewed elsewhere previously (35). From the early 1990s onwards, many reports describing the antimicrobial activity of TTO appeared in the scientific literature. Although there was still a degree of discrepancy between the methods used in the different studies, the MICs reported were often relatively similar. A broad range of bacteria have now been tested for their susceptibilities to TTO, and some of the published susceptibility data are summarized in Table 3. While most bacteria are susceptible to TTO at concentrations of 1.0% or less, MICs in excess of 2% have been reported for organisms such as commensal skin staphylococci and micrococci, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* (13,

TABLE 3. Susceptibility data for bacteria tested against
M. alternifolia oil

| Bacterial species | % (vol/vol) | | Reference(s) |
	MIC	MBC	
Acinetobacter baumannii	1	1	79
Actinomyces viscosus	0.6	>0.6	134
Actinomyces spp.	1	1	80
Bacillus cereus	0.3		61
Bacteroides spp.	0.06–0.5	0.06–0.12	75
Corynebacterium sp.	0.2–2	2	42, 61, 79
Enterococcus faecalis	0.5–>8	>8	13, 61
E. faecium (vancomycin resistant)	0.5–1	0.5–1	115
Escherichia coli	0.08–2	0.25–4	13, 32, 67, 104
Fusobacterium nucleatum	0.6–>0.6	0.25	134, 144
Klebsiella pneumoniae	0.25–0.3	0.25	61, 79
Lactobacillus spp.	1–2	2	75, 80
Micrococcus luteus	0.06–0.5	0.25–6	79
Peptostreptococcus anaerobius	0.2–0.25	0.03–>0.6	75, 134
Porphyromonas endodentalis	0.025–0.1	0.025–0.1	80
P. gingivalis	0.11–0.25	0.13–>0.6	134, 144
Prevotella spp.	0.03–0.25	0.03	75
Prevotella intermedia	0.003–0.1	0.003–0.1	80
Propionibacterium acnes	0.05–0.63	0.5	37, 61, 126
Proteus vulgaris	0.08–2	4	13, 42, 61, 104
Pseudomonas aeruginosa	1–8	2–>8	13, 61, 79
Staphylococcus aureus	0.5–1.25	1–2	13, 32, 126
S. aureus (methicillin resistant)	0.04–0.35	0.5	31, 42, 104, 115
S. epidermidis	0.45–1.25	4	42, 79, 126
S. hominis	0.5	4	79
Streptococcus pyogenes	0.12–2	0.25–4	13, 33
Veillonella spp.	0.016–1	0.03–1	80

79). TTO is for the most part bactericidal in nature, although it may be bacteriostatic at lower concentrations.

The activity of TTO against antibiotic-resistant bacteria has attracted considerable interest, with methicillin-resistant *Staphylococcus aureus* (MRSA) receiving the most attention thus far. Since the potential to use TTO against MRSA was first hypothesized (153), several groups have evaluated the activity of TTO against MRSA, beginning with Carson et al. (31), who examined 64 MRSA isolates from Australia and the United Kingdom, including 33 mupirocin-resistant isolates. The MICs and minimal bactericidal concentrations (MBCs) for the Australian isolates were 0.25% and 0.5%, respectively, while those for the United Kingdom isolates were 0.312% and 0.625%, respectively. Subsequent reports on the susceptibility of MRSA to TTO have similarly not shown great differences compared to antibiotic-sensitive organisms (39, 58, 68, 106, 115).

For the most part, antibacterial activity has been determined using agar or broth dilution methods. However, activity has also been demonstrated using time-kill assays (34, 48, 80, 106), suspension tests (107), and "ex vivo"-excised human skin (108). In addition, vaporized TTO can inhibit bacteria, including *Mycobacterium avium* ATCC 4676 (105), *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* (85). There are anecdotal reports of aerosolized TTO reducing hospital-acquired infections (L. Bowden, Abstr. Infect. Control Nurses Assoc. Annu. Infect. Control Conf., p. 23, 2001) but no scientific data.

Mechanism of antibacterial action. The mechanism of action of TTO against bacteria has now been partly elucidated. Prior to the availability of data, assumptions about its mechanism of action were made on the basis of its hydrocarbon structure and attendant lipophilicity. Since hydrocarbons partition preferentially into biological membranes and disrupt their vital functions (138), TTO and its components were also presumed to behave in this manner. This premise is further supported by data showing that TTO permeabilizes model liposomal systems (49). In previous work with hydrocarbons not found in TTO (90, 146a) and with terpenes found at low concentrations in TTO (4, 146), lysis and the loss of membrane integrity and function manifested by the leakage of ions and the inhibition of respiration were demonstrated. Treatment of *S. aureus* with TTO resulted in the leakage of potassium ions (49, 69) and 260-nm-light-absorbing materials (34) and inhibited respiration (49). Treatment with TTO also sensitized *S. aureus* cells to sodium chloride (34) and produced morphological changes apparent under electron microscopy (127). However, no significant lysis of whole cells was observed spectrophotometrically (34) or by electron microscopy (127). Furthermore, no cytoplasmic membrane damage could be detected using the lactate dehydrogenase release assay (127), and only modest uptake of propidium iodide was observed (50) after treatment with TTO.

In *E. coli*, detrimental effects on potassium homeostasis (47), glucose-dependent respiration (47), morphology (67), and ability to exclude propidium iodide (50) have been observed. A modest loss of 280-nm-light-absorbing material has also been reported (50). In contrast to the absence of whole-cell lysis seen in *S. aureus* treated with TTO, lysis occurs in *E. coli* treated with TTO (67), and this effect is exacerbated by cotreatment with EDTA (C. Carson, unpublished data). All of these effects confirm that TTO compromises the structural and functional integrity of bacterial membranes.

The loss of viability, inhibition of glucose-dependent respiration, and induction of lysis seen after TTO treatment all occur to a greater degree with organisms in the exponential rather than the stationary phase of growth (67; S. D. Cox, J. L. Markham, C. M. Mann, S. G. Wyllie, J. E. Gustafson, and J. R. Warmington, Abstr. 28th Int. Symp. Essential Oils, p. 201–213, 1997). The increased vulnerability of actively growing cells was also apparent in the greater degree of morphological changes seen in these cells by electron microscopy (S. D. Cox et al. Abstr. 28th Int. Symp. Essential Oils, p. 201–213). The differences in susceptibility of bacteria in different phases of growth suggest that targets other than the cell membrane may be involved.

When the effects of terpinen-4-ol, α-terpineol, and 1,8-cineole on *S. aureus* were examined, none was found to induce autolysis but all were found to cause the leakage of 260-nm-light-absorbing material and to render cells susceptible to sodium chloride (34). Interestingly, the greatest effects were seen with 1,8-cineole, a component often considered to have marginal antimicrobial activity. This raises the possibility that while cineole may not be one of the primary antimicrobial components, it may permeabilize bacterial membranes and facilitate the entry of other, more active components. Little work on the effects of TTO components on cell morphology has been reported. Electron microscopy of terpinen-4-ol-treated *S. aureus* cells (34) revealed lesions similar to those seen after TTO

TABLE 4. Susceptibility data for fungi tested against
M. alternifolia oil

| Fungal species | % (vol/vol) | | Reference(s) |
	MIC	MFC	
Alternaria spp.	0.016–0.12	0.06–2	74
Aspergillus flavus	0.31–0.7	2–4	61, 74, 116, 137
A. fumigatus	0.06–>2	1–2	74, 148
A. niger	0.016–0.4	2–8	15, 61, 74
Blastoschizomyces capitatus	0.25		117
Candida albicans	0.06–8	0.12–1	13, 42, 52, 59, 77, 111, 116, 117, 148
C. glabrata	0.03–8	0.12–0.5	13, 52, 59, 77, 111, 117, 148
C. parapsilosis	0.03–0.5	0.12–0.5	52, 77, 111, 117
C. tropicalis	0.12–2	0.25–0.5	52, 59, 148
Cladosporium spp.	0.008–0.12	0.12–4	74
Cryptococcus neoformans	0.015–0.06		111
Epidermophyton flocossum	0.008–0.7	0.12–0.25	42, 74
Fusarium spp.	0.008–0.25	0.25–2	74
Malassezia furfur	0.03–0.12	0.5–1.0	73
M. sympodialis	0.016–0.12	0.06–0.12	73
Microsporum canis	0.03–0.5	0.25–0.5	52, 74, 116
M. gypseum	0.016–0.25	0.25–0.5	52
Penicillium spp.	0.03–0.06	0.5–2	74
Rhodotorula rubra	0.06	0.5	71
Saccharomyces cerevisiae	0.25	0.5	71
Trichophyton mentagrophytes	0.11–0.44	0.25–0.5	52, 61, 116
T. rubrum	0.03–0.6	0.25–1	42, 52, 74, 116
T. tonsurans	0.004–0.016	0.12–0.5	74
Trichosporon spp.	0.12–0.22	0.12	71, 116

treatment (127), including mesosome-like structures.

Mechanism of action studies analogous to those described above have not been conducted with *P. aeruginosa*. Instead, research has concentrated on how this organism is able to tolerate higher concentrations of TTO and/or components. These studies have indicated that tolerance is associated with the outer membrane by showing that when *P. aeruginosa* cells are pretreated with the outer membrane permeabilizer polymyxin B nonapeptide or EDTA, cells become more susceptible to the bactericidal effects of TTO, terpinen-4-ol, or γ-terpinene (99, 103).

In summary, the loss of intracellular material, inability to maintain homeostasis, and inhibition of respiration after treatment with TTO and/or components are consistent with a mechanism of action involving the loss of membrane integrity and function.

Antifungal Activity

Comprehensive investigations of the susceptibility of fungi to TTO have only recently been completed. Prior to this, data were somewhat piecemeal and fragmentary. Early data were also largely limited to *Candida albicans*, which was a commonly chosen model test organism. Data now show that a range of yeasts, dermatophytes, and other filamentous fungi are susceptible to TTO (14, 42, 52, 61, 116, 128, 140) (Table 4). Although

test methods differ, MICs generally range between 0.03 and 0.5%, and fungicidal concentrations generally range from 0.12 to 2%. The notable exception is *Aspergillus niger*, with minimal fungicidal concentrations (MFCs) of as high as 8% reported for this organism (74). However, these assays were performed with fungal conidia, which are known to be relatively impervious to chemical agents. Subsequent assays have shown that germinated conidia are significantly more susceptible to TTO than nongerminated conidia (74), suggesting that the intact conidial wall confers considerable protection. TTO vapors have also been demonstrated to inhibit fungal growth (86, 87) and affect sporulation (88).

Mechanism of antifungal action. Studies investigating the mechanism(s) of antifungal action have focused almost exclusively on *C. albicans*. Similar to results found for bacteria, TTO also alters the permeability of *C. albicans* cells. The treatment of *C. albicans* with 0.25% TTO resulted in the uptake of propidium iodide after 30 min (50), and after 6 h significant staining with methylene blue and loss of 260-nm-light-absorbing materials had occurred (72). TTO also alters the permeability of *Candida glabrata* (72). Further research demonstrating that the membrane fluidity of *C. albicans* cells treated with 0.25% TTO is significantly increased confirms that the oil substantially alters the membrane properties of *C. albicans* (72).

TTO also inhibits respiration in *C. albicans* in a dose-dependent manner (49). Respiration was inhibited by approximately 95% after treatment with 1.0% TTO and by approximately 40% after treatment with 0.25% TTO. The respiration rate of *Fusarium solani* is inhibited by 50% at a concentration of 0.023% TTO (88). TTO also inhibits glucose-induced medium acidification by *C. albicans*, *C. glabrata*, and *Saccharomyces cerevisiae* (72). Medium acidification occurs largely by the expulsion of protons by the plasma membrane ATPase, which is fuelled by ATP derived from the mitochondria. The inhibition of this function suggests that the plasma and/or mitochondrial membranes have been adversely affected. These results are consistent with a proposed mechanism of antifungal action whereby TTO causes changes or damage to the functioning of fungal membranes. This proposed mechanism is further supported by work showing that the terpene eugenol inhibits mitochondrial respiration and energy production (46).

Additional studies have shown that when cells of *C. albicans* are treated with diethylstilbestrol to inhibit the plasma membrane ATPase, they then have a much greater susceptibility to TTO than do control cells (72), suggesting that the plasma membrane ATPase has a role in protecting cells against the destabilizing or lethal effects of TTO.

TTO inhibits the formation of germ tubes, or mycelial conversion, in *C. albicans* (52, 78). Two studies have shown that germ tube formation was completely inhibited in the presence of 0.25 and 0.125% TTO, and it was further observed that treatment with 0.125% TTO resulted in a trend of blastospores changing from single or singly budding morphologies to multiply budding morphologies over the 4-h test period (78). These cells were actively growing but were not forming germ tubes, implying that morphogenesis is specifically inhibited, rather than all growth being inhibited. Interestingly, the inhibition of germ tube formation was shown to be reversible, since cells were able to form germ tubes after the removal of the

TTO (78). However, there was a delay in germ tube formation, indicating that TTO causes a postantifungal effect.

Antiviral Activity

The antiviral activity of TTO was first shown using tobacco mosaic virus and tobacco plants (18). In field trials with *Nicotiniana glutinosa*, plants were sprayed with 100, 250, or 500 ppm TTO or control solutions and were then experimentally infected with tobacco mosaic virus. After 10 days, there were significantly fewer lesions per square centimeter of leaf in plants treated with TTO than in controls (18). Next, Schnitzler et al. (132) examined the activity of TTO and eucalyptus oil against herpes simplex virus (HSV). The effects of TTO were investigated by incubating viruses with various concentrations of TTO and then using these treated viruses to infect cell monolayers. After 4 days, the numbers of plaques formed by TTO-treated virus and untreated control virus were determined and compared. The concentration of TTO inhibiting 50% of plaque formation was 0.0009% for HSV type 1 (HSV-1) and 0.0008% for HSV-2, relative to controls. These studies also showed that at the higher concentration of 0.003%, TTO reduced HSV-1 titers by 98.2% and HSV-2 titers by 93.0%. In addition, by applying TTO at different stages in the virus replicative cycle, TTO was shown to have the greatest effect on free virus (prior to infection of cells), although when TTO was applied during the adsorption period, a slight reduction in plaque formation was also seen (132). Another study evaluated the activities of 12 essential oils, including TTO, for activity against HSV-1 in Vero cells (110). Again, TTO was found to exert most of its antiviral activity on free virus, with 1% oil inhibiting plaque formation completely and 0.1% TTO reducing plaque formation by approximately 10%. Pretreatment of the Vero cells prior to virus addition or posttreatment with 0.1% TTO after viral absorption did not significantly alter plaque formation.

Some activity against bacteriophages has also been reported, with exposure to 50% TTO at 4°C for 24 h reducing the number of SA and T7 plaques formed on lawns of *S. aureus* and *E. coli*, respectively (41).

The results of these studies indicate that TTO may act against enveloped and nonenveloped viruses, although the range of viruses tested to date is very limited.

Antiprotozoal Activity

Two publications show that TTO has antiprotozoal activity. TTO caused a 50% reduction in growth (compared to controls) of the protozoa *Leishmania major* and *Trypanosoma brucei* at concentrations of 403 mg/ml and 0.5 mg/ml, respectively (109). Further investigation showed that terpinen-4-ol contributed significantly to this activity. In another study, TTO at 300 mg/ml killed all cells of *Trichomonas vaginalis* (151). There is also anecdotal in vivo evidence that TTO may be effective in treating *Trichomonas vaginalis* infections (120).

Antimicrobial Components of TTO

Considerable attention has been paid to which components of TTO are responsible for the antimicrobial activity. Early

indications from RW coefficients were that much of the activity could be attributed to terpinen-4-ol and α-terpineol (121). Data available today confirm that these two components contribute substantially to the oil's antibacterial and antifungal activities, with MICs and MBCs or MFCs that are generally the same as, or slightly lower than values obtained for TTO (36, 42, 48, 71, 117, 126). However, α-terpineol does not represent a significant proportion of the oil. Additional components with MICs similar to or lower than those of TTO include α-pinene, β-pinene, and linalool (36, 71), but, similar to the case for α-terpineol, these components are present in only relatively low amounts. Of the remaining components tested, it seems that most possess at least some degree of antimicrobial activity (36, 71, 126), and this is thought to correlate with the presence of functional groups, such as alcohols, and the solubility of the component in biological membranes (63, 138). While some TTO components may be considered less active, none can be considered inactive. Furthermore, methodological issues have been demonstrated to have a significant influence on assay outcomes (48, 71).

The possibility that components in TTO may have synergistic or antagonistic interactions has been explored in vitro (48), but no significant relationships were found. The possibility that TTO may act synergistically with other essential oils, such as lavender (38), and other essential oil components, such as β-triketones from manuka oil (43, 44), has also been investigated. Given the numerous components of TTO, the scope for such effects is enormous, and much more work is required to examine this question.

Resistance to TTO

The question of whether true resistance to TTO can be induced in vitro or may occur spontaneously in vivo has not been examined systematically. Clinical resistance to TTO has not been reported, despite the medicinal use of the oil in Australia since the 1920s. There has been one short report of induced in vitro resistance to TTO in *S. aureus* (114). Stepwise exposure of five MRSA isolates to increasing concentrations of TTO yielded three isolates with TTO MICs of 1% and one isolate each with TTO MICs of 2% and 16%, respectively. All isolates showed initial MICs of 0.25%. There has also been one report suggesting that *E. coli* strains harboring mutations in the multiple antibiotic resistance (*mar*) operon, so-called Mar mutants, may exhibit decreased susceptibility to TTO (66). Minor changes in TTO and α-terpineol susceptibilities have also been seen in *S. aureus* isolates with reduced susceptibility to household cleaners (53). However, in these last two studies the changes in susceptibility were marginal and do not represent strong evidence of resistance (53, 66). With regard to fungi, an attempt to induce resistance to TTO in two clinical isolates of *Candida albicans* was largely unsuccessful, with isolates failing to grow in 2% (vol/vol) TTO after serial passage in increasing concentrations of TTO (111).

Resistance to conventional antibiotics has not been demonstrated to influence susceptibility to TTO, suggesting that cross-resistance does not occur. For example, antimicrobial-resistant isolates of *S. aureus* (31, 58), *C. albicans* and *C. glabrata* (148), *P. aeruginosa* (106), and *Enterococcus faecium*

(106, 115) have in vitro susceptibilities to TTO that are similar to those of nonresistant isolates.

Overall, these studies provide little evidence to suggest that resistance to TTO will occur, either in vitro or in vivo, although minimal data are available. It is likely that the multicomponent nature of TTO may reduce the potential for resistance to occur spontaneously, since multiple simultaneous mutations may be required to overcome all of the antimicrobial actions of each of the components. Furthermore, since TTO is known to affect cell membranes, it presumably affects multiple properties and functions associated with the cell membrane, similar to the case for membrane-active biocides. This means that numerous targets would have to adapt to overcome the effects of the oil. Issues of potential resistance are important if TTO is to be used more widely, particularly against antibiotic-resistant organisms.

CLINICAL EFFICACY

In parallel with the characterization of the in vitro antimicrobial activity of TTO, the clinical efficacy of the oil has also been the subject of investigation. Early clinical studies attempting to characterize the clinical efficacy of TTO (60, 120, 152) are not considered scientifically valid by today's standards and will therefore not be discussed further. Data from some of the more recent clinical investigations are summarized in Table 5.

One of the first rigorous clinical studies assessed the efficacy of 5% TTO in the treatment of acne by comparing it to 5% benzoyl peroxide (BP) (14). The study found that both treatments reduced the numbers of inflamed lesions, although BP performed significantly better than TTO. The BP group showed significantly less oiliness than the TTO group, whereas the TTO group showed significantly less scaling, pruritis, and dryness. Significantly fewer overall side effects were reported by the TTO group (27 of 61 patients) than by the BP group (50 of 63 patients).

The efficacy of TTO in dental applications has been assessed. An evaluation of the effect of a 0.2% TTO mouthwash and two other active agents on the oral flora of 40 volunteers suggested that TTO used once daily for 7 days could reduce the number of mutans streptococci and the total number of oral bacteria, compared to placebo treatment. The data also indicated that these reductions were maintained for 2 weeks after the use of mouthwash ceased (64). In another study, comparison of mouthwashes containing either approximately 0.34% TTO, 0.1% chlorhexidine, or placebo on plaque formation and vitality, using eight volunteers (9), showed that after TTO treatment, both plaque index and vitality did not differ from those of subjects receiving placebo mouthwash on any day, whereas the results for the chlorhexidine mouthwash group differed significantly from those for the placebo group on all days (9). Lastly, a study comparing a 2.5% TTO gel, a 0.2% chlorhexidine gel, and a placebo gel found that although the TTO group had significantly reduced gingival index and papillary bleeding index scores, their plaque scores were actually increased (139). These studies indicate that although TTO may cause decreases in the levels of oral bacteria, this does not necessarily equate to reduced plaque levels. However, TTO may have a role in the treatment of gingivitis, and there is also

some evidence preliminary suggesting that TTO reduces the levels of several compounds associated with halitosis (144).

Two studies have assessed the efficacy of TTO for the eradication of MRSA carriage. The effectiveness of a 4% TTO nasal ointment and a 5% TTO body wash was compared to that of conventional treatment with mupirocin nasal ointment and Triclosan body wash in a small pilot study (28). Of the 15 patients receiving conventional treatment, 2 were cleared and 8 remained colonized at the end of therapy; in the TTO group of 15, 5 were cleared and 3 remained colonized. The remainder of patients did not complete therapy. Differences in clearance rates were not statistically significant, most likely due to the low patient numbers. Stronger evidence for the efficacy of TTO in decolonizing MRSA carriage comes from a recent trial in which 236 patients were randomized to receive standard or TTO treatment regimens (56). The standard regimen consisted of 2% mupirocin nasal ointment applied three times a day, 4% chlorhexidine gluconate soap applied at least once a day, and 1% silver sulfadiazine cream applied to skin lesions, wounds, and leg ulcers once a day, all for 5 days. The TTO regimen consisted of 10% TTO nasal cream applied three times a day, 5% TTO body wash applied at least once daily and 10% TTO cream applied to skin lesions, wounds, and leg ulcers once a day, all for 5 days. The 10% TTO cream was allowed to be used as an alternative to the body wash. Follow-up swabs were taken at 2 and 14 days posttreatment, with the exception of 12 patients who were lost to follow-up. Evaluation of the remaining 224 patients showed no significant differences between treatment regimens, with 49% of patients receiving standard therapy cleared versus 41% of patients in the TTO group.

For many years there has been considerable interest in the possibility of using TTO in handwash formulations for use in hospital or health care settings. It is well known that handwashing is an effective infection control measure and that lack of compliance is related to increased rates of nosocomial infections. The benefits of using TTO in a handwash formulation include its antiseptic effects and increased handwashing compliance. A recent handwash study using volunteers showed that either a product containing 5% TTO and 10% alcohol or a solution of 5% TTO in water performed significantly better than soft soap, whereas a handwash product containing 5% TTO did not (108).

Occasional case reports of the use of TTO have also been published. In one, a woman self-treated successfully with a 5-day course of TTO pessaries after having been clinically diagnosed with bacterial vaginosis (19). In a second, a combination of plant extracts of which TTO was a major component was inserted percutaneously into bone to treat an intractable MRSA infection of the lower tibia, which subsequently resolved (136). This same essential oil solution has now been shown to aid in the healing of malodorous malignant ulcers (154).

With regard to fungal infections, TTO has been clinically evaluated for the treatment of onychomycosis (26, 143), tinea pedis (131, 145), dandruff (130), and oral candidiasis (92, 149). Although much has been made of the potential for TTO to be used in the treatment of vaginal candidiasis, no clinical data have been published. However, results from an animal (rat) model of vaginal candidiasis support the use of TTO for the treatment of this infection (111).

In the first of the onychomycosis trials (26), 60% of patients

TABLE 5. Summary of clinical studies using TTO

Study population	Study type	Treatment groups (no. of evaluable patients)	Administration of treatment	Outcomes	Adverse events	Reference
124 patients with mild to moderate acne	RCT[a], investigator blinded[b]	5% TTO gel (58), 5% benzoyl peroxide (61)	3 mo	Both significantly reduced inflamed lesions ($P < 0.001$) but BP better than TTO ($P < 0.05$); BP better at reducing oiliness ($P < 0.05$); less scaling ($P < 0.02$), pruritis ($P < 0.05$), dryness ($P < 0.001$) with TTO; treatments equivalent for noninflamed lesions, erythema	27 (44%) in TTO group, 50 (79%) in BP group (e.g., dryness, stinging, burning, redness); significantly fewer events in TTO group ($P < 0.001$)	14
18 patients with recurrent herpes labialis (cold sores)	RCT, investigator blinded[b]	6% TTO gel (9), placebo gel (9)	5 times daily	Median time to repithelization of 9 days for TTO vs 12.5 days for placebo (not significant)	1 in TTO group (event not stated)	30
126 patients with mild to moderate dandruff	RCT, investigator blinded[b]	5% TTO shampoo (63), placebo (62)	Daily for 4 wk	Whole scalp lesion score significantly improved in TTO group (41.2%) compared to placebo group (11.2%) ($P < 0.001$)	3 (5%) in TTO group, 8 (13%) in placebo group (e.g., mild burning, stinging, itching)	130
30 hospital inpatients colonized or infected with MRSA	Randomized, controlled pilot study	4% TTO nasal ointment + 5% TTO body wash (15), 2% mupirocin nasal ointment + Triclosan body wash (15)	Frequency not stated, minimum of 3 days	For TTO, 33% cleared, 20% chronic, 47% incomplete; for routine treatment, 3% cleared, 53% chronic, 33% incomplete (no significant differences)	With TTO nasal ointment (no. not stated), mild swelling of nasal mucosa to acute burning	28
236 hospital patients colonized with MRSA	RCT	10% TTO cream + 5% TTO body wash (110), 2% mupirocin nasal ointment + 4% Triclosan body wash + 1% silver sulfadiazine cream (114)	Once daily for 5 days	For TTO, 41% cleared; for routine treatment, 49% cleared; treatment regimens did not differ significantly ($P = 0.0286$); mupirocin significantly better than TTO at clearing nasal carriage ($P = 0.0001$)	None	56
117 patients with culture-positive onychomycosis	RCT, double blind	100% TTO (64), 1% clotrimazole (53)	Twice daily for 6 mo	Full or partial resolution for 60% of TTO and 61% of clotrimazole patients after 6 months of therapy (not significant; $P > 0.05$)	5 (7.8%) in TTO group, 3 (5.7%) in clotrimazole group (erythema, irritation, edema)	26
60 outpatients with a clinical diagnosis of onychomycosis	RCT, double blind	2% butenafine hydrochloride with 5% TTO cream (40), 5% TTO cream (20)	3 times daily for 8 wk	Cure in 80% of butenafine/TTO group and 0% of TTO group ($P < 0.0001$)	4 (10%) in butenafine/TTO group (mild inflammation)	143
13 patients with AIDS and fluconazole-refractory oral candidiasis	Case series	Melaleuca oral solution (15 ml) (12)	4 times daily for 2–4 wk	Clinical response rate of 67% after 4 weeks (cure in 2 patients, improvement in 6 patients, no response in 4 patients, 1 deterioration)	None	92
27 patients with AIDS and fluconazole-refractory oral candidiasis	Open-label trial	Melaleuca oral solution (15 ml) (12), alcohol-free melaleuca oral solution (5 ml)[c] (13)	4 times daily for 2–4 wk	Mycological and clinical response in 58% (alcohol-based solution) and 54% (alcohol-free solution) of patients after 4 wk	8 (66.7%) in alcohol-based solution group, 2 (15.4%) in alcohol-free solution group (mild to moderate burning)	149
121 patients with clinically diagnosed tinea pedis	RCT, double blind	10% TTO in sorbolene (37), 1% tolnaftate (33), placebo (sorbolene) (34)	Twice daily for 4 wk	Mycological cure and clinical improvement in 46% (tolnaftate), 22% (TTO), and 9% (placebo) of patients; tolnaftate significantly better than placebo ($P = 0.003$) but not TTO ($P = 0.59$); TTO not different from placebo ($P = 0.3$)	None	145
137 patients with culture-positive tinea pedis	RCT, double blind	25% TTO (36), 50% TTO (38), placebo (46)	Twice daily for 4 wk	Effective cure in 48% (25% TTO), 50% (50% TTO), and 13% (placebo) of patients; TTO significantly better than placebo ($P < 0.0005$)	1 (2.8%) in 25% TTO group, 3 (7.9%) in 50% TTO group (moderate to severe dermatitis)	131

[a] RCT, randomized controlled trial.
[b] The distinctive odor of TTO was stated as preventing patient blinding.
[c] The alcohol-free solution was more concentrated, and thus a smaller volume was used.

treated with TTO and 61% of patients treated with 1% clotrimazole had full or partial resolution. There were no statistically significant differences between the two treatment groups for any parameter. The second onychomycosis trial (143) compared two creams, one containing 5% TTO alone and the other containing 5% TTO and 2% butenafine, both applied three times daily for 8 weeks. The overall cure rate was 0% for patients treated with 5% TTO alone, compared to 80% for patients treated with both butenafine and TTO. Unfortunately, butenafine alone was not evaluated. The observation that TTO may be useful adjunct therapy for onychomycosis has been made by Klimmek et al. (95). However, onychomycosis is considered to be largely unresponsive to topical treatment of any kind, and a high rate of cure should therefore not be expected.

The effectiveness of TTO in treating tinea pedis has been evaluated in two trials. In the first trial, patients were treated with 10% TTO in sorbolene, 1% tolnaftate, or placebo (sorbolene) (145). At completion of treatment, patients treated with TTO had mycological cure and clinical improvement rates of 30% and 65%, respectively. This compares to mycological cure rates of 21% in patients receiving placebo and 85% in patients receiving tolnaftate. Similarly, clinical improvement was seen in 41% of patients receiving placebo and 68% of patients receiving tolnaftate. In a second tinea trial, the efficacy of solutions of 25% and 50% TTO in ethanol and polyethylene glycol was compared to treatment with placebo (vehicle) (131). Marked clinical responses were seen in 72% and 68% of patients in the 25% and 50% TTO treatment groups, respectively, compared to 39% of patients in the placebo group. Similarly, there were mycological cures of 55% and 64% in the 25% and 50% TTO treatment groups, respectively, compared to 31% in the placebo group. Dermatitis occurred in one patient in the 25% TTO group and in three patients in the 50% TTO group. This led to the recommendation that 25% TTO be considered an alternative treatment for tinea, since it was associated with fewer adverse reactions than but was just as effective as 50% TTO. These studies highlight the importance of considering the formulation of the TTO product when conducting in vivo work, since it is likely that the sorbolene vehicle used in the first tinea trial may have significantly compromised the antifungal activity of the oil.

The evaluation of a 5% TTO shampoo for mild to moderate dandruff demonstrated statistically significant improvements in the investigator-assessed whole scalp lesion score, total area of involvement score, and total severity score, as well as in the patient-assessed itchiness and greasiness scores, compared to placebo. Overall, the 5% TTO was well tolerated and appeared to be effective in the treatment of mild to moderate dandruff.

TTO has been evaluated as a mouthwash in the treatment of oropharyngeal candidiasis. In a case series, 13 human immunodeficiency virus-positive patients who had already failed treatment with a 14-day course of oral fluconazole were treated with an alcohol-based TTO solution for up to 28 days (92). After treatment, of the 12 evaluable patients, 2 were cured, 6 were improved, 4 were unchanged, and 1 had deteriorated. Overall, eight patients had a clinical response and seven had a mycological response. In subsequent work the same TTO solution was compared with an alcohol-free TTO solution (149). Of patients receiving the alcohol-based solution, two were cured, six improved, four were unchanged, and

one had deteriorated. Of patients receiving the alcohol-free solution, five were cured, two improved, two were unchanged, and one had deteriorated. Three patients were lost to follow-up and were considered nonresponders.

Support for TTO possessing in vivo antiviral activity comes from a pilot study investigating the treatment of recurrent herpes labialis (cold sores) with a 6% TTO gel or a placebo gel (30). Comparison of the patient groups (each containing nine evaluable patients) at the end of the study showed that reepithelialization after treatment occurred after 9 days for the TTO group and after 12.5 days for the placebo group. Other measures, such as duration of virus positivity by culture or PCR, viral titers, and time to crust formation, were not significantly different, possibly due to small patient numbers. Interestingly, when TTO was evaluated for its protective efficacy in an in vivo mouse model of genital HSV type 2 infection, it did not perform well (21). In contrast, the oil component 1,8-cineole performed well, protecting 7 of 16 animals from disease.

There are a number of limitations to the clinical studies described above. Several had low numbers of participants, meaning that statistical analyses could not be performed or differences did not reach significance. Many studies had ambiguous and/or equivocal outcomes. Of those studies with larger numbers of patients, few reported 95% confidence intervals or relative risk values. While most studies compared the efficacy of TTO to a placebo, many did not compare TTO to a conventional therapy or treatment regimen, again limiting the conclusions that could be drawn about efficacy. Several publications noted that patient blinding was compromised or impracticable due to the characteristic odor of TTO (14, 30, 130, 131). These studies, while perhaps conducted as double blinded, are technically only single blinded, which is not ideal. Perhaps most importantly, few studies have been replicated independently. Therefore, although some of these data indicate that TTO has potential as a therapeutic agent, confirmatory studies are required. In addition, factors such as the final TTO concentration, product formulation, and length and frequency of treatment undoubtedly influence clinical efficacy, and these factors must be considered in future studies. The cost-effectiveness of any potential TTO treatments must also be considered. For example, TTO therapy may offer no cost advantage over the azoles in the treatment of tinea but is probably more economical than treatment with the allylamines.

ANTI-INFLAMMATORY ACTIVITY

Numerous recent studies now support the anecdotal evidence attributing anti-inflammatory activity to TTO. In vitro work over the last decade has demonstrated that TTO affects a range of immune responses, both in vitro and in vivo. For example, the water-soluble components of TTO can inhibit the lipopolysaccharide-induced production of the inflammatory mediators tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and IL-10 by human peripheral blood monocytes by approximately 50% and that of prostaglandin E_2 by about 30% after 40 h (81). Further examination of the water-soluble fraction of TTO identified terpinen-4-ol, α-terpineol, and 1,8-cineole as the main components, but of these, only terpinen-4-ol was able to diminish the production of TNF-α, IL-1β, IL-8, IL-10, and prostaglandin E_2 by lipopolysaccharide-activated

monocytes. The water-soluble fraction of TTO, terpinen-4-ol, and α-terpineol also suppressed superoxide production by agonist-stimulated monocytes but not neutrophils (22). In contrast, similar work found that TTO decreases the production of reactive oxygen species by both stimulated neutrophils and monocytes and that it also stimulates the production of reactive oxygen species by nonprimed neutrophils and monocytes (29). TTO failed to suppress the adherence reaction of neutrophils induced by TNF-α stimulation (2) or the casein-induced recruitment of neutrophils into the peritoneal cavities of mice (1). These studies identify specific mechanisms by which TTO may act in vivo to diminish the normal inflammatory response. In vivo, topically applied TTO has been shown to modulate the edema associated with the efferent phase of a contact hypersensitivity response in mice (23) but not the development of edema in the skin of nonsensitized mice or the edematous response to UVB exposure. This activity was attributed primarily to terpinen-4-ol and α-terpineol. When the effect of TTO on hypersensitivity reactions involving mast cell degranulation was examined in mice, TTO and terpinen-4-ol applied after histamine injection reduced histamine-induced skin edema, and TTO also significantly reduced swelling induced by intradermal injection of compound 48/80 (24). Human studies on histamine-induced wheal and flare provided further evidence to support the in vitro and animal data, with the topical application of neat TTO significantly reducing mean wheal volume but not mean flare area (97). Erythema and flare associated with nickel-induced contact hypersensitivity in humans are also reduced by neat TTO but not by a 5% TTO product, product base, or macadamia oil (119). Work has now shown that terpinen-4-ol, but not 1,8-cineole or α-terpineol, modulates the vasodilation and plasma extravasation associated with histamine-induced inflammation in humans (94).

SAFETY AND TOXICITY

Despite the progress in characterizing the antimicrobial and anti-inflammatory properties of tea tree oil, less work has been done on the safety and toxicity of the oil. The rationale for continued use of the oil rests largely on the apparently safe use of the oil for almost 80 years. Anecdotal evidence over this time suggests that topical use is safe and that adverse events are minor, self-limiting, and infrequent. More concrete evidence such as published scientific work is scarce, and much information remains out of the public domain in the form of reports from company-sponsored work. The oral and dermal toxicities of TTO are summarized briefly below.

Oral Toxicity

TTO can be toxic if ingested, as evidenced by studies with animals and from cases of human poisoning. The 50% lethal dose for TTO in a rat model is 1.9 to 2.6 ml/kg (129), and rats dosed with ≤1.5 g/kg TTO appeared lethargic and ataxic (D. Kim, D. R. Cerven, S. Craig, and G. L. De George, Abstr. Amer. Chem. Soc. **223:**114, 2002). Incidences of oral poisoning in children (55, 91, 112) and adults (57, 133) have been reported. In all cases, patients responded to supportive care and recovered without apparent sequelae. No human deaths due to TTO have been reported in the literature.

Dermal Toxicity

TTO can cause both irritant and allergic reactions. A mean irritancy score of 0.25 has been found for neat TTO, based on patch testing results for 311 volunteers (10). Another study, in which 217 patients from a dermatology clinic were patch tested with 10% TTO, found no irritant reactions (150). Since irritant reactions may frequently be avoided through the use of lower concentrations of the irritant, this bolsters the case for discouraging the use of neat oil and promoting the use of well-formulated products. Allergic reactions have been reported (54, 147), and although a range of components have been suggested as responsible, the most definitive work indicates that they are caused mainly by oxidation products that occur in aged or improperly stored oil (82). There is little scientific support for the notion that 1,8-cineole is the major irritant in TTO. No evidence of irritation was seen when patch testing was performed on rabbits with intact and abraded skin (118), guinea pigs (82), and humans (118, 141), including those who had previous positive reactions to TTO (96). Rarely, topically applied tea tree oil has been reported to cause systemic effects in domestic animals. Dermal application of approximately 120 ml of undiluted TTO to three cats with shaved but intact skin resulted in symptoms of hypothermia, uncoordination, dehydration, and trembling and in the death of one of the cats (17).

PRODUCT FORMULATION ISSUES

The physical characteristics of TTO present certain difficulties for the formulation and packaging of products. Its lipophilicity leads to miscibility problems in water-based products, while its volatility means that packaging must provide an adequate barrier to volatilization. Since TTO is readily absorbed into plastics, packaging must cater to and minimize this effect. Consideration must also be given to the properties of the finished product. Early suggestions that the antimicrobial activity of TTO may be compromised by organic matter came from disk diffusion studies in which the addition of blood to agar medium decreased zone sizes (8). This observation contrasts sharply with historical claims that the activity of TTO may in fact be enhanced in the presence of organic matter such as blood and pus. A thorough investigation of this claim comprehensively refuted this idea (76) and also showed that product excipients may compromise activity.

Some work on the characteristics and behavior of TTO within formulations has been conducted. Caboi et al. (27) examined the potential of a monoolein/water system as a carrier for TTO and terpinen-4-ol. The activity of TTO products in vitro has also been investigated (16, 77, 107). However, very little work has been conducted in this area, and if stable, biologically active formulations of TTO are going to be developed, much remains to be done.

CONCLUSIONS

A paradigm shift in the treatment of infectious diseases is necessary to prevent antibiotics becoming obsolete, and where appropriate, alternatives to antibiotics ought to be considered. There are already several nonantibiotic approaches to the treatment and prevention of infection, including probiotics,

phages, and phytomedicines. Alternative therapies are viewed favorably by many patients because they are often not being helped by conventional therapy and they believe there are fewer detrimental side effects. In addition, many report significant improvement while taking complementary and alternative medicines. Unfortunately, the medical profession has been slow to embrace these therapies, and good scientific data are still scarce. However, as we approach the "postantibiotic era" the situation is changing. A wealth of in vitro data now supports the long-held beliefs that TTO has antimicrobial and anti-inflammatory properties. Despite some progress, there is still a lack of clinical evidence demonstrating efficacy against bacterial, fungal, or viral infections. Large randomized clinical trials are now required to cement a place for TTO as a topical medicinal agent.

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