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Survey of the Antimicrobial Activity of Commercially Available Australian Tea Tree (*Melaleuca alternifolia*) Essential Oil Products *In Vitro*

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Abstract

Objectives: The aim of this study was to investigate the antimicrobial activity of a range of commercially available tea tree oil (TTO) products and to evaluate whether formulation plays a significant part in their antiseptic activity.

Methods: The antimicrobial activity of the purchased products and control TTO solutions was assessed against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Candida albicans* using well diffusion, broth microdilution, and broth macrodilution assays.

Results: Zone sizes obtained by the agar well diffusion assay ranged from 0 to 49.8 mm, with the more viscous and lipophilic products producing the smallest zones. Micro- and macrodilution methods showed that eight products had minimum inhibitory concentrations that were lower than the nonformulated TTO control. The remaining three products showed activity equivalent to the TTO control.

Conclusions: In general, the commercially available antiseptic TTO products showed antimicrobial activity that was equivalent to, or greater than the nonformulated TTO control. This suggests that the TTO within these products has retained its antimicrobial activity. Furthermore, the enhanced activity of the products may be attributed to other antimicrobial excipients within the products such as preservatives, or to synergistic antimicrobial interactions between the TTO and other product excipients. The observation that the commercially available antiseptic TTO products tested in this study retained adequate antimicrobial activity emphasizes the importance of considering how product bases and excipients may interact with the active compound during formulation to ensure efficacy of the final product. Finally, the current data suggest that these TTO products may also be active *in vivo*. However, this can only be determined through further studies and in clinical trials.

Introduction

The oil of *Melaleuca alternifolia*, also known as tea tree oil (TTO), has been used as an antiseptic remedy for decades. Although there is no published documentation of specific medicinal applications of the *M. alternifolia* plant or oil by Aboriginals prior to white colonization of Australia,[1] the Bundjalung Aboriginals of New South Wales used the plant for medicinal purposes and told of the wound healing properties of the water from a lake into which *M. alternifolia* leaves had fallen.[2]

Extensive medicinal use of TTO did not begin until its antiseptic and disinfectant properties were reported in the 1920s by Penfold and Grant.[3] It has been claimed that TTO was used by Australian munitions factories during World War II. Also during the war, maintaining production of TTO was considered so vital that bush cutters of *M. alternifolia* were exempt from national service.[2,4]

Until the beginning of the 1960s, when Peña[5] successfully treated trichomonal vaginitis and other vaginal infections with TTO, the oil was not mentioned further in the scientific literature. After this, TTO use remained uncommon until a natural product renaissance occurred in the early 1980s. A study in 1990 examining the effect of a 5% TTO product versus 5% benzoylperoxide for the treatment of acne revealed that TTO and benzoylperoxide were equally effective

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in reducing acne lesions, although TTO use resulted in fewer side-effects.[6] Since then, a wide variety of TTO products have been formulated, and further studies on the effects of TTO against a broad range of micro-organisms and superficial clinical conditions have been conducted.[7–10] One such study has demonstrated that hand washes containing 5% TTO are more effective at removing contaminating bacteria from hands than regular nonmedicated soap.[11]

Despite the popularity of TTO and TTO products, only very few studies of the appropriateness and *in vitro* efficacy of commercial TTO formulations that claim to have antiseptic activity have been undertaken.[11] In two of these studies, the release of terpinen-4-ol from several different topical formulations was found to depend on both the formulation of the preparation and the concentration of TTO.[12,13] This is particularly important since terpinen-4-ol has been claimed to be one of the main components responsible for the antimicrobial activity of TTO.[14,15] Other natural products that have shown antimicrobial activity, such as propolis, have been tested for synergism with topical antimicrobials or compared to standard treatments with good outcomes.[16,17] When antiseptic TTO products are formulated, the activity of the preparations should be attributed to the active ingredient, namely, the TTO. Having a product with optimal TTO solubility in the base and optimal delivery of TTO to the affected skin area in appropriate concentrations must be considered paramount for marketing a successful antiseptic TTO preparation. The potential exists for poorly formulated products, where the antiseptic activity of TTO has been supplemented by the presence of preservatives such as parabens, or co-solvents such as alcohols that have antimicrobial activity themselves.

Materials and Methods

Aims and objectives

The aim of this study was to investigate the antimicrobial activity of a range of commercially available TTO products *in vitro* and to evaluate whether formulation plays a significant part in their overall activity.

Tea tree oil

TTO (batch W/ES04) was supplied by Australian Plantations Pty. Ltd. (Wyrallah, NSW, Australia). Levels of components were assessed by gas chromatography mass spectroscopy, performed by the Wollongbar Agricultural Institute (Table 1). The Composition of the TTO was compliant with the International Organization for Standardization ISO 4730:2004.[18] Control solutions of TTO were prepared at 5%, 10%, and 15% (vol/vol) in sterile distilled water with 0.001% (vol/vol) Tween 80 to solubilize the TTO. These solutions were tested in parallel with the purchased products.

Tea tree oil products

A total of 11 products containing TTO and claiming antiseptic or antimicrobial activity were obtained either locally through health care outlets or over the Internet (Table 2). Four (4) of the products were of liquid consistency and the remaining seven were semisolid. TTO concentrations in the products varied from 0.1% to 15% (Table 2). For some products, the amount of TTO or how the amount of TTO was measured (e.g., wt/vol, vol/vol) was not stated. Most products did not state the amounts of preservatives, and a few did not list any excipients other than the TTO. Manufacturers were contacted by e-mail or telephone in an attempt to obtain this information.

Table 1. Chromatographic Profile of Tea Tree Oil Batch W/ES04

Component	*% Composition*	*ISO standard range (%)*[18]
Terpinen-4-ol	40.3	30–48
γ-Terpinene	19.7	10–28
α-Terpinene	8.6	5–13
1,8-Cineole (eucalyptol)	3.2	Traces–15
Terpinolene	3.2	1.5–5
α-Terpineol	3.1	1.5–8
α-Pinene	2.4	1–6
p-Cymene	2.4	0.5–8
Aromadendrene	1.6	Trace–3
δ-Cadinene	1.2	Trace–3
Limonene	1.0	0.5–1.5
Globulol	0.5	Trace–1
Sabinene	0.1	Trace–3.5
Viridiflolol	0.4	Trace–1

ISO, International Organization for Standardization.

Microorganisms

The reference isolates *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571, *Salmonella enterica* subsp. *enterica* serovar *typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 10662, and *Candida albicans* ATCC 10231 were obtained from the Division of Microbiology and Infectious Diseases at PathWest Laboratory Medicine WA, Perth, Western Australia. All isolates were maintained on horse blood agar during the study.

Antimicrobial activity study protocol

Inoculum preparation. Inocula were prepared by inoculating one to two colonies of each organism into 10 mL of Mueller Hinton broth (MHB) and incubating for 24 hours at 35°C. The prepared cultures were then adjusted with 0.85% saline using a Vitek Colorimeter (Hach Company, Loveland CO) to approximately 10^8 cfu/mL for bacteria and 10^7 cfu/mL for *C. albicans*. Adjusted suspensions were used to surface-inoculate agar plates for the well diffusion assay or were further diluted to approximately 10^6 cfu/mL in either single or double-strength MHB and used as inocula for the broth dilution assays.[19,20] Inocula concentrations were confirmed by viable counts.

Agar well diffusion assay. The agar well diffusion assay was used to assess the antimicrobial activity of the products based on the diffusion of antimicrobial compounds from the formulation through the hydrophilic agar. Although not approved by the Clinical Laboratory Standards Institute,[21] this method has been used previously to determine the antimicrobial effects of other essential oils[22] and antimicrobials in food.[23] The assay was carried out and zones of growth inhibition were determined according to the method developed by Davidson and Parish.[23] Briefly, one well (11 mm in diameter) was punched into each inoculated agar plate.

Table 2. Tea Tree Oil (TTO) and Products Tested *In Vitro* for Antimicrobial Activity

Product name	*Manufacturer*	*TTO content*	*Formulation consistency*	*Other excipients as stated on packaging*
TTO (nonformulated)	Australian Plantations, Australia	100%	Liquid	—
On-the-spot stick	Australian Bodycare, Denmark	10%	Liquid	Alcohol denat (>10%) PEG-40, hydrogenated castor oil, polysorbate 60, *Camelia sinensis*, ceteareth-25, *Triticum vulgare*, silica
Goanna TTO solution	Herron Pharmaceuticals Pty. Ltd., QLD, Australia	15% (wt/vol)	Liquid	Water, polysorbate 20, isopropyl alcohol
TTO antiseptic solution	Thursday Plantation, NSW, Australia	15% (vol/vol)	Liquid	Ethanol (28.8%)
Antiseptic mouthwash	Thursday Plantation	0.1% (wt/vol)	Liquid	Sorbitol, xylitol, polysorbate 20, spearmint oil, peppermint oil, anise oil, cinnamon leaf oil, fennel oil, nutmeg oil, sage oil (Spanish), coriander oil
Derma E tea tree and antiseptic creme	Derma E Products, USA	5.0%	Semisolid	Safflower oil, stearic acid, glyceryl stearate, cetyl alcohol, tocopheryl acetate, glycerin, allantoin, panthenol, aloe vera powder, almond oil, avocado oil, sesame seed oil, horsetail extract (herb), methylparaben, imidazolidinyl urea
Tea tree herbal cream	Martin & Pleasance Pty. Ltd., VIC, Australia	5.0% (vol/wt)	Semisolid	1% (wt/wt) Phenoxyethanol, aqueous cream base
Metique™ sensitive skin wash	Novasel, QLD, Australia	5.0%	Semisolid	Water, lauryl glucoside, cocamidopropyl betaine, laureth-4, tocopheryl acetate, lactic acid, fragrance, undecylenic acid (0.2%)
Metique™ after wax lotion	Novasel	5.0%	Semisolid	Glycerol, capric/caprylic triglyceride, glycerin, stearic acid, octyl palmitate, glyceryl stearate S.E., cetyl alcohol, ceteth 20, phenoxyethanol, dimethicone, tocopherol acetate, methyl hydroxybenzoate, carbomer, potassium hydroxide, ethyl hydroxybenzoate, butyl hydroxybenzoate, propyl hydroxybenzoate, iso-butyl *p*-hydroxybenzoate
Tea tree cream	Oil Garden, VIC, Australia	5.0% (wt/wt)	Semisolid	Not stated
Tea tree antiseptic cream	Thursday Plantation	5.0% (wt/vol)	Semisolid	Diazolidinyl urea, methylhydroxybenzoate, propylhydroxybenzoate
Tea tree antiseptic cream	Treemenda,™ Canada	5.0%	Semisolid	Palm kernel oil, palm oil, extra virgin olive oil, chamomile, nettle stem and leaf, red clover leaf and blossom, dandelion leaf, cera alba, avocado oil, pumpkinseed oil

Wells were then filled with 200 μL of product or control TTO solution and plates were incubated for 24 hours at 35°C. After incubation, the diameter of the zone of inhibition was measured to the nearest millimeter. All trials were carried out in duplicate on at least two separate occasions. The mean and standard deviation of zone size was then calculated.

Broth microdilution assay. The broth microdilution assay[21,24] was used for testing liquid formulations. A minor modification was made to the method whereby a final concentration of 0.001% Tween 80 was included in assays with TTO (but not assays with products) to aid in oil solubilization. This concentration of Tween 80 was selected to minimize potential interference with the activity of TTO. Briefly, the amounts of product were measured out in such a way that it would result in final TTO concentrations of 2%, 1%, 0.5%, and 0.25% wt/vol for each product. These concentrations were chosen, because four of the five test organisms have reported minimum inhibitory concentrations (MICs) in this concentration interval. A positive growth control of growth medium without any product was also included. Each row of a 96-well microtiter tray was inoculated with the relevant test organism and the tray was incubated for 24h at 37°C. After this time, MICs were determined visually as the lowest concentration resulting in the absence of turbidity. Minimum bactericidal concentrations (MBCs) or minimum fungicidal concentrations (MFCs) for the liquid TTO formulations were determined by subculturing 10-μL aliquots from nonturbid wells, spot inoculating onto pre-dried agar plates, and incubating for 24 hours at 37°C. The MBC/MFC was determined as the lowest concentration resulting in no growth of subculture following incubation. All tests were carried out in duplicate on at least two separate occasions and were repeated if results differed by more than one doubling dilution. The means were then calculated.

Broth macrodilution assay. Semisolid TTO products were tested using the broth macrodilution assay. Dilutions of product were prepared by weighing specific amounts of each product into glass McCartney bottles. Product dilutions were prepared as described under the broth microdilution assay, except that the quantities were weighed out and not measured with a pipette. Appropriate volumes of sterile distilled water were then added to obtain a total volume of 2.5 mL in each McCartney bottle. Product and diluent were vortexed for 10 seconds prior to inoculation. A 2.5-mL volume of inocula was then added to a final volume of 5 mL. The positive growth control consisted of sterile water with added inocula. Nonformulated TTO was prepared in corresponding concentrations and tested in parallel. Dilution of the products resulted in opaque solutions and as such, endpoints could not be determined visually. MBCs were therefore determined, after 24-hour incubation at 35°C, by subculturing 100-μL volumes from each of the four dilutions, spreading onto Mueller Hinton agar and incubating for another 24 hours at 35°C. All tests were carried out in duplicate on at least 2 separate days and were repeated a third time if results differed by more than one doubling dilution to eliminate intra- and interday variations, and the means were subsequently calculated.

Results

Agar well diffusion assay

All TTO control solutions produced zones against the test organisms with the exception of the 5% solution against *P. aeruginosa* (Table 3). As a generalization, the largest zones for the control TTO solutions were seen for *S. aureus* NCTC 6571, followed by *S. typhimurium*, *C. albicans*, *E. coli*, and *P. aeruginosa*. For products, the largest zones of inhibition were produced by the liquid formulations with the exception of the mouthwash, which did not produce a zone of inhibition. Several of the semisolid formulations also produced no zones or only small zones of inhibition. Similar to the control TTO solutions, the largest zone sizes were generally seen for *S. aureus*.

Micro- and macrodilution assays

The majority of liquid (Table 4) and semisolid products (Table 5) had MIC or MBC values that were either equivalent

Table 3. Zones of Inhibition (Mean mm ± Standard Deviation) for Tea Tree Oil (TTO) and Products

TTO/product		E. coli *NCTC 10418*	S. aureus *NCTC 6571*	S. typhimurium *ATCC 13311*	P. aeruginosa *NCTC 10662*	C. albicans *ATCC 10231*
TTO (nonformulated)	5% (vol/vol)	22.5 ± 0.5	34.0 ± 2.0	14.0 ± 2.0	—	17.3 ± 3.3
	10%	27.3 ± 0.3	39.8 ± 0.8	29.3 ± 0.3	14.8 ± 0.3	28.3 ± 2.3
	15%	28.5 ± 0.5	42.3 ± 1.3	32.0 ± 0.0	18.5 ± 0.5	30.5 ± 2.5
Australian Bodycare On-the-spot stick		23.8 ± 1.8	25.5 ± 1.5	20.3 ± 0.3	13.5 ± 0.5	30.0 ± 1.0
Goanna TTO solution		43.3 ± 2.8	30.5 ± 0.5	44.8 ± 0.3	16.5 ± 0.0	49.8 ± 0.3
Thursday Plantation TTO solution		29.8 ± 0.3	41.3 ± 0.3	32.8 ± 1.3	16.0 ± 1.0	36.0 ± 1.0
Thursday Plantation Antiseptic Mouthwash		—[a]	—	—	—	-
Derma E creme		24.8 ± 0.8	39.5 ± 2.3	33.5 ± 1.5	21.0 ± 0.0	13.0 ± 0.0
Martin & Pleasance Tea tree herbal cream		—	—	—	—	-
Metique™ sensitive skin wash		14.8 ± 0.8	21.0 ± 1.0	16.8 ± 0.3	13.0 ± 0.0	21.5 ± 0.5
Metique™ after wax lotion		12.5 ± 0.5	—	13.0 ± 0.0	—	17.5 ± 0.0
Oil Garden Tea tree cream		23.0 ± 0.0	31.5 ± 0.5	30.5 ± 1.5	18.5 ± 0.5	25.5 ± 1.5
Thursday Plantation Tea tree antiseptic cream		19.5 ± 0.5	34.8 ± 0.3	28.5 ± 0.5	17.0 ± 0.0	17.5 ± 1.5
Treemenda™ Tea tree Antiseptic Cream		13.0 ± 0.0	17.3 ± 1.3	13.0 ± 0.0	—	—

[a]No zone of inhibition.

E. coli, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *S. typhimurium*, *Salmonella typhimurium*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *C. albicans*, *Candida albicans*.

Table 4. MICs and MBC/MFCs for Liquid TTO Products

	E. coli		S. aureus		S. typhimurium		P. aeruginosa		C. albicans	
	NCTC 10418		*NCTC 6571*		*ATCC 13311*		*NCTC 10662*		*ATCC 10231*	
TTO/product	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
TTO (nonformulated)	0.37	0.37	0.75	1.50	0.37	0.63	>8	>8	0.75	1.50
Australian Bodycare on-the-spot stick	0.11	0.11	0.21	0.34	0.11	0.11	0.57	1.13	0.21	0.33
Goanna TTO solution	0.06	0.13	0.13	0.38	0.06	0.19	0.37	1.5	0.19	0.38
Thursday Plantation TTO solution	0.10	0.25	0.25	0.5	0.06	0.20	1.50	4	0.13	0.75
Thursday Plantation antiseptic mouthwash	0.04	0.04	>0.05	>0.05	0.03	0.04	>0.05	>0.05	>0.05	0.05

All values are expressed as the final concentration (% vol/vol) of tea tree oil present.

MICs, minimum inhibitory concentrations; MBC, minimum bactericidal concentration; MFCs, minimum fungicidal concentrations; TTO, tea tree oil.

E. coli, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *S. typhimurium*, *Salmonella typhimurium*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *C. albicans*, *Candida albicans*.

to, or lower than, those of the control TTO solution. Products with MICs that were severalfold lower than the TTO control solution for one or more organisms were Herron TTO solution, Thursday Plantation TTO solution and mouthwash, derma E cream, and Metique™ sensitive skin wash (*C. albicans* only). In a few instances, the MICs obtained for products were greater than those obtained for the TTO control. In particular, the Martin & Pleasance TTO cream had elevated MICs against *S. aureus*, *S. typhimurium*, and *C. albicans*. However, elevations in MIC were less than twofold and were therefore considered negligible.

Discussion

This study has provided a quantitative evaluation of the antimicrobial activity of a range of commercially available antiseptic TTO products. Values obtained for the nonformulated TTO, which was used in all assays and served as a positive control, corresponded well to literature values for all tested microorganisms.[13,24–26] However, any discussion of the relative activity of each of the products must be considered in terms of the method used to assess activity because the method undoubtedly influences the results obtained.

The well diffusion assay relies on the diffusion of the active components of the TTO and any other antimicrobial excipients out of the product base and into the agar, thereby inhibiting microbial growth. The less viscous the formulation is, the more readily the TTO will be able to diffuse out of the product and into the agar, creating high enough concentrations in the agar to inhibit microbial growth. However, it is apparent from the data obtained in this study that the zone of inhibition did not correlate well between products with the equivalent concentrations of TTO. Furthermore, some products such as the mouthwash, Martin & Pleasance cream, and Metique™ after wax lotion showed little or no activity using the well diffusion assay, yet had demonstrable activity by broth dilution assays. The diffusion of TTO is also likely to be either enhanced or impaired by other product excipients such as alcohols or surfactants. In summary, the agar well diffusion assay is not an accurate method for assessing the antimicrobial activity of TTO products.

Although standardized methods for the assessment of antiseptic products have not been published, the broth dilution assays used in the current study have been adapted from widely accepted methods.[21] The assays are relatively simple and straightforward to perform and allow for the quantitative evaluation of the antimicrobial activity of various pharmaceutical preparations. With regard to the liquid products, all had MICs several concentrations lower than the TTO control. However, the On-the-spot stick and both formulated TTO solutions contain 10%–15% alcohol in addition to TTO. This is likely to contribute to the low MICs, because

Table 5. MBCs or MFCs of Semisolid TTO products

TTO/product	E. coli *NCTC 10418*	S. aureus *NCTC 6571*	S. typhimurium *ATCC 13311*	P. aeruginosa *NCTC 10662*	C. albicans *ATCC 10231*
TTO (nonformulated)	0.37	0.75	0.37	>8	0.75
Derma E crème	0.13	0.19	0.13	0.13	1.50
Martin & Pleasance tea tree herbal cream	0.37	>2	0.75	2	2
Metique™ sensitive skin wash	0.19	0.37	0.19	1.50	0.06
Metique™ after wax lotion	0.25	>2	0.25	1.50	1.00
Oil Garden tea tree cream	0.19	0.25	0.13	0.37	0.50
Thursday Plantation tea tree antiseptic cream	0.13	0.25	0.13	0.50	0.50
Treemenda™ tea tree antiseptic cream	0.25	1.50	0.50	>2	2

All values are expressed as the final concentration (%) of TTO present.

MBCs, minimum bactericidal concentrations; MFCs, minimum fungicidal concentrations; TTO, tea tree oil.

E. coli, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *S. typhimurium*, *Salmonella typhimurium*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *C. albicans*, *Candida albicans*.

it has been suggested previously that the combined antimicrobial effect of the TTO and alcohol is greater than for the TTO alone.[11] The remaining liquid product, the mouthwash, contained only 0.1% TTO, but also contained the surface active agent Tween-20 and other essential oils such as spearmint, nutmeg, and fennel oils, which were likely to contribute to the overall antimicrobial activity of this product.

The majority of semisolid products showed activity that was either better than, or equivalent to, the control TTO solutions. This activity is likely to be a result of several factors including the TTO, any other antimicrobial excipients, and possibly synergy between TTO and some of the excipients. For example, the derma E cream was one of the most antimicrobially active products, with greater activity than the TTO control against almost all microorganisms. This product contains the surfactants sodium lauryl sulphate and alkyl dimethyl betaine, which have been shown to enhance the antimicrobial effect of TTO.[26] It also contained the preservatives methylparaben and imidazolidinyl urea, which are mostly active against gram-positive bacteria but also act against a few gram-negative bacteria.[27] Imidazolidinyl urea is claimed to exert synergy with parabens, especially against fungi.[28] However, a better effect against *C. albicans* compared to the TTO control was not apparent in the current study. In summary, the presence of preservatives and surfactants is likely to have contributed to the overall antimicrobial activity of the product. Similarly, the Metique™ after wax lotion and Thursday Plantation antiseptic cream both had relatively good antimicrobial activity but also contained preservatives, which were likely to contribute to overall activity. Lastly, whereas the Oil Garden cream showed good activity, the excipients of this cream were not stated. As such, it cannot be conclusively stated whether excipients other than the TTO also contribute to overall activity.

The product that performed the most poorly overall was the Martin & Pleasance cream, which performed either equivalent to, or slightly poorer than the TTO control. It may therefore contain one or more excipients that exert an inhibitory effect on the release or activity of TTO. However, the only excipient listed was 1% phenoxyethanol, and without additional excipient information it is not possible to postulate why this product was comparatively less active. The MBCs for the cream indicated fair activity against *E. coli*, *S. typhimurium*, and *P. aeruginosa* compared to the control. Since phenoxyethanol is active against *P. aeruginosa* and other gram-negative organisms,[28] this could explain the activity against *P. aeruginosa*, *E. coli*, and *S. typhimurium*, and its relatively poorer activity against the gram-positive *S. aureus* and the yeast *C. albicans.* Interestingly, the Treemenda™ antiseptic cream had activity approximately equivalent to the TTO control but also contained the preservatives imidazolidinyl urea, methyl- and propylparaben, which would theoretically be exerting an additional antimicrobial action. It is therefore possible that one or more excipients in the cream had an inhibiting or antagonizing effect on both the TTO and the preservatives. Although it is not known which excipient(s) could be causing this effect, it may be caused by the lipophilic excipients in the formulation.

Product excipients have a great influence on the activity of TTO in formulation. Alcohols themselves exhibit antimicrobial activity and can act as a co-solvent in aqueous solutions of TTO thereby increasing the overall antimicrobial activity of the formulation. In addition, surfactants such as sodium lauryl sulphate, lauryl glucoside, and alkyl dimethyl betaine are commonly used to solubilize essential oils in aqueous solutions. Most surfactants do not usually have any antimicrobial activity on their own, but when used to solubilize TTO in an aqueous solution they may display either synergism or antagonism with TTO. Alternatively, in certain conditions surfactants are also known to compromise the activity of essential oils.[26] During the current study, it was found that the antiseptic activity of the products may have declined with increasing amounts of lipophilic excipients in the formulations, as has been shown previously.[12] More investigations into the effects of excipients on the activity of TTO are required to clarify this.

Although the majority of products tested in this study showed promising *in vitro* activity, efficacy *in vivo* can only be determined in clinical trials. Since TTO products are now being incorporated into standard therapies, such as a decolonization regimen for *S. aureus* carriage,[29] it is critical that decreased activity due to improper formulation be scrutinized. This study has illustrated that the assessment of antimicrobial activity of commercial TTO products *in vitro* is an effective way to evaluate product formulations relatively quickly and quantitatively and is an important initial step in the development of clinically effective TTO products.

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Disclosure Statement

No competing financial interests exist.

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