Properties and Acne Treatment of Tea Tree Oil

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Tea tree oil is an essential oil extracted from *Melaleuca alternifolia* (Maiden & Betch) Cheel with known antibacterial, anti-inflammatory, and antioxidant properties and widely used in cosmetic products to treat acne vulgaris.

Keywords: antioxidant properties ; antibacterial properties ; tea tree oil ; acne vulgaris

1. Components of Tea Tree Oil (TTO) and Their Variability

Melaleuca alternifolia (Maiden & Betch) Cheel is an evergreen Australian native tree and belongs to the Myrtaceae family. The leaves and branches of this species are rich in EOs, which along with other *Melaleuca* species (*M. dissitiflora* Smith, *M. linariifolia* F. Mueller, and *M. uncinata* R. Br.), EOs are known as tea tree oil (TTO). This TTO is used in the pharmaceutical, cosmetic, and food industries due to its antimicrobial, antioxidant, anti-inflammatory, and antineoplastic properties [1][2][3]. Adulteration of TTO happens, which may be related to confusion with the common name (tea tree) of some species in the genus *Leptospermum*, and species in the genera *Kunzea* and *Baeckea* from Australia and New Zealand, which also have the same common name. Nevertheless, the financial gain can be the most important factor in the adulteration of TTO [2], through the addition of lower-cost byproducts derived from *Eucalyptus* essential oil or even through the addition of pure chemical compounds obtained by chemical synthesis or fermentation [4].

The Australian tea tree industry exports 90% of its production to international markets, around 900 metric tonnes of oil per annum. The production is mainly based on genetically improved populations resulting from a long-term breeding program [5].

There are six oil chemotypes in *M. alternifolia* identified by the presence of distinct percentages of terpinen-4-ol, 1,8-cineole, and terpinelone: terpinen-4-ol chemotype containing 30 to 40% of this monoterpenoid (used in commercial TTO production); terpinolene chemotype; 1,8-cineole chemotype; and three chemotypes all dominated by 1,8-cineole but differing in either terpinen-4-ol or terpinolene content [1][6]. According to the International Standard Organization (ISO 4730:2017) [7], TTO must have a minimum terpinen-4-ol content of 35% and a maximum 1,8-cineole content of 10%, nevertheless market prefers a TTO with the highest terpinen-4-ol (higher than 38%) and the lowest 1,8-cineole content (less than 3%) possible [7][8][9]. Ten monoterpene/monoterpenoids and five sesquiterpene/sesquiterpenoids can be found in TTO (**Figure 1**).

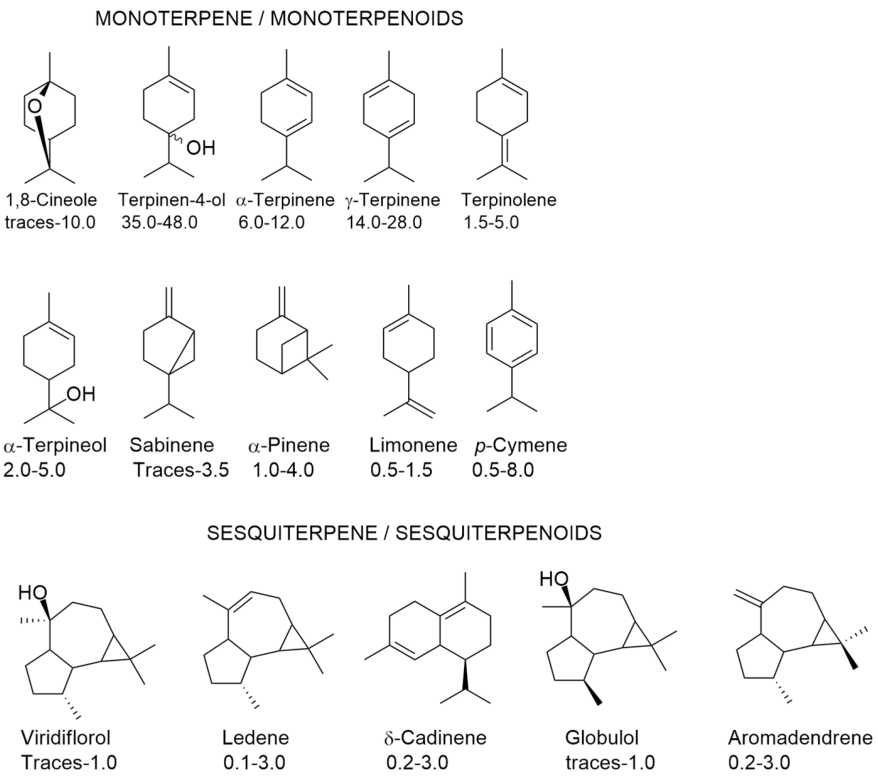

Figure 1. Chemical structures of the monoterpene/monoterpenoids and sesquiterpene/sesquiterpenoids present in the TTO and their concentrations in percentages [8].

To avoid adulterated TTO with other compounds, the revised international standard, ISO 4730-2017 [7] included a chiral ratio parameter, indicating that oil from the terpinen-4-ol chemotype must have a chiral ratio of 67–71% for (+)-terpinen-4-ol and 29–33% for (−)-terpinen-4-ol. Southwell et al. [10] characterized the enantiomeric ratios of the key chiral monoterpenes limonene, terpinen-4-ol, and α-terpineol in the 6 most common chemotypes of *M. alternifolia*. The study found that there was a difference among the average chiral ratios of the 6 chemotypes of this species. So, the authors concluded that oil blended from more than one chemotype has the potential to change the chiral ratio from a chemotype average.

In the same chemotype of TTO, there is chemical variability, as can be observed in **Figure 1**, since it is permitted an interval of percentages for each volatile compound. This variability can be attributed to genetic [11][12] and environmental factors [6][11], leaf age and mechanical damage [13], and harvesting season [14], hydrodistillation-induced artifact formation [15]. Other isolation procedures [16][17] have been used for isolating the volatiles of tea trees, which may contribute to the chemical variability, but they are not considered in the present entry, due to the definition of ISO [18] for EOs.

2. Biological Properties of TTO

According to the review made by Carson and Riley [19], TTO was applied for many purposes: perionychia, empyema, gynaecological conditions, epidermophyton infections, impetigo contagiosum, pediculosis, ringworm, tinea, throat, psoriasis, and mouth conditions. A systematic review to assess preclinical and clinical studies focused on the antiparasitic activity of TTO against *Demodex* mites, scabies mites, house dust mites, lice, fleas, chiggers, and bed bugs revealed the efficacy of TTO and its components against ectoparasites of medical importance. Such results can justify the use of TTO in the pharmacotherapy of ectoparasitic infections [20] with the advantages to be simple to use and having better side effects than the current treatments [21].

In Australian traditional medicine, Aboriginal people used *M. alternifolia* to treat bruises, insect bites, and skin infections [22]. During World War II, TTO was used as an insect repellant to reduce the infection rate following skin injuries [19][22]. However, after that period, a declining use was observed owing to unreliable supply and variable quality [19]. Currently, to prevent this variability, the ISO has established limits for the proportions of volatile constituents that must be present in any product(s) marketed as the terpinen-4-ol chemotype of TTO. This norm also includes the enantiomeric ratio of terpinen-4-ol [7][23].

The ethnopharmacological studies made on the utilization of TTO may disclose their antimicrobial, antioxidant, and anti-inflammatory activities.

2.1. Antimicrobial Activity of TTO

Melaleuca alternifolia essential oil has antibacterial, antifungal, and antiviral activities, and such properties have been attributed to terpinen-4-ol. For example, May et al. [24] reported that a terpinen-4-ol-rich TTO was more effective against several multi-drug-resistant organisms, including MRSA, glycopeptide-resistant enterococci, aminoglycoside-resistant *Klebsiella*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, and also against sensitive microorganisms than a TTO with a lower percentage of that monoterpenoid and higher percentage of 1,8-cineole. However, the antimicrobial activity of TTO that can be attributed to the loss of membrane integrity and function, letting the intracellular material out, disabling to maintain homeostasis, and inhibiting respiration [1][25] is not only attributed to the amount of terpinen-4-ol, but from to the complex interaction among different components such as 1,8-cineole, α-terpinene, γ-terpinene, terpinolene, among other ones of the TTO [9][26]. The antimicrobial activity of ten EOs of terpinen-4-ol chemotype assessed against *Candida glabrata*, *Herpes simplex* virus type 1 (HSV-1), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa* grown in planktonic mode or biofilms showed that only five had significant antimicrobial activity by reducing bacterial survival in biofilms, generating oxidative damage in *C. glabrata*, and decreasing HSV-1 infectivity [26]. The authors were not able to correlate such activities to the amounts of terpinen-4-ol in the sample oils.

In *Escherichia coli*, *S. aureus*, and *C. albicans*, Cox et al. [27] concluded that the antimicrobial activity of TTO results from its ability to disrupt the permeability barrier of microbial membrane structures, that is, similar to that of other disinfectants and preservatives, such as phenol derivatives, and chlorhexidine. Nevertheless, this observation was more evident when *E. coli* cells were in the exponential phase because, at the stationary phase, *E. coli* showed an increased TTO tolerance [28][29]. According to the authors, such results can be partially explained by alterations in membrane/structure that occur during the stationary phase. The disruption of the cell walls and membranes were also reported by Li et al. [30]. They observed that TTO penetrated through the cell walls and cytoplasmic membranes of *E. coli*, *S. aureus*, *C. albicans,* and *Aspergillus niger*, damaging these structures with subsequent loss of cytoplasm content and cell death. Cuaron et al. [31] reported that TTO was able to denature proteins, alter the membrane, cell wall structure, and function of *S. aureus*. In addition, TTO was able to down-regulate the genes involved with energy-intensive transcription and translation and alter the regulation of genes involved with heat shock and cell wall metabolism in *S. aureus*. For example, the inactivation of those heat shock genes, which encodes a regulatory system that responds to peptidoglycan biosynthesis inhibition in *S. aureus*, led to an increase in TTO susceptibility [31].

Although the absence of a correlation between the terpinen-4-ol amount and the biological activity of TTO, the majority of the antimicrobial activities are made with the terpinen-4-ol chemotype against several microorganisms: **Table 1** depicts the antimicrobial activity of TTO found in diverse works and against several microorganisms.

Table 1. Antimicrobial activity, biofilm reduction, or biofilm inhibition induced by TTO, according to several works made by diverse research teams.

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Terpinen-4-ol, 42 Terpinen-4-ol standard	*Legionella pneumophila*	-	0.25–0.5% (*v/v*)	0.06–0.125% (*v/v*)	-	[32]
β-Pinene, 9 β-Terpineol, 6 Terpinen-4-ol, 10 α-Terpineol, 20	Planktonic *Enterococcus faecalis* *E. faecalis* biofilm inhibition	-	0.5%	0.25%	≥0.25% (I)	[33]

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
α-Terpinene, 9 γ-Terpinene, 20 Terpinen-4-ol, 43	*Escherichia coli* 22BT *E. coli* 45DT *Enterococcus faecium A29* *E. faecalis* VAN3 *Staphylococcus aureus* C3 *S. aureus* O	-	-	128 µg/mL 1 µg/mL 1 µg/mL 64 µg/mL 8 µg/mL 8 µg/mL	128 µg/mL (R) 1 µg/mL (R) 1 µg/mL (R) 64 µg/mL (R) 8 µg/mL (R) 8 µg/mL (R)	[34]
α-Terpinene, 11 γ-Terpinene, 19 Terpinen-4-ol, 33	14 Clinical and 2 references *S. aureus* strains	Liquid 8–30 Volatile 0–15	-	Liquid 0.1–0.8% (*v/v*) Liquid (biofilm)	Liquid (biofilm) 0.8–6.3% (*v/v*) (minimal biofilm eradication)	[35]

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Area by standard GC-MS α-Pinene, 12 1,8-Cineole, 15 γ-Terpinene, 10 *o*-Cymene, 6 Terpinen-4-ol, 35 Area by Head Space GC-MS α-Pinene, 23 1,8-Cineole, 17 γ-Terpinene, 11 *o*-Cymene, 9 Terpinen-4-ol, 29	Methicillin-susceptibility *Staphylococcus aureus* Methicillin-resistant *Staphylococcus aureus* *Escherichia coli* Extended Spectrum Beta-Lactamases Carbapenem-Susceptible Kp Extended Spectrum Beta-Lactamases Carbapenem-Resistant *Acinetobacter baumannii* *Pseudomonas aeruginosa* Methicillin-susceptibility *Staphylococcus aureus* + oxacillin Methicillin-resistant *Staphylococcus aureus* + oxacillin			2% (*v/v*) 2% (*v/v*) 0.25 0.50 0.25 0.25 1	1% (*v/v*) 0.50% (*v/v*) 0.25% (*v/v*) 0.50% (*v/v*) 0.25% (*v/v*) 0.25% (*v/v*) 1% (*v/v*) Fractional inhibitory concentration index 0.32 (synergism) 0.32 (synergism)	[36]

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Terpinen-4-ol, 40 γ-Terpinene, 12 1,8-Cineole, 7 *p*-Cymene, 6	*Bacillus subtilis*			MIC 90 (µL/mL)		[37]
	Enterococcus faecalis					
	Micrococcus luteus	9.33		18.36		
	Staphylococcus aureus	10.67		18.45		
	Pseudomonas aeruginosa	7.67		18.68		
	Yersinia enterocolitica	7.33		14.26		
	Salmonella enterica	6.00		12.32		
	Serratia marcescens	6.00		15.46		
	Pseudomonas fluorescens (biofilm)	6.67		16.36		
	Salmonella enterica (biofilm)	6.00		16.24		
	Candida albicans	6.00		28.59		
	C. glabrata	10.67		25.43		
	C. krusei	7.67		26.76		
	C. tropicalis	6.33		29.85		
		8.33		26.32		
				27.46		

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Terpinen-4-ol, 36	*Staphylococcus aureus*		1	0.5		[38]
	Coliform bacilli		2	1–2		
	Proteus spp.		4	2		
	Klebsiella spp.		2	1		
	Escherichia coli		2	1		
	Citrobacter spp.		2	1		
	Enterobacter spp.		2	2		
	E. coli (NCTC 11560)		4	2		
	Fecal streptococci		4	2		
	Fecal streptococci		4	2		
	β-Hemolytic streptococci GP.2		>8	>8		
	Enterococcus faecalis (ATC29212)		>8	8		
	β-Hemolytic streptococci		>8	>8		
	Streptococcus pyogenes		1–4	0.5–2		
	Coagulase-negative staphylococci		4	2		
	MRSA		4	2–4		
	Staphylococcus aureus (NCTC 6571)		2	2		
	Candida spp.		1	0.5		
	P. aeruginosa		1–5	2–6		
	P. aeruginosa (NCTC10662)		>8	8		

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Without a chemical profile, only with the following information: TTO complied with the ISO 4730 and European Pharmacopoeia standards	Twenty-seven clinical isolates of *S. aureus* and the reference strain *S. aureus* NCTC 8325-4		0.25–1% (*v/v*) 0.5% (*v/v*)	0.125–0.5% (*v/v*) 0.5% (*v/v*)	2% (*v/v*) 1% (*v/v*)	[39]
TTO (enterprise 1)	Thirty MRSA isolates		1–8% (*v/v*)	0.125–1% (*v/v*)		[40]
TTO (enterprise 2)			1->8% (*v/v*)	0.125–1% (*v/v*)		
Terpinen-4-ol (racemic)			0.125–1% (*v/v*)	0.0625–0.5 (*v/v*)		
L-Terpinen-4-ol			0.125–1% (*v/v*)	0.0625–0.5 (*v/v*)		
TTO (enterprise 1)	Twenty-eight CoNS isolates		0.5–2% (*v/v*)	0.25–0.5% (*v/v*)		
TTO (enterprise 2)			0.5–2% (*v/v*)	0.125–0.5% (*v/v*)		
Terpinen-4-ol (racemic)			0.25–0.5% (*v/v*)	0.0625–0.25% (*v/v*)		
L-Terpinen-4-ol			0.25–0.5% (*v/v*)	0.0625–0.25% (*v/v*)		

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Terpinen-4-ol, 40 α-Terpinene, 9 γ-Terpinene, 21	*Staphylococcus aureus*	1 mg/mL (16.57) 0.5 mg/mL (15.54) 0.01 mg/mL (11.08)				[41]
	Escherichia coli	1 mg/mL (16.75) 0.5 mg/mL (15.13)				
	Candida albicans	0.01 mg/mL (9.87) 0.01 mg/mL (12.21)				

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Terpinen-4-ol, 44 γ-Terpinene, 22 α-Terpinene, 7 α-Terpineol, 6	*C. albicans* *Trichophyton mentagrophytes* *S. aureus* *S. epidermidis* *Streptococcus pyogenes* MRSA *Klebsiella pneumoniae* *P. aeruginosa* *C. albicans* *Trichophyton mentagrophytes* *S. aureus* *S. epidermidis* *Streptococcus pyogenes* MRSA *Klebsiella pneumoniae* *P. aeruginosa*	TTO 20.3 21.1 19.2 21.7 19.2 19.5 18.1 13.2 $AgNO_3$ 17.7 19.2 18.2 19.2 22.4 17.6 24.2 15.3				[42]
Not determined	*Bacteroides* *Prevotella* *Fusobacterium* *Peptostreptococcus anaerobius* Other gram-positive anaerobic cocci			0.03–0.5% (*v/v*) 0.03–0.25% (*v/v*) 0.06–0.55% (*v/v*) 0.06–0.25% (*v/v*) 0.03–0.25% (*v/v*)		[43]

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Three batches Terpinen-4-ol, 41–44 α-Terpinene, 10–11 γ-Terpinene, 21–23	*Cutibacterium acnes*			0.25% (*v/v*)		[44]
Terpinen-4-ol, 40 1,8-Cineole, 5	*Trichophyton rubrum* *T. mentagrophytes* *Microsporum canis* *Candida albicans* *Candida* sp. *Trichosporon cutaneum* *Malassezia furfur* isolated from patients with Dandruff Seborrheic dermatitis Pityriasis versicolor			0.11–0.22% (*m/v*) 0.11–0.44% (*m/v*) 0.11% (*m/v*) 0.44% (*m/v*) 0.22–0.44% (*m/v*) 0.22% (*m/v*) 0.05–0.44% (*m/v*) 0.11–0.22% (*m/v*) 0.05–0.22% (*m/v*)		[45]
α-Terpinene, 9 γ-Terpinene, 19 Terpinen-4-ol, 46	*Chromobacterium violaceum* CV026	At MIC 0.25 mg/mL: 14.3		2 mg/mL		[46]

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
α-Terpinene, 10 *p*-Cymene, 24 Terpinen-4-ol, 25 β-Fenchyl alcohol, 9 Oregano + TTO TTO + Cinamom TTO + Lavender TTO + Laurel Oregano + TTO TTO + Cinamom TTO + Lavender TTO + Laurel Oregano + TTO TTO + Cinamom TTO + Lavender TTO + Laurel	*Streptococcus pyogenes* ATCC 19625 *Staphylococcus aureus* ATCC 25923 *Streptococcus agalactiae* ATCC 12386 *Streptococcus pyogenes* ATCC 19625 *S. aureus* ATCC 25923 *Streptococcus agalactiae* ATCC 12386	15.00 29.50 26.50 No interaction Additive effect Synergic effect No interaction Synergic effect No interaction No interaction Additive effect Synergic effect No interaction Synergic effect No interaction	2.00 0.25 Growth	1.00 0.125 1.00		[47]

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
Cupressus sempervirens + TTO *Myrtus communis* + TTO *Origanum marjorana* + TTO *Origanum vulgare* + TTO	*Streptococcus agalactiae* ATCC 55618 *S. pneumoniae* ATCC 49619 *S. pyogenes* ATCC 12344 *Mycobacterium smegmatis* ATCC 19420 *Moraxella catarrhalis* ATCC 23246 *Cryptococcus neoformans* ATCC 14116 *Staphylococcus aureus* ATCC 25924 *Streptococcus agalactiae* ATCC 55618 *S. pneumoniae* ATCC 49619 *S. pyogenes* ATCC 12344 *Mycobacterium smegmatis* ATCC 19420 *Klebsiella pneumoniae* ATCC 13883 *Moraxella catarrhalis* ATCC 23246 *Cryptococcus neoformans* ATCC 14116 *Staphylococcus aureus* ATCC 25924	Additive effect Additive effect Additive effect Synergic effect Additive effect Synergic effect Synergic effect Additive effect Synergic effect Synergic effect Synergic effect Synergic effect Synergic effect Synergic effect Additive effect Additive effect Additive effect Synergic effect Synergic effect		1.00 mg/mL 2.00 mg/mL 1.50 mg/mL 1.00 mg/mL 2.00 mg/mL 0.09 mg/mL 2.00 mg/mL 1.00 mg/mL 1.00 mg/mL 2.00 mg/mL 1.00 mg/mL 1.00 mg/mL 0.25 mg/mL 2.00 mg/mL 1.00 mg/mL 1.50 mg/mL 3.00 mg/mL 1.00 mg/mL 1.00 mg/mL 0.25 mg/mL 1.00 mg/mL 1.00 mg/mL 2.00 mg/mL 1.00 mg/mL 0.50 mg/mL	[48]

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
	Streptococcus agalactiae ATCC 55618	Synergic effect				
	S. pneumoniae ATCC 49619	Additive effect				
	Mycobacterium smegmatis ATCC 19420	Synergic effect				
	Klebsiella pneumoniae ATCC 13883	Synergic effect				
	Moraxella catarrhalis ATCC 23246	Synergic effect				
	Cryptococcus neoformans ATCC 14116					
	Streptococcus agalactiae ATCC 55618					
	S. pneumoniae ATCC 49619					
	Mycobacterium smegmatis ATCC 19420					
	Klebsiella pneumoniae ATCC 13883					
	Cryptococcus neoformans ATCC 14116					
γ-Terpinene, 17 4-Terpinenyl acetate, 67	*Cutibacterium acnes*		0.053 g/mL	0.053 g/mL	R: No effect	[49]
	Staphylococcus epidermidis		0.053 g/mL	0.053 g/mL	I: 0.107 g/mL	

Main Components, Percentage (>5%)	Microorganism	Diameters of Inhibition Zones (mm)	Minimal Bactericidal Concentration (MBC) TTO	Minimal Inhibitory Concentration (MIC) TTO	Biofilm Reduction (R) or Inhibition (I) (Concentration)	Reference
The quantification of the components were not provided	*Staphylococcus aureus* strain EG-AE1	15.5	78 mg/mL	78 mg/mL		[50]
	Staphylococcus epidermidis strain EG-AE2	21.02	78 mg/mL	78 mg/mL		
	Cutibacterium acnes Strain EG-AE1	20.85	39 mg/mL	39 mg/mL		

Legionella pneumophila is responsible for severe pneumonia named Legionnaires' disease [31]. Mondello et al. [32] tested TTO and terpinen-4-ol against diverse strains of *L. pneumophila* and reported that both had activity against this microorganism (**Table 1**). In addition, the authors also found that the activity was temperature-dependent, that is, the values of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) decreased with increasing temperature (36 to 45 °C).

One of the main causes of endodontic failure is persistent root canal infection and *Enterococcus faecalis,* a Gram-positive facultative anaerobe, is associated with this persistent endodontic infection. Chlorhexidine (CHX) has been used to eliminate *E. faecalis* from superficial layers and dentinal tubules if applied for seven days. Even so, the microorganism is able to survive harsh environments and develop resistance to CHX and antibiotics as well [33]. Qi et al. [33] evaluate the antimicrobial effects of TTO on planktonic *E. faecalis* and biofilms compared with 0.2% CHX. The results are compiled in **Table 1**. In what concerns the biofilm formation, the effects of TTO on pre-formed *E. faecalis* biofilm, at 12, 24, and 48 h time points there was no difference between 2%, 1%, 0.5% TTO, and the CHX group for12 h, 24 h or 48 h. However, when the TTO concentration was ≤0.25%, the activity was statistically different from the CHX group at all time points [33]. The results obtained by these authors permitted them to conclude that TTO could inhibit *E. faecalis* by destroying cell membranes, inhibiting the formation of *E. faecalis* biofilms, and eliminating mature-formed biofilms.

The biofilm is a structured community of microorganisms, enclosed in a self-produced polymeric matrix, able to adhere to an inert or living surface in an aqueous medium. In hospital-acquired infections, many times the bacteria involved in the infection are found within biofilms. These may have a single microbial species or a set of microorganisms (including fungi, algae, and protozoa) [34]. The authors [34] studied the effect of two EOs being one of them TTO, against both mature biofilms and biofilms in the process of formation, produced by vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA) and broad-spectrum-lactamase-producing *Escherichia coli* (ESBL). The MIC values are presented in **Table 1**. However, the authors also determined the MIC of the associations of TTO and other EO, and the combinations of TTO and antibiotic (not depicted in **Table 1**). Their results confirmed a synergistic effect among the EOs and antibiotics. The same synergistic effect among the EOs and antibiotics was also observed for the biofilm formation and the anti-mature biofilm activity [34].

Not only liquid EOs but also volatile forms of EOs show antimicrobial activity. The utilization of the volatile form of EOs has the advantage to provide higher concentrations of active compounds to the infection site and avoid the side effects of the systemic administration of EOs [35]. Taking this in mind, the authors [35] evaluated the antimicrobial and antibiofilm in vitro activity of seven volatile and liquid EOs against MRSA and methicillin-sensitive (MSSA) clinical and reference strains, generally responsible for wound and bone infection. **Table 1** only depicts the results of TTO. Liquid and volatile thyme EO had the highest antibiofilm activity in contrast to the vapor phases of tea tree and lavender. The antimicrobial activity of the vapor phase of TTO was also performed and compared with the liquid phase against methicillin-sensitive *Staphylococcus aureus* (MSSA), *Escherichia coli*, and clinical strains of methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamases producer carbapenem-sensitive *Klebsiella pneumoniae* (ESBL-CS-Kp), carbapenem-resistant *K. pneumoniae* (CR-Kp), *Acinetobacter baumannii* (CR-Ab), and *Pseudomonas aeruginosa* (CR-Pa) [36]. Moreover, synergistic activity between TTO and different antimicrobials were also determined. TTO showed bactericidal activity against all the tested

microorganisms (**Table 1**). TTO in combination with oxacillin showed a high level of synergism at sub-inhibitory concentrations, against MRSA. The vapor phase assay showed high activity of TTO against CR-Ab. According to these results, the authors [36] suggest that TTO in the vapor phase might represent a promising option for local therapy of pneumonia caused by CR-Ab.

Pseudomonas fluorescens can form biofilm in soil and water habitats, can influence food spoilage, water quality, and plant diseases, and create nosocomial infections [37]. *Salmonella enterica* is the main cause of acute foodborne illness and is a source of infection from poultry meat, and can form biofilm even in low temperatures. Moreover, the biofilm can be formed on glass and wooden surfaces, which can cause cross-contamination of vegetables [37]. Antibiofilm activity was observed against *P. fluorescens* and *enterica* through the observation of degradation of the protein spectra using the matrix-assisted laser desorption/ionization—time-of-flight mass spectrometer (MALDI-TOF MS) and under the effect of TTO [37]. TTO was also used on Gram-positive, and Gram-negative bacteria, and yeasts, and the values found for the growth inhibitions are depicted in **Table 1**.

Banes-Marshall et al. [38] investigated the effect of TTO on diverse isolates from leg ulcers, pressure sores, skin, and vagina. The MIC and MBC values are resumed in **Table 1**. According to the results, *S. aureus* and *Candida* spp. were particularly sensitive to the action of TTO, and therefore, it may have a positive role in the growth inhibition of the commonly isolated wound pathogens, and in the frequent infection in immunosuppressed and antibiotic-treated patients [38].

The activity of TTO against twenty-seven clinical isolates of *S. aureus* and the reference strain *S. aureus* NCTC 8325-4 were assayed [37] either in planktonic cells or biofilm (**Table 1**). The killing rate for stationary phase cells was less affected by increasing TTO concentration than that for exponential phase cells. Moreover, the fastest killing of biofilm occurred during the first 15 min of contact with TTO, and concentrations above 1% did not affect the results [39].

Loughlin et al. [40] compared the bactericidal activity of the racemic terpinen-4-ol and the L-isomer terpinen-4-ol with TTO manufactured by two enterprises, against clinical skin isolates of MRSA and coagulase-negative staphylococci (CoNS). The MIC values are depicted in **Table 1**. Terpinen-4-ol was a more potent antimicrobial against MRSA and CoNS isolates than the TTO. In any case, the concentrations tested displayed toxicity to human fibroblast cells [40].

The antimicrobial effect of TTO against *S. aureus*, *E. coli*, and *C. albicans* was also studied by Blejan et al. [41] (**Table 1**). These authors compared the activity of TTO with other EOs and concluded that oregano and basil EOs had a similar antimicrobial effect against *S. aureus*, while against *E. coli*, the antimicrobial activity was similar for oregano, basil EO, and TTO. Against *C. albicans*, TTO and basil EO showed strong antifungal properties [41]. According to these authors, linalool might be responsible for the activity against *S. aureus*, whereas, for *E. coli*, the effect could be attributed to linalool, 1,8-cineole, terpinen-4-ol, α-pinene, *p*-cymene, α/γ-terpinene, whereas for *C. albicans*, 1,8-cineole, and terpinen-4-ol could be responsible for the activities found.

Silver nanoparticles (AgNPs) have displayed antimicrobial effects against a wide range of microorganisms, including antibiotic-resistant strains. Several methods have been used to synthesize AgNPs. The green synthesis method, in which aqueous plant extracts are used, provides a simple, cheap, fast, energy-efficient, and eco-friendly alternative to the traditional chemical and physical methods of nanoparticle synthesis [42]. Ramadan et al. [42] studied the antimicrobial potential of TTO and AgNPs (obtained by using an aqueous extract of *M. alternifolia*) against selected skin-infecting pathogens, including bacteria, fungi, and viruses (**Table 1**). AgNPs had better antiviral activity against both herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) than TTO at its maximal noncytotoxic concentration (0.043%, *v/v*).

The activities of TTO against lactobacilli and a range of organisms associated with bacterial vaginosis were evaluated by Hammet et al. [43]. **Table 1** lists MIC data. It is possible to observe that all lactobacilli tested were appreciably more resistant to TTO than organisms known to be associated with bacterial vaginosis, with at least a twofold difference in MIC values. Three batches of TTO were able to inhibit the growth of *C. acnes*, with a MIC value of 0.25% (*v/v*) (**Table 1**) [44].

Nenoff et al. [45] evaluated the antifungal activity of TTO against several dermatophyte and yeast strains, among the latter 31 strains of the *Malassezia furfur,* and determined the MIC values (**Table 1**).

Ngeng et al. [46] evaluated the anti-quorum sensing activity of TTO through the violacein production in *Chromobacterium violaceum*. The quorum sensing inhibition diameter zone in *C. violaceum* was 14.3 ± 0.5 mm at MIC concentration (**Table 1**). However, *Citrus sinensis,* also studied by these authors, presented better activity than TTO, since it could inhibit violacein production right down MIC/16 whereas TTO did not inhibit violacein production beyond MIC/4 [46]. Violacein is an

antioxidant that protects the bacterial membrane against oxidative stress. When TTO inhibits violacein production and swarming motility, it can suppress virulence and reduce biofilm risks during infections [46].

The synergistic effect between some EOs has also been performed through the Fractional Inhibitory Concentration Index (FICI) of the binary combinations of EOs determined by the checkerboard method. Following this method, and evaluating the MIC and Minimum Bactericidal Concentration (MBC), the authors concluded that TTO/lavender oil mixtures showed a synergistic effect against *Streptococcus pyogenes* and *Streptococcus agalactiae*; TTO/oregano oil had a synergistic effect against *Staphylococcus aureus* and *S. agalactiae*. According to these results, the authors [47] concluded that combination against pathogens should be preferred as potential antimicrobial agents. Combinations of commercial EOs mainly applied in aromatherapy for respiratory tract infections were also studied by [48] aiming the antimicrobial, anti-inflammatory, and toxicity properties. Five combinations were found presenting antimicrobial activity, reduced cytotoxicity, and improved anti-inflammatory effects, having four the presence of TTO: *Cupressus sempervirens* L. + *M. alternifolia*; *Origanum marjorana* L. + *M. alternifolia*; *Myrtus communis* L. + *M. alternifolia*; *Origanum vulgare* L. + *M. alternifolia* at 1:1 ratios. The microorganisms included the Gram-positive strains *Staphylococcus aureus* (ATCC 25924), *Streptococcus agalactiae* (ATCC 55618), *Streptococcus pneumoniae* (ATCC 49619), and *Streptococcus pyogenes* (ATCC 12344); the Gram-negative strains *Haemophilus influenzae* (ATCC 19418), *Klebsiella pneumoniae* (ATCC 13883) and *Moraxella catarrhalis* (ATCC 23246); and the non-pathogenic *Mycobacterium* strain M. *smegmatis* (ATCC 19420) and yeast strain *Cryptococcus neoformans* (ATCC 14116) [48].

Abdelhamed et al. [49] reported that TTO (**Table 1**), thyme and clove EOs have the capacity for inhibiting the growth of *C. acnes* and *Staphylococcus epidermidis*, although thyme essential oil was more effective since it was able to eliminate the initial bacterial inoculum after 10 h and 6 h of exposure for *C. acnes* and *S. epidermidis*, respectively. In contrast, Esmael et al. [50] reported that TTO was more effective than rosemary oil as a growth inhibitor of three groups of the acne-inducing bacteria *C. acnes* EG-AE1, *S. epidermidis* EG-AE2 and *S. aureus* EG-AE1, from Egypt. The chemical composition of TTO described by both teams was distinct, Abdelhamed et al. [49] described that 4-terpinenyl acetate dominated the EO extracted from the buds and terpinen-4-ol was absent. TTO used by Esmael et al. [50] for the determination of antimicrobial activity did not present those compounds.

Invasive fungal wound infections reported in trauma patients cause considerable morbidity and mortality despite the standard of care treatment in trauma centers [43]. Homeyer et al. [51] assessed the activity of various concentrations of TTO (without chemical composition provided) against 13 clinical filamentous fungal isolates comprising nine species (*Exophiala* sp., *Apophysomyces* sp., *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, *Actinomucor* sp., *Mucor* sp., *Fusarium* sp., and *Absidia* sp.). Seven concentrations of TTO were assayed (100%, 75%, 50%, 25%, 10%, 5%, and 1%). For the majority of isolates, fungicidal activity was observed for all of the concentrations tested, 1, 10, and 100% (*v/v*), following 12–24 h exposures. *Aspergillus terreus* and *Absidia* spp. were more tolerant to the activity of TTO requiring higher concentrations for greater log-reductions. Cell viability assays were also performed in vitro using human fibroblasts, keratinocytes, osteoblasts, and umbilical vein endothelial cells. The activities found were dose-dependent with significant cytotoxicity at concentrations of ≥10% [51]. Despite these results, a recent meta-analysis showed that TTO had a limited antifungal effect (*Trichophyton rubrum*, *Trichophyton mentagrophytes* complex, and *C. albicans*) when compared to other EOs (*Cinnamomum zeylanicum* and *Thymus vulgaris*) [52]. Thus, the use of TTO against dermatophytes would not be advisable.

As can be seen in **Table 1** there was a wide range of MIC values for the antimicrobial potency, which can be due to the diverse bacterial species and strains used, their culture methods, and the chemical composition of the TTO samples, and previously reported [53] in a compilation about the utilization of fifteen plant-based natural compounds on the antimicrobial activities.

2.1.1. Tea Tree Oil Formulations with Antimicrobial Activity

Carson et al. [54] in a revision, and May et al. [24] reported that microorganisms colonizing skin transiently were more susceptible to TTO than commensal microorganisms. Such results would be promising since the commensal microorganisms would constitute a barrier against the development of pathogenic microorganisms. The in vitro antimicrobial activities of TTO have led to the development of formulations to achieve better biological activities, as compiled below in a brief way.

The antimicrobial activity of TTO is widely described, but allergic contact dermatitis in susceptible individuals can occur, particularly if the oil is old, which can happen during the daily opening of bottles where TTO is kept [55]. So, an alternative to overcome this inconvenience could be of interest. Minghetti et al. [55] developed a patch (monolayer device) prepared by using methacrylic copolymers, Eudragit E100 (EuE100) or Eudragit NE (EuNE), and a silicone resin, BioPSA7-4602

(Bio-PSA). TTO and oleic acid (skin penetration enhancer in patches) contents were fixed at 10% *w/w* and 3% *w/w*, respectively. The patches were prepared by a casting method and characterized in terms of terpinen-4-ol amounts and skin permeability. The patches were self-adhesive controlled release matrix with TTO and a removable protecting foil [55]. The skin permeation of terpinen-4-ol enhanced when TTO was used in combination (1:1) with oleic acid, being up to 10-fold higher than pure TTO. This ability of oleic acid of enhancing the penetration of TTO can be a kick-off for the optimization of the efficacy and safety of TTO patches [55].

A TTO lipid-based nano-formulation (TTO-LNF) was developed [56] using a quality-by-design (QbD) approach. Using a mixture experimental design, TTO-LNF was optimized with 5% TTO, 10% surfactant (Kolliphor. RH40:Tween-80 = 50:50, *w/w*), 5% co-surfactant (Transcutol P), and 80% water. To make easier the topical administration, it was formulated a TTO-LNF gel adding xanthan gum. The in vitro antibacterial tests of TTO-LNF, TTO-LNF, TTO-LNF, and 5% TTO solution against *Staphylococcus epidermidis* ATCC 35984 and *Pseudomonas aeruginosa* PAO1 were performed. The results showed that *P. aeruginosa* was more susceptible than *S. epidermidis* to the TTO-LNF gel. The respective inhibition zones were 7.8 mm and 4.2 mm. Similarly, the TTO-LNF showed 6.4 mm for P. aeruginosa and 5 mm for *S. epidermidis*. The formulation was also shown to be more effective than the 5% TTO solution. The bacterial growth curve conducted over time showed that the treatment of *S. epidermis* with TTO-LNF gel and TTO-LNF had a notable suppression of bacteria growth for 24 h, even better than the antibiotic kanamycin. The antibacterial effects of blank LNF and blank LNF gel (without TTO) can be explained by the antimicrobial activity of Tween 80 (5%) [56].

Hydrogels are biodegradable three-dimensional crosslinked polymer networks able to assimilate large amounts of water or biological fluids and provide controlled release of drugs [57]. The remarkable water absorption capacity of these systems is due to the high content of functional hydrophilic groups (carboxyl, hydroxyl, and amino groups) contained in the polymer [58]. The polymers can be synthetic or natural (collagen, gelatin, polydopamine, elastin, chitosan, hyaluronic acid, alginate, and cellulose) [58]. Low et al. [57] used chitosan to fabricate hydrogels combined with TTO and silver ions (Ag^+) to treat common wound-infecting pathogens. Silver ions are recognized as possessing antibacterial, antiviral, antiprotozoal, and antifungal activity [57]. The hydrogels loaded with TTO and Ag^+ displayed antimicrobial activity against *P. aeruginosa*, *S. aureus*, and *C. albicans*. The combination lowered the effective concentrations needed for the antimicrobial activity. According to the results obtained, the authors [57] proposed that the relationship between the variables in the fabrication of hydrogels requires deeper studies to be used as antimicrobials in the treatment of acute wounds. This approach will achieve smarter delivery systems [57].

Ghosh et al. [59] fabricated a hydrogel scaffold with β-cyclodextrin and chitosan along with pectin, carboxymethyl cellulose, and polyethylene glycol 400 (PEG 400) as a plasticizer, and copper sulfate as crosslinker loaded with TTO, to enhance the antimicrobial activity in the treatment of acne and other skin infections. These scaffolds could be used as sheet masks or patches. Two hydrogels were formulated differing in the ratio of pectin, carboxymethyl cellulose, chitosan, and β-cyclodextrin (1:1:0.8:0.8 or 1:1:0.8:0.1) plus the plasticizer and the crosslinker. At the moment of the antimicrobial assay, 10 µL of TTO were infused in the scaffold which was in the hole of a plate. *Pseudomonas* sp. was the microorganism used in the assay. Only the first formulation with TTO presented better physical characteristics along with the best antibacterial activity [59]. It could also deliver TTO in a more efficient way enhancing, therefore, the antibacterial activity. The authors [59] concluded that this hydrogel can be used as a sheet mask to deliver TTO, for treating infection and acne.

Antimicrobial in situ-forming alginate wound dressing with TTO microemulsions was assayed in which alginate hydrogels were prepared by a layer-by-layer spray deposition. This formulation would be useful as an advanced dressing for infected wounds [60]. Diverse combinations of TTO, water, polysorbate 80, and ethanol were tested and through the pseudoternary phase diagrams, it was possible to find a stable spherical microemulsion with TTO at 20%, with good antimicrobial activity. The antimicrobial effect of alginate/TTO microemulsion hydrogels on *Escherichia coli* strains was remarkable. Catanzano et al. [60] considered that such a formulation has the potential to as a bioactive wound dressing.

Emulgels with jelly-like consistency are used in dermatological products due to their better applicability, thixotropic behavior, greaseless nature, improved spreadability, and controlled rheological properties. They present the properties of both emulsions and gels [61]. Sinha et al. [61] optimize a nanoemulsion-based emulgel formulation as a vehicle for topical delivery of TTO. The central composite design was used to choose the best processing conditions for nanoemulsion preparation by high energy emulsification method, namely surfactant concentration (Tween® 20), co-surfactant concentration (Cremophor EL®), and stirring speed. The TTO concentration used was 5% (*v/v*) dissolved in Cremophor EL®. After optimization, the nanoemulsion was converted into emulgel using the polymer Carbopol 940 and triethanolamine as an alkalizer. The antimicrobial activity of the emulgel was assayed against the following microorganisms: *Staphylococcus aureus* MTCC-96, *Streptococcus mutans* MTCC-890, *Pseudomonas aeruginosa* MTCC-741, *Escherichia coli* MTCC-723, *Candida albicans* (wild) MTCC-1637, and *Candida albicans* (CA) AIIMS. The results

were compared with those assays using a conventional gel and pure TTO. The emulgel revealed broader zones of growth inhibitions than conventional gel or pure TTO [61].

Other TTO delivery system was studied [62] for combating Gram-positive and Gram-negative bacteria and to be used as an antimicrobial and healing agent in skin wounds. Semisolid bicontinuous microemulsions containing TTO is an example, in which a formulation consisting of Kolliphor® HS 15 (31.05%), Span® 80 (3.45%), isopropyl myristate (34.5%), and distilled water (31%) with TTO incorporated in the proportion of 3.45% (*v/v*) was selected after optimization through diagram construction [62]. In vivo studies using male and female Swiss mice (*Mus musculus* Linnaeus, 1758) showed that the TTO-loaded bicontinuous microemulsion was effective in the healing process of skin wounds because it promotes a higher percentage of wound edge contraction. Antibacterial activity for Gram-positive and Gram-negative bacteria was also observed. According to these results, Assis et al. [62] suggest that this new formulation can be an alternative for topical application in skin wounds as a healing and antimicrobial agent.

Pickering emulsions are those stabilized by solid particles which possess higher biosafety and biocompatibility than classical emulsions stabilized by conventional surfactants. Pickering emulsions are able to spray and recover to high viscosity after spraying onto the wounds. High viscosity can be desired to provide a long residence time and intimate contact with the wounds [63]. A sprayed Pickering emulsion stabilized by chitosan nanoparticles was developed and the TTO was used as its oil phase. Curcumin was added to the oil phase to enhance the antioxidant and anti-inflammatory effects of the emulsion. Pickering emulsions were characterized and their antibacterial activities were evaluated, and the wound healing test was also performed. The authors also formulated a classical emulsion for comparing the results of both emulsion types. The classical emulsion also had the surfactants Tween® 80 and Span® 85. After injecting Balb/c mice, 6–8 weeks of age, with the mixed bacteria suspension subcutaneously, the wounds of the group treated with chitosan nanoparticles and the blank group presented purulence in contrast to the wounds treated with the Pickering emulsion. Moreover, it was observed that the wounds treated with the Pickering emulsion could heal normally and had the smallest area on the fifth day, suggesting that the Pickering emulsion displayed an excellent killing ability to bacteria avoiding wound infections. The wound healing rate in percentage and on day 10 was significantly higher (95.06%) in the group treated with the Pickering emulsion than those treated with classical emulsion (82.93%), chitosan nanoparticles (80.28%), and TTO (84.31%). Those results can be partially explained by the synergistic effects of TTO, curcumin, and chitosan nanoparticles. TTO had antimicrobial activity, anti-inflammatory properties, and scar prevention effects, while curcumin had great antioxidant and anti-inflammatory effects reducing the production of ROS during the inflammatory phase and promoting wound repair [63]. According to these results, the authors suggested that Pickering emulsion is a promising candidate for sprayed wound dressings.

Liposomes are phospholipid bilayer (unilamellar) and/or a concentric series of multiple (multilamellar) vesicles with a large aqueous inner core. They are constituted by synthetic and/or natural phospholipids (soybean lecithin, egg yolk, sunflower) and other membrane components (cholesterol). The size of liposomes ranges from 20 nm (nanoliposomes) to the micrometer scale with the phospholipid bilayer being 4–5 nm thick. Liposomes can encapsulate hydrophobic and hydrophilic drugs in their structure, being effective in drug delivery [64][65][66]. Aguilar-Pérez et al. [66] formulated and characterized nanoliposomes comprising various TTO concentrations (1.2–6.2 mM) and tested the antifungal activity against *Trichophyton rubrum* through the mycelial growth inhibition test. The same procedure was carried out for clove essential oil. Soybean and cholesterol were used as lipids, and Tween® 80 as surfactant. The mycelial growth inhibition results of essential oil-loaded nanoliposomes were compared with pure EOs. The concentrations used for the assays were 0.25, 0.5, 1.0, and 1.5 µL/mL. The maximum encapsulation efficacy was observed for clove essential oil-loaded nanoliposomes. Nanoliposomes' anti-fungal potential demonstrates their capability to inhibit mycelial growth at lower concentrations than the pure EOs against *T. rubrum* [66].

TTO-loaded nanoliposomes were also fabricated by Ge and Ge [67] using soybean phosphatidylcholine, cholesterol, and Tween® 80. They characterized them and the antimicrobial activity was also assayed against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Candida albicans* ATCC 10231. Liposomes encapsulated TTO to form a stable liposome suspension, and the TTO-loaded nanoliposomes showed a significant increase in antimicrobial activity after encapsulation.

Liposomes may be part of nanofibers such as, for example, chitosan/poly(ethylene oxide) nanofiber mats containing TTO liposomes fabricated by using an electrospinning process [68]. These nanofibers had long-term and better antimicrobial activity against *S. aureus*, *E. coli,* and *C. albicans* than chitosan/poly(ethylene oxide) nanofiber mats. The combination of TTO-loaded liposomes and chitosan nanofiber mats act synergistically destroying the cell membrane, preventing cell adhesion, and causing the irregular aggregation of cytoplasm, according to the transmission electron microscope observation [68].

Ethosomes' composition is based on phospholipids (such as soybean phosphatidylcholine), water, and ethanol content comprised between 20 and 45% *v/v* [69]. Similar to liposomes, ethosomes are able to solubilize both lipophilic and hydrophilic drugs inside the vesicles; nevertheless, ethanol has a higher loading capacity for lipophilic drugs than liposomes [69]. Azelaic acid, a dicarboxylic acid analog inhibits follicular keratinization and can be used topically to treat mild to moderate acne [70] Bisht et al. [70] fabricated ethosomes with azelaic acid and TTO to achieve a system with synergistic anti-acne properties within carbopol hydrogel. This hydrogel would make an easier application, adhesion, and drug penetration. The physical characterization was made and the authors also tested antibacterial activity against *S. aureus*, *S. epidermidis,* and *C. acnes.* The developed optimized ethanolic vesicle formulation of azelaic acid and TTO was compared with the topical marketed formulation in the testosterone-induced acne model in Swiss Albino mice [70]. The new hydrogel formulation with azelaic acid and TTO within ethosomes had significantly lower MIC values than TTO alone, maybe to the synergistic effect of azelaic acid and TTO, associated with the improved contact with the bacteria cell walls, increased contact time, and sustained drug delivery of the hydrogel [70]. Moreover, the new formulation-treated animals had a significant decrease in lesions, sebaceous gland hyperplasia, and seborrhea induced by testosterone in Swiss Albino mice [70].

Nanocapsules are polymeric nanocarriers prepared with poly ε-caprolactone, which is biocompatible, and biodegradable, of low toxicity, high stability, and low cost. The surface of nanocapsules can be modified to develop delivery systems able to interact more specifically with biological targets and enhance the intended biological activity [71]. In the pharmaceutical area, the modification has been conducted by using chitosan owing to its biocompatibility and biodegradability, and low toxicity [71]. Silva et al. [71] fabricated TTO-loading poly ε-caprolactone nanocapsules coated by chitosan for the topical acne treatment acting against anti-*C. acnes*. Chitosan with bioadhesive capacity would favor the drug retention in the skin surface and the positive charge would also contribute to the antimicrobial activity in combination with TTO, which would act synergistically. This hypothesis was confirmed by the authors after their experiment in which the coating of TTO-nanocapsules with chitosan presented higher anti-*C. acnes* activity (MIC = 0.14%, *v/v*) than the pure TTO (MIC = 0.56%, *v/v*) [71]. The poly ε-caprolactone nanocapsules did not present antimicrobial activity [71].

TTO has been also used for hand hygiene. For example, Youn et al. [72] compared the hand disinfection effects of TTO (5 mL of 10% tea tree oil disinfectant mixed in a ratio of 2:2:1:15 of *M. alternifolia* oil, solubilizer, glycerin, and sterile distilled water) with alcohol (2 mL of a gel-type hand sanitizer comprising 83% ethanol used without water), and benzalkonium chloride group receiving 0.8 mL of a foam-type hand sanitizer containing benzalkonium chloride used without water, and a control group with no treatment. The results were followed through subjective skin condition, transepidermal water loss, adenosine triphosphate, and a microbial culture test. The TTO group showed a remarkably higher disinfection effect, whereas the benzalkonium chloride group exhibited no disinfection effect based on adenosine triphosphate measurements. The control group demonstrated similar results to the benzalkonium chloride group. In view of the results, Youn et al. [72] suggest that TTO disinfectants should be introduced to nursing practice to prevent and reduce healthcare-associated infections.

The activity of different concentrations of TTO in diverse TTO-containing products such as a hygienic skin wash (HSW), an alcoholic hygienic skin wash (AHSW), and an alcoholic hand rub (AHR) was investigated against *S. aureus*, *Acinetobacter baumannii*, *E. coli* and *P. aeruginosa* [73]. The activity of the same formulations without TTO was used as a control. The efficacy of TTO was dependent on the formulation and the concentration tested, the concentration of interfering substances and the organism tested.

A complex based on natural antimicrobial and anti-irritant compounds, constituted by TTO (0.3%), eucalyptol (0.15%), α-bisabolol (0.10%), and silver citrate (0.01%) in specific ratio 30:15:10:1, respectively was mixed with soap base ingredients and tested for skin hygiene [74]. The antimicrobial activity was determined against the following microorganisms: *Bacillus cereus* ATCC 10702, *S. epidermidis* ATCC 14990, *S. aureus* ATCC 6538-P or ATCC 29213, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 9027, *Micrococcus luteus* 10240a, and *C. albicans* ATCC 10231. The combination displayed additive or synergistic activities against most strains studied, and a balanced performance between antimicrobial activity and biological safety, without skin irritant potential [74].

The antimicrobial activity of lavender, TTO, and lemon in washing liquid (1% alone or in mixtures) and O/W soft body balm (0.5% alone), was evaluated as well as combined with the synthetic preservative 1,3-dimethylol-5,5-dimethylhydantoin and 3-iodo-2-propynyl butyl carbamate mixture (0.1 and 0.3%) against *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *Candida* sp. ŁOCK 0008 and *A. niger* ATCC 16404 [75]. In soft body balm formulations, oils at a concentration of 0.5% did not present any activity. The introduction of a solubilizer (polysorbate 80) to a system containing 0.5% TTO induced a substantial increase in bacteriostatic activity. A combination of 0.5% TTO, 5% solubilizer (polysorbate 80), and 0.3% synthetic preservative warranted the microbiological stability of soft body balm [75].

In antimicrobial terms, the comparison of three soaps for hand hygiene: 2.0% TTO, 0.5% triclosan, and 2.0% chlorhexidine was evaluated along with the perception of healthcare professionals about TTO [71]. For this, a determination of the logarithmic reduction of *E. coli* K12 colony-forming units before and after the hand hygiene of 15 volunteers was done, and interviews with 23 health professionals were performed. All the soaps demonstrated antimicrobial activity (a $\log_{10}$ reduction factor of 4.18 for TTO, 4.31 for triclosan, 3.89 for chlorhexidine, and 3.17 for reference soap), nevertheless, the TTO soap had the advantage to present a pleasant aroma and did not cause skin dryness [76].

Sgorbini et al. [77] investigated the permeation and release kinetics of the main constituents (terpinen-4-ol, α-terpineol, and 1,8-cineole) of TTO at different percentages (5–30% *w/w*) from several semisolid formulations (creams, ointments, and gels). The gel formulation had the highest percentages of those monoterpenoids' release and permeation than creams and ointments, even at lower TTO concentrations, which means the possibility of using less concentrated formulations. By decreasing order, terpinen-4-ol, α-terpineol and 1,8-cineole were the most released and permeated compounds. In all cases, their skin retention was negligible, below 0.1% of the total amount in the formulation [77].

3. Efficacy in acne vulgaris

Various studies have demonstrated the efficacy of TTO in several human pathologies, such as dentistry, infectious diseases, ophthalmology, and dermatology, amongst others [78]. In dermatology, and specifically, in acne vulgaris, several essential oils have shown beneficial results. Tea tree oil is one of the oils described in tests in vivo as having biological activity on acne, thanks to its antimicrobial, anti-inflammatory, and antioxidant properties [79].

Several human studies have been found evaluating the efficacy of TTO in topical preparations for acne vulgaris [80][81][82][83][84][85][86][87][88][89]. All of them have shown efficacy in the treatment of this pathology, namely in inflammatory lesions. In addition to this demonstrated efficacy, the low incidence of adverse effects described is also an advantage for the use of this oil in topical products for the treatment of acne vulgaris.

In 1990, a single-blind random clinical trial (RCT) was carried out on subjects with mild-moderate acne, for three months, comparing the use of a TTO (5%) water-based gel and benzoyl peroxide (BP) 5% water-based lotion. Although both groups showed a reduction in the inflammatory lesions, the group that applied BP showed a significantly greater improvement than the group that applied TTO ($p < 0.001$). Although the results point towards greater efficacy of the BP in reducing acne lesions, the results regarding safety point to better results with the TTO gel. In this group, the adverse events reported were less (44%) than in the BP group (79%), with a statistically significant difference ($p < 0.001$) [80].

Compared with the placebo (carbomer gel), the TTO 5% gel demonstrated good anti-inflammatory and antibacterial capacities, leading to a significant reduction in inflammatory lesions (papules 46.06%; pustules 47.45%) in a double-blind RCT developed by Enshaieh et al. [81], on subjects with mild-moderate acne, for 45 days. This RCT also showed a significant decrease in the number of comedones (40.24%), the total number of lesions (43.64%), and the acne severity index (ASI) (40.49%) [81].

The use of TTO in cleansing and moisturizing products for acne was evaluated in a dual-center, open-label, phase II pilot study, which assessed the efficacy and safety of two products containing TTO, a Face Wash (7 mg/g) and a Gel (200 mg/g), on mild-moderate acne vulgaris, for 12 weeks. The results showed a significant 54% decrease in the total number of lesions ($p < 0.001$), as well as in the investigator's global assessment score ($p < 0.05$). Furthermore, skin oiliness decreased significantly ($p < 0.01$). Even with a twice-daily application, no serious adverse reactions were reported [83].

Compared with *Lactobacillus-fermented Chamaecyparis obtusa* (LFCO), TTO demonstrated less efficacy in reducing the number of inflammatory and non-inflammatory lesions [82]. However, the anti-inflammatory properties of TTO were enhanced in this research with a significant decrease in inflammation-related proteins, namely IL-8 and TLR-2 mRNA. Adverse reactions such as dryness, erythema, and desquamation were once again described as the most prevalent in the use of topical TTO [82].

The efficacy of the association of adapalene and TTO vs. adapalene was evaluated in a triple-blind RCT in subjects with mild-moderate acne vulgaris for 12 weeks, The efficacy was evaluated demonstrating good results of the association of TTO with adapalene in reducing the number of total lesions, inflammatory and non-inflammatory lesions. The association between adapalene and TTO makes it possible to obtain a topical formulation that covers the four main factors in the development of acne: hyperseborrhoea, hyperkeratinization, inflammation, and bacterial colonization. Despite the good results obtained, further studies should be carried out to obtain more consistent results that corroborate those already obtained in the work developed by Najafi-Taher et al. [85].

The anti-inflammatory and antibacterial properties of TTO have been described and are mainly due to terpinen-4-ol [90], being widely used and studied in the treatment of acne. However, this compound does not have excellent antiseborrheic and keratolytic properties, and it may therefore be necessary to associate it with other compounds that not only reinforce but also complement its activity in the treatment of acne, as described above. Other studies have associated TTO with various plant extracts with known activity on acne, also showing good results in reducing acne lesions [84][86][87][88][89].

In general, the studies found in the literature demonstrate the good properties of TTO in treating acne, both in isolation and associated with other ingredients with complementary activities.

References

1. Carson, C.F.; Hammer, K.A.; Riley, T.V. Melaleuca Alternifolia (Tea Tree) Oil: A Review of Antimicrobial and Other Medicinal Properties. Clin. Microbiol. Rev. 2006, 19, 50.
2. Zhang, X.; Chen, S.; Zhang, Y.; Xiao, Y.; Qin, Y.; Li, Q.; Liu, L.; Liu, B.; Chai, L.; Yang, H.; et al. Draft Genome of the Medicinal Tea Tree Melaleuca alternifolia. Mol. Biol. Rep. 2023, 50, 1545–1552.
3. Caldefie-Chézet, F.; Fusillier, C.; Jarde, T.; Laroye, H.; Damez, M.; Vasson, M.P.; Guillot, J. Potential Anti-Inflammatory Effects of Melaleuca alternifolia Essential Oil on Human Peripheral Blood Leukocytes. Phytother. Res. 2006, 20, 364–370.
4. American Botanical Council. Botanical Adulterants Program Publishes Bulletin on Tea Tree Oil Adulteration. Available online: https://www.herbalgram.org/news/press-releases/2017/botanical-adulterants-program-publishes-bulletin-on-tea-tree-oil-adulteration/ (accessed on 20 February 2023).
5. Voelker, J.; Mauleon, R.; Shepherd, M. The Terpene Synthase Genes of Melaleuca alternifolia (Tea Tree) and Comparative Gene Family Analysis Among Myrtaceae Essential Oil Crops. Plant Syst. Evol. 2023, 309, 13.
6. Homer, L.E.; Leach, D.N.; Lea, D.; Slade Lee, L.; Henry, R.J.; Baverstock, P.R. Natural Variation in the Essential Oil Content of Melaleuca alternifolia Cheel (Myrtaceae). Biochem. Syst. Ecol. 2000, 28, 367–382.
7. ISO 4730:2017; Essential Oil of Melaleuca, Terpinen-4-Ol Type (Tea Tree Oil). Technical Committee ISO/TC 54: Geneva, Switzerland, 2017.
8. Gloerfelt-Tarp, F.; Micog, J.C.; Bigland, M.; Wheeler, S.; Palmer, W.M.; Kretzschmar, T. Predicting Tea Tree Oil Distillate Composition Using Portable Spectrometric Technology. J. Raman Spectrosc. 2022, 53, 771–784.
9. Shelton, D.; Aitken, K.; Doimo, L.; Leach, D.; Baverstock, P.; Henry, R. Genetic Control of Monoterpene Composition in the Essential Oil of Melaleuca alternifolia (Cheel). Theor. Appl. Genet. 2002, 105, 377–383.
10. Southwell, I.; Dowell, A.; Morrow, S.; Allen, G.; Savins, D.; Shepherd, M. Monoterpene Chiral Ratios: Chemotype Diversity and Interspecific Commonality in Melaleuca alternifolia and M. linariifolia. Ind. Crops Prod. 2017, 109, 850–856.
11. Butcher, P.A.; Doran, J.C.; Slee, M.U. Intraspecific Variation in Leaf Oils of Melaleuca alternifolia (Myrtaceae). Biochem. Syst. Ecol. 1994, 22, 419–430.
12. Butcher, P.A.; Matheson, A.C.; Slee, M.U. Potential for Genetic Improvement of Oil Production in Melaleuca alternifolia and M. linariifolia. New For. 1996, 11, 31–51.
13. Zabaras, D.; Spooner-hart, R.N.; Wyllie, S.G. Effects of Mechanical Wounding on Concentration and Composition of Essential Oil from Melaleuca alternifolia Leaves. Biochem. Syst. Ecol. 2002, 30, 399–412.
14. Figueiredo, M. Chemical Composition and oil Concentration of Tea Tree Leaf Oil Grown in South Africa During a One-Year Vegetative Cycle. J. Essent. Oil Res. 2006, 18, 52–53.
15. Silva, C.J.; Barbosa, L.C.A.; Maltha, C.R.A.; Pinheiro, A.L.; Ismail, F.M.D. Comparative Study of the Essential Oils of Seven Melaleuca (Myrtaceae) Species Grown in Brazil. Flavour Fragr. J. 2007, 22, 474–478.
16. Baker, G.R.; Lowe, R.F.; Southwell, I.A. Comparison of Oil Recovered from Tea Tree Leaf by Ethanol Extraction and Steam Distillation. J. Agric. Food Chem. 2000, 48, 4041–4043.
17. Wong, V.; Wyllie, S.G.; Cornwell, C.P.; Tronson, D. Supercritical Fluid Extraction (SFE) of Monoterpenes from the Leaves of Melaleuca alternifolia (Tea Tree). Molecules 2001, 6, 92–103.
18. ISO 9235: 2021; Aromatic Natural Raw Materials—Vocabulary. Technical Committee ISO: Geneva, Switzerland, 2021.
19. Carson, C.F.; Riley, T.V. Antimicrobial Activity of the Essential Oil of Melaleuca alternifolia. Lett. Appl. Microbiol. 1993, 16, 49–55.

20. Bezabh, S.A.; Tesfaye, W.; Christenson, J.K.; Carson, C.F.; Thomas, J. Antiparasitic Activity of Tea Tree Oil (TTO) and Its Components against Medically Important Ectoparasites: A Systematic Review. Pharmaceutics 2022, 14, 1587.
21. Lam, N.S.K.; Long, X.X.; Griffin, R.C.; Chen, M.K.; Doery, J.C.G. Can the Tea Tree Oil (Australian Native Plant: Melaleuca alternifolia Cheel) Be an Alternative Treatment for Human Demodicosis on Skin? Parasitology 2018, 145, 1510–1520.
22. Sharifi-Rad, J.; Salehi, B.; Varoni, E.M.; Sharopov, F.; Yousaf, Z.; Ayatollahi, S.A.; Kobarfard, F.; Sharifi-Rad, M.; Afdjei, M.H.; Iriti, M. Plants of the Melaleuca Genus as Antimicrobial Agents: From Farm to Pharmacy. Phytother. Res. 2017, 31, 1475–1494.
23. Johnson, J.B.; Thani, P.R.; Mani, J.S.; Cozzolino, D.; Naiker, M. Mid-Infrared Spectroscopy for the Rapid Quantification of Eucalyptus Oil Adulteration in Australian Tea Tree Oil (Melaleuca alternifolia). Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2022, 283, 121766.
24. May, J.; Chan, C.H.; King, A.; Williams, L.; French, G.L. Time-Kill Studies of Tea Tree Oils on Clinical Isolates. J. Antimicrob. Chemother. 2000, 45, 639–643.
25. Cox, S.D.; Mann, C.M.; Markham, J.L.; Gustafson, J.E.; Warmington, J.R.; Wyllie, S.G. Determining the Antimicrobial Actions of Tea Tree Oil. Molecules 2001, 6, 87–91.
26. Brun, P.; Bernabè, G.; Filippini, R.; Piovan, A. In Vitro Antimicrobial Activities of Commercially Available Tea Tree (Melaleuca alternifolia) Essential Oils. Curr. Microbiol. 2019, 76, 108–116.
27. Cox, S.D.; Mann, C.M.; Markham, J.L.; Bell, H.C.; Gustafson, J.E.; Warmington, J.R.; Wyllie, S.G. The Mode of Antimicrobial Action of the Essential Oil of Melaleuca alternifolia (Tea Tree Oil). J. Appl. Microbiol. 2000, 88, 170–175.
28. Cox, S.D.; Gustafson, J.E.; Mann, C.M.; Markham, J.L.; Liew, Y.C.; Hartland, R.P.; Bell, H.C.; Warmington, J.R.; Wyllie, S.G. Tea Tree Oil Causes K+ Leakage and Inhibits Respiration in Escherichia coli. Lett. Appl. Microbiol. 1998, 26, 355–358.
29. Gustafson, J.E.; Liew, Y.C.; Chew, S.; Markham, J.; Bell, H.C.; Wyllie, S.G.; Warmington, J.R. Effects of Tea Tree Oil on Escherichia coli. Lett. Appl. Microbiol. 1998, 26, 194–198.
30. Li, W.R.; Li, H.L.; Shi, Q.S.; Sun, T.L.; Xie, X.B.; Song, B.; Huang, X.M. The Dynamics and Mechanism of the Antimicrobial Activity of Tea Tree Oil against Bacteria and Fungi. Appl. Microbiol. Biotechnol. 2016, 100, 8865–8875.
31. Cuaron, J.A.; Dulal, S.; Song, Y.; Singh, A.K.; Montelongo, C.E.; Yu, W.; Nagarajan, V.; Jayaswal, R.K.; Wilkinson, B.J.; Gustafson, J.E. Tea Tree Oil-Induced Transcriptional Alterations in Staphylococcus aureus. Phytother. Res. 2013, 27, 390–396.
32. Mondello, F.; Fontana, S.; Scaturro, M.; Girolamo, A.; Colone, M.; Stringaro, A.; Di Vito, M.; Ricci, M.L. Terpinen-4-Ol, the Main Bioactive Component of Tea Tree Oil, as an Innovative Antimicrobial Agent against Legionella pneumophila. Pathogens 2022, 11, 682.
33. Qi, J.; Gong, M.; Zhang, R.; Song, Y.; Liu, Q.; Zhou, H.; Wang, J.; Mei, Y. Evaluation of the Antibacterial Effect of Tea Tree Oil on Enterococcus faecalis and Biofilm in Vitro. J. Ethnopharmacol. 2021, 281, 114566.
34. Iseppi, R.; Mariani, M.; Benvenuti, S.; Truzzi, E.; Messi, P. Effects of Melaleuca alternifolia Chell (Tea Tree) and Eucalyptus globulus Labill. Essential Oils on Antibiotic-Resistant Bacterial Biofilms. Molecules 2023, 28, 1671.
35. Brożyna, M.; Paleczny, J.; Kozłowska, W.; Chodaczek, G.; Dudek-Wicher, R.; Felińczak, A.; Gołębiewska, J.; Górniak, A.; Junka, A. The Antimicrobial and Antibiofilm in Vitro Activity of Liquid and Vapour Phases of Selected Essential Oils against Staphylococcus aureus. Pathogens 2021, 10, 1207.
36. Oliva, A.; Costantini, S.; de Angelis, M.; Garzoli, S.; Božović, M.; Mascellino, M.T.; Vullo, V.; Ragno, R. High Potency of Melaleuca alternifolia Essential Oil Against Multi-Drug Resistant Gram-Negative Bacteria and Methicillin-Resistant Staphylococcus aureus. Molecules 2018, 23, 2584.
37. Borotová, P.; Galovičová, L.; Vukovic, N.L.; Vukic, M.; Tvrdá, E.; Kačániová, M. Chemical and Biological Characterization of Melaleuca alternifolia Essential Oil. Plants 2022, 11, 558.
38. Banes-Marshall, L.; Cawley, P.; Phillips, C.A. In Vitro Activity of Melaleuca alternifolia (Tea Tree) Oil against Bacterial and Candida spp. Isolates from Clinical Specimens. Br. J. Biomed. Sci. 2001, 58, 139–145.
39. Kwieciński, J.; Eick, S.; Wójcik, K. Effects of Tea Tree (Melaleuca alternifolia) Oil on Staphylococcus aureus in Biofilms and Stationary Growth Phase. Int. J. Antimicrob. Agents 2009, 33, 343–347.
40. Loughlin, R.; Gilmore, B.F.; McCarron, P.A.; Tunney, M.M. Comparison of the Cidal Activity of Tea Tree Oil and Terpinen-4-Ol against Clinical Bacterial Skin Isolates and Human Fibroblast Cells. Lett. Appl. Microbiol. 2008, 46, 428–433.

41. Blejan, E.I.; Popa, D.E.; Costea, T.; Cioacă, A.; Olariu, L.; Ghica, M.; Georgescu, M.; Stancov, G.; Arsene, A.L. The in Vitro Antimicrobial Activity of Some Essential Oils from Aromatic Plants. Farmacia 2021, 69, 290–298.
42. Ramadan, M.A.; Shawkey, A.E.; Rabeh, M.A.; Abdellatif, A.O. Promising Antimicrobial Activities of Oil and Silver Nanoparticles Obtained from Melaleuca alternifolia Leaves against Selected Skin-Infecting Pathogens. J. Herb. Med. 2020, 2020, 100289.
43. Hammer, K.A.; Carson, C.F.; Riley, T.V. In Vitro Susceptibilities of Lactobacilli and Organisms Associated with Bacterial Vaginosis to Melaleuca alternifolia (Tea Tree) Oil. Antimicrob. Agents Chemother. 1999, 43, 196.
44. Ossa-Tabares, J.C.; Llanos, C.J.; García, A.M. Evaluation of Tea Tree Oil Physicochemical Features and Its Antimicrobial Activity against Cutibacterium acnes (Propionibacterium acnes) ATCC 6919. Biomedica 2020, 40, 693–694.
45. Nenoff, P.; Haustein, U.F.; Brandt, W. Antifungal Activity of the Essential Oil of Melaleuca alternifolia (Tea Tree Oil) against Pathogenic Fungi in vitro. Ski. Pharmacol. 1996, 9, 388–394.
46. Ngenge, A.T.; Kucukaydin, S.; Ceylan, O.; Duru, M.E. Evaluation of Enzyme Inhibition and Anti-Quorum Sensing Potentials of Melaleuca alternifolia and Citrus sinensis Essential Oils. Nat. Prod. Commun. 2021, 16, 1934578X211044565.
47. Altun, M.; Yapici, B.M. Determination of Chemical Compositions and Antibacterial Effects of Selected Essential Oils against Human Pathogenic Strains. An. Acad. Bras. Cienc. 2022, 94, e20210074.
48. Leigh-de Rapper, S.; Viljoen, A.; van Vuuren, S. Essential Oil Blends: The Potential of Combined Use for Respiratory Tract Infections. Antibiotics 2021, 10, 1517.
49. Abdelhamed, F.M.; Abdeltawab, N.F.; ElRakaiby, M.T.; Shamma, R.N.; Moneib, N.A. Antibacterial and Anti-Inflammatory Activities of Thymus vulgaris Essential Oil Nanoemulsion on Acne Vulgaris. Microorganisms 2022, 10, 1874.
50. Esmael, A.; Hassan, M.G.; Amer, M.M.; Abdelrahman, S.; Hamed, A.M.; Abd-raboh, H.A.; Foda, M.F. Antimicrobial Activity of Certain Natural-Based Plant Oils against the Antibiotic-Resistant Acne Bacteria. Saudi J. Biol. Sci. 2020, 27, 448–455.
51. Homeyer, D.C.; Sanchez, C.J.; Mende, K.; Beckius, M.L.; Murray, C.K.; Wenke, J.C.; Akers, K.S. In Vitro Activity of Melaleuca alternifolia (Tea Tree) Oil on Filamentous Fungi and Toxicity to Human Cells. Med. Mycol. 2015, 53, 285–294.
52. Villar Rodríguez, J.; Pérez-Pico, A.M.; Mingorance-Álvarez, E.; Mayordomo Acevedo, R. Meta-Analysis of the Antifungal Activities of Three Essential Oils as Alternative Therapies in Dermatophytosis Infections. J. Appl. Microbiol. 2022, 133, 241–253.
53. Pormohammad, A.; Hansen, D.; Turner, R.J. Antibacterial, Antibiofilm, and Antioxidant Activity of 15 Different Plant-Based Natural Compounds in Comparison with Ciprofloxacin and Gentamicin. Antibiotics 2022, 11, 1099.
54. Carson, C.F.; Riley, T.V.; Cookson, B.D. Efficacy and Safety of Tea Tree Oil as a Topical Antimicrobial Agent. J. Hosp. Infect. 1998, 40, 175–178.
55. Minghetti, P.; Casiraghi, A.; Cilurzo, F.; Gambaro, V.; Montanari, L. Formulation Study of Tea Tree Oil Patches. Nat. Prod. Commun. 2009, 4, 133–137.
56. Muta, T.; Parikh, A.; Kathawala, K.; Haidari, H.; Song, Y.; Thomas, J.; Garg, S. Quality-by-Design Approach for the Development of Nano-Sized Tea Tree Oil Formulation-Impregnated Biocompatible Gel with Antimicrobial Properties. Pharmaceutics 2020, 12, 1091.
57. Low, W.L.; Kenward, K.; Cairul, M.; Amin, I.M.; Martin, C.; Nahar, L.; Basar, N.; Sarker, S.D.; Adams, J.D. Ionically Crosslinked Chitosan Hydrogels for the Controlled Release of Antimicrobial Essential Oils and Metal Ions for Wound Management Applications. Medicines 2016, 3, 8.
58. Sánchez-Cid, P.; Jiménez-Rosado, M.; Romero, A.; Pérz-Puyana, V. Novel Trends in Hydrogel Development for Biomedical Applications: A Review. Polymers 2022, 14, 3023.
59. Ghosh, B.; Bhattacharya, D.; Mukhopadhyay, M. A Hydrogel Sheet Mask with Tea Tree Essential Oil Entrapment and Targeted Dose Delivery Capability. Mater. Today Proc. 2022, 57, 77–83.
60. Catanzano, O.; Straccia, M.C.; Miro, A.; Ungaro, F.; Romano, I.; Mazzarella, G.; Santagata, G.; Quaglia, F.; Laurienzo, P.; Malinconico, M. Spray-by-Spray in Situ Cross-Linking Alginate Hydrogels Delivering a Tea Tree Oil Microemulsion. Eur. J. Pharm. Sci. 2015, 66, 20–28.
61. Sinha, P.; Srivastava, S.; Mishra, N.; Singh, D.K.; Luqman, S.; Chanda, D.; Yadav, N.P. Development, Optimization, and Characterization of a Novel Tea Tree Oil Nanogel Using Response Surface Methodology. Drug Dev. Ind. Pharm. 2016, 42, 1434–1445.

62. de Assis, K.M.A.; da Silva Leite, J.M.; de Melo, D.F.; Borges, J.C.; Santana, L.M.B.; dos Reis, M.M.L.; Moreira, V.M.; da Rocha, W.R.V.; Catão, R.M.R.; dos Santos, S.G.; et al. Bicontinuous Microemulsions Containing Melaleuca alternifolia Essential Oil as a Therapeutic Agent for Cutaneous Wound Healing. Drug Deliv. Transl. Res. 2020, 10, 1748–1763.

63. Bao, X.; Wu, J.; Ma, G. Sprayed Pickering Emulsion with High Antibacterial Activity for Wound Healing. Prog. Nat. Sci. Mater. Int. 2020, 30, 669–676.

64. Liu, P.; Chen, G.; Zhang, J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. Molecules 2022, 27, 1372.

65. Low, W.L.; Martin, C.; Hill, D.J.; Kenward, M.A. Antimicrobial Efficacy of Liposome-Encapsulated Silver Ions and Tea Tree Oil against Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans. Lett. Appl. Microbiol. 2013, 57, 33–39.

66. Aguilar-Pérez, K.M.; Medina, D.I.; Parra-Saldívar, R.; Iqbal, H.M.N. Nano-Size Characterization and Antifungal Evaluation of Essential Oil Molecules-Loaded Nanoliposomes. Molecules 2022, 27, 5728.

67. Ge, Y.; Ge, M. Distribution of Melaleuca alternifolia Essential Oil in Liposomes with Tween 80 Addition and Enhancement of in Vitro Antimicrobial Effect. J. Exp. Nanosci. 2015, 11, 345–358.

68. Ge, Y.; Tang, J.; Fu, H.; Fu, Y.; Wu, Y. Characteristics, Controlled-Release and Antimicrobial Properties of Tea Tree Oil Liposomes-Incorporated Chitosan-Based Electrospun Nanofiber Mats. Fibers Polym. 2019, 20, 698–708.

69. Esposito, E.; Calderan, L.; Galvan, A.; Cappellozza, E.; Drechsler, M.; Mariani, P.; Pepe, A.; Sguizzato, M.; Vigato, E.; Pozza, E.D.; et al. Ex Vivo Evaluation of Ethosomes and Transethosomes Applied on Human Skin: A Comparative Study. Int. J. Mol. Sci. 2022, 23, 15112.

70. Bisht, A.; Hemrajani, C.; Upadhyay, N.; Nidhi, P.; Rolta, R.; Rathore, C.; Gupta, G.; Dua, K.; Chellappan, D.K.; Dev, K.; et al. Azelaic Acid and Melaleuca alternifolia Essential Oil Co-Loaded Vesicular Carrier for Combinational Therapy of Acne. Ther. Deliv. 2022, 13, 13–29.

71. da Silva, N.P.; Pereira, E.D.C.R.L.; Duarte, L.M.; de Oliveira Freitas, J.C.; de Almeida, C.G.; da Silva, T.P.; de Melo, R.C.N.; Morais Apolônio, A.C.; de Oliveira, M.A.L.; de Mello Brandão, H.; et al. Improved Anti-Cutibacterium acnes Activity of Tea Tree Oil-Loaded Chitosan-Poly(ε-Caprolactone) Core-Shell Nanocapsules. Colloids Surf. B Biointerfaces 2020, 196, 111371.

72. Youn, B.H.; Kim, Y.S.; Yoo, S.; Hur, M.H. Antimicrobial and Hand Hygiene Effects of Tea Tree Essential Oil Disinfectant: A Randomised Control Trial. Int. J. Clin. Pract. 2021, 75, e14206.

73. Messager, S.; Hammer, K.A.; Carson, C.F.; Riley, T.V. Assessment of the Antibacterial Activity of Tea Tree Oil Using the European EN 1276 and EN 12054 Standard Suspension Tests. J. Hosp. Infect. 2005, 59, 113–125.

74. Filatov, V.A.; Kulyak, O.Y.; Kalenikova, E.I. In Vitro and in Vivo Antimicrobial Activity of an Active Plant-Based Quadrocomplex for Skin Hygiene. J. Pharm. Pharmacogn. Res. 2022, 10, 905–921.

75. Kunicka-Styczyńska, A.; Sikora, M.; Kalemba, D. Lavender, Tea Tree and Lemon Oils as Antimicrobials in Washing Liquids and Soft Body Balms. Int. J. Cosmet. Sci. 2011, 33, 53–61.

76. Gnatta, J.R.; de Brito Poveda, V.; Padoveze, M.C.; Graziano, K.U.; Turrini, R.N.T.; da Silva, M.J.P. Melaleuca alternifolia Essential Oil Soap: A Potential Alternative for Hand Hygiene. Eur. J. Clin. Microbiol. Infect. Dis. 2021, 40, 1517–1520.

77. Sgorbini, B.; Cagliero, C.; Argenziano, M.; Cavalli, R.; Bicchi, C.; Rubiolo, P. In Vitro Release and Permeation Kinetics of Melaleuca alternifolia (Tea Tree) Essential Oil Bioactive Compounds from Topical Formulations. Flavour Fragr. J. 2017, 32, 354–361.

78. Kairey, L.; Agnew, T.; Bowles, E.J.; Barkla, B.J.; Wardle, J.; Lauche, R.; Efficacy and safety of Melaleuca alternifolia (tea tree) oil for human health—A systematic review of randomized controlled trials. *Front. Pharmacol.* **2023**, *14*, 31, doi.org/10.3389/fphar.2023.1116077.

79. Abozeid, D.; Fawzy, G.; Issa, M.; Abdeltawab, N.; Soliman, F.; Medicinal Plants and Their Constituents in the Treatment of Acne Vulgaris. . *Biointerface Res. Appl. Chem.* **2023**, *13*, 189, .

80. Bassett, I.B.; Pannowitz, D.L.; Barnetson, R.S.C. A Comparative Study of Tea-Tree Oil versus Benzoylperoxide in the Treatment of Acne. Med. J. Aust. 1990, 153, 455–458.

81. Enshaieh, S.; Jooya, A.; Siadat, A.H.; Iraji, F. The Efficacy of 5% Topical Tea Tree Oil Gel in Mild to Moderate Acne Vulgaris: A Randomized, Double-Blind Placebo-Controlled Study. Indian J. Dermatol. Venereol. Leprol. 2007, 73, 22–25.

82. Kwon, H.H.; Yoon, J.Y.; Park, S.Y.; Min, S.; Suh, D.H. Comparison of Clinical and Histological Effects between Lactobacillus-Fermented Chamaecyparis obtusa and Tea Tree Oil for the Treatment of Acne: An Eight-Week Double-

Blind Randomized Controlled Split-Face Study. Dermatology 2014, 229, 102–109.

83. Malhi, H.K.; Tu, J.; Riley, T.V.; Kumarasinghe, S.P.; Hammer, K.A. Tea Tree Oil Gel for Mild to Moderate Acne; a 12 Week Uncontrolled, Open-Label Phase II Pilot Study. Australas. J. Dermatol. 2017, 58, 205–210.
84. Lubtikulthum, P.; Kamanamool, N.; Udompataikul, M. A Comparative Study on the Effectiveness of Herbal Extracts vs 2.5% Benzoyl Peroxide in the Treatment of Mild to Moderate Acne Vulgaris. J. Cosmet. Dermatol. 2019, 18, 1767–1775.
85. Najafi-Taher, R.; Jafarzadeh kohneloo, A.; Eslami Farsani, V.; Mehdizade Rayeni, N.; Moghimi, H.R.; Ehsani, A.; Amani, A. A Topical Gel of Tea Tree Oil Nanoemulsion Containing Adapalene versus Adapalene Marketed Gel in Patients with Acne Vulgaris: A Randomized Clinical Trial. Arch. Dermatol. Res. 2022, 314, 673–679.
86. Mazzarello, V.; Donadu, M.G.; Ferrari, M.; Piga, G.; Usai, D.; Zanetti, S.; Sotgiu, M.A. Treatment of Acne with a Combination of Propolis, Tea Tree Oil, and Aloe Vera Compared to Erythromycin Cream: Two Double-Blind Investigations. Clin. Pharmacol. 2018, 10, 175–181.
87. Yadav, N.; Singh, A.; Chatterjee, A.; Belemkar, S. Evaluation of Efficacy and Safety of Perfact Face Gel and Perfact Face Tablets in Management of Acne. J. Clin. Exp. Dermatol. 2011, 2, 1000118.
88. Kim, B.Y.; Shin, S. Antimicrobial and Improvement Effects of Tea Tree and Lavender Oils on Acne Lesions. J. Converg. Inf. Technol. 2013, 8, 339–345.
89. Lupu, M.; Malciu, A.M.; Voiculescu, V.M. Feasibility of Reflectance Confocal Microscopy Monitoring in Oily, Acne-Prone Facial Skin Treated with a Topical Combination of Alpha and Beta-Hydroxy Acids, Anti-Inflammatory Molecules, and Herculane Thermal Water: A Blinded, One-Month Study. Life 2022, 12, 1973.
90. Pazyar, N.; Yaghoobi, R.; Bagherani, N.; Kazerouni, A. A Review of Applications of Tea Tree Oil in Dermatology. Int. J. Dermatol. 2013, 52, 784–790.