Tea Tree Oil: Antifungal Potential and Applications

[1]Devesh, [2]Lovekesh, [3]Ajay
[123]Pharmaceutical Science and Pharmacology
[123]BDM College of Pharmacy, Jhajjar, Haryana 124106

***Abstract*—As antimicrobial resistance levels soar, accompanied by frequent relapses and negative side effects of available synthetic antifungals, fungal infections pose an ever-increasing global concern for public health. Among numerous broadly acting, broad-spectrum, natural agents, tea tree oil (TTO) extracted from Melaleuca alternifolia has proved promising. Undergoing standardized evaluation to comply with the ISO 4730 criteria, namely a minimum 30% terpinen-4-ol content and 1,8-cineole max 15%, it is a bioactive, monoterpenoid-rich, chemically complicated, well-tolerated natural oil. Lipophilic disruption of the membrane lipid structure is its prevalent antifungal property, leading to altered electrical potential, cytoplasmic leakage, suppression of biofilm genesis, and cell destruction. Clinical studies with topical application have further confirmed the useful application of TTO for superficial skin and mucosal fungal infection sites, namely tinea pedis and subungual mycosis as well as mucosal candidiasis. Although ~10% formulations ameliorate skin conditions for better symptomatology with the advantage of significant anti-inflammatory activities, 25–50% preparations or combinations with existing synthetic drugs are generally recommended for the entire eradication of mycosis. Recent nanotechnological approaches, including solid lipid nanoparticles (SLNs), nanoemulsions, and microcapsules, have been introduced to tackle with the issues of the inherent low aqueous solubility of TTO, skin irritation upon external use, and volatility and oxidation properties. The current article reports botanical sources, chemical standardization guidelines and characteristics, cell damaging mechanism, clinical data, modern developments in nanovehicular delivery systems of TTO and a safety perspective on antifungal treatments.**

***Index Terms*—Tea tree oil, Antifungal activity, Nanoemulgel, Solid lipid nanoparticles**

I. Introduction

Fungal infections represent an alarming increase as a global public health concern owing to growing incidences of resistant pathogenic fungal organisms, increasing numbers of immunocompromised patients, the lack of sufficiently effective synthetic antifungal drugs, as well as limitations on conventional treatment of these patients with standard azole antifungals (systemic), allylamines(systemic), owing to their hepatotoxicity, poor drug-drug interaction potential, expense, and patients compliance during long-term treatment [1] . Therefore, plant natural products (antimicrobials derived from plants) as renewable and wide-spectrum alternative treatments have greatly interested researchers [2].

Of all the plant-based extracts available, Tea Tree Oil (TTO) essential oil produced from Melaleuca alternifolia is considered to be a particularly effective phytotherapeutic. Already in use by indigenous Australians, TTO has been included within conventional therapy for over a century, and has a chemical nature largely constituted by lipophilic monoterpenes. Pharmaceutical research has shown TTO has broad-range antibacterial, anti-viral, anti-inflammatory and anti-fungicidal action [3].

The inherent high potency in vitro of TTO against dermatophytes, yeasts and filamentous fungi remains limited when evaluated for its clinical applications owing to it being prone to volatility, solubility in aqueous medium and, oxidative breakdown and the possibility of the emergence of skin toxicity [4]. Advances made in the field of nano-medicine, solid lipid nanoparticles, nanoemulsion and hydrogel matrices have helped in the formulation of efficient delivery systems, which are a potent approach towards overcoming those restrictions. In this review, chemical constituents and mechanisms of TTO, clinical trials conducted, novel

delivery systems, safety profiles, etc are reviewed and their suitability is evaluated by the experts in dermatology and mycology [5].

II. Tea Tree Oil: Source, Composition and Properties

Botanical Origin and Extraction

Tea tree oil is isolated mainly from the leaves and ultimate twigs of *Melaleuca alternifolia* (Maiden & Betche) Cheel, an Australian belonging to the family Myrtaceae. It is obtained commercially solely through steam distillation, a process where steam is passed through the harvested plant material and the volatile lipophilic secondary metabolites are carried up with the steam, subsequently condensed, and separated from the hydrosol layer. The resulting product is an oil and ranges in color from clear to pale yellow with a characteristic camphoraceous odor [6,7].

Chemical Profiling

The chemical composition of tea tree oil (TTO) is multifaceted, with the presence of > 100 individual compounds, the majority of which are mono- and sesquiterpenes and their corresponding terpene alcohols. International regulatory systems have thus defined specific composition limits so that the consumer benefits of a consistent preparation and safety is assured [8]. The international benchmark to ensure quality and standardise composition is that laid down in the standard of the International Organization for Standardization (ISO) standard ISO 4730 [9]. ISO 4730 defines terpinen-4-ol to be the predominant pharmacologically active marker; 30% must be present in the oil (typically ranging between 35% and 48% in commercial preparations) and it is a monocyclic monoterpenol predominantly involved in conferring antibacterial and anti-inflammatory activity on the oil. Under the standard, a maximum of 15% of 1,8-cineole (eucalyptol) is established as the upper limit (with typical values falling between 3% and 5% for high-quality oil). The latter is important to consider from a therapeutic perspective since, although 1,8-cineole may aid absorption into the skin, a higher concentration contributes greatly to irritation and contact allergy of the skin [10,11]. Also defined as majors by ISO standards are γ-terpinene (10-28%), α-terpinene (5-13%), ρ-cymene (0.5-12%), terpinolene (1.5-5%), α-terpineol (1.5-8%), α-pinene (1-6%). In modern testing methods, the ratios of the optical isomers are also analysed using chiral gas chromatography to indicate adulteration with synthetic terpenes, and under ISO 16128 criteria, those meeting the compositional standards have a Natural Index value of 1, indicating the oil to be derived from a natural resource in its entirety [12,13].

Component	Chemical Class	Chemical Formula	ISO 4730 Spec. (%)	Typical Profile (%)	Water Solubility (ppm)	Octanol-Water Partition (logKOW)

Terpinen-4-ol	Monocyclic terpene alcohol	$C_{10}H_{18}O$	≥30.0	40.1–42.1	1,491	3.26
γ-Terpinene	Monocyclic terpene	$C_{10}H_{16}$	10.0–28.0	19.7–23.0	1.0	4.36
α-Terpinene	Monocyclic terpene	$C_{10}H_{16}$	5.0–13.0	9.1–10.4	8.2	4.25
1,8-Cineole	Monocyclic terpene ether	$C_{10}H_{18}O$	≤15.0	3.4–5.1	907	2.84
Terpinolene	Monocyclic terpene	$C_{10}H_{16}$	1.5–5.0	3.0–3.1	4.3	4.24
***p*-Cymene**	Monocyclic terpene	$C_{10}H_{14}$	0.5–12.0	2.5–2.9	6.2	4.10
α-Pinene	Dicyclic terpene	$C_{10}H_{16}$	1.0–6.0	2.0–2.6	0.57	4.44
α-Terpineol	Monocyclic terpene alcohol	$C_{10}H_{18}O$	1.5–8.0	2.4–2.9	1,827	3.28
Aromadendrene	Sesquiterpene	$C_{15}H_{24}$	Trace–7.0	1.3–1.5	Low	> 5.00
δ-Cadinene	Sesquiterpene	$C_{15}H_{24}$	Trace–8.0	1.3–1.6	Low	> 5.00

Table 1: Chemical standards (ISO 4730), typical composition, and physicochemical properties of key Melaleuca alternifolia constituents.

Physicochemical Properties and Stability Dynamics

Its high lipophilicity is another physical property responsible for TTOs physical behaviour with the values of octanol-water partition coefficient (log K_{OW}) between 2.84 and 4.44. The fact that water insolubility of hydrocarbons like the monoterpene γ-terpinene (log K_{OW} = 4.36) is in the order of ~1 ppm but monoterpene alcohols terpinen-4-ol (log K_{OW} = 3.26) and α-terpineol (log K_{OW} = 3.28) exhibit water solubility of 1,491 ppm and 1,827 ppm, respectively. While lipophilicity facilitates transport of TTO across membranes and uptake through skin contact, direct TTO has undesirable properties of volatility and instability. Exposure of airborne oxygen, UV radiation or heat leads to autoxidation of the unsaturated monoterpenes (mainly α-terpinene and terpinolene) [14,15].

Aromatic terpenes degrade to sensitizing products such as peroxides, epoxides, endoperoxides (e.g. Ascaridole), or ρ-cymene. There is significantly higher contact allergenic potential in aged oxidized TTO and it is imperative that the TTO is stored in opaque amber-coloured bottles, refrigerated and with added antioxidants when in preparation stage [16] .

III. Antifungal Potential of Tea Tree Oil

Susceptibility of Dermatophytes

The number one cause for these superficial skin diseases (tinea pedis, tinea corporis, onychomycosis, etc.) are dermatophytes; however, *Melaleuca alternifolia* has demonstrated remarkable in vitro activity against key dermatophytes including: *Trichophyton rubrum*; *T. mentagrophytes; T. schoenleinii*; and *T. tonsurans*. Specifically, *T. rubrum* showed very acute sensitivity to TTO as demonstrated by EUCAST-AFST broth microdilution trials [17,18]. Clinical isolates of *Trichophyton rubrum* revealed effective Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) at 0.03% v/v (approximately 0.27-0.30 mg/ mL); whereas *T. mentagrophytes* experienced complete growth inhibition when concentration reached 0.02% to 0.07% v/v (which is an MIC) [19]. *Trichophyton schoenleinii* was much more resistant when it appeared to take 13x the concentration (0.4% v/v or 3.6mg/mL) and all other dermatophytes experienced the same MFC as MIC, showing fungicidal properties [20].

Activity Against Yeasts and Filamentous Fungi

TTO demonstrated a broad spectrum of antifungal activity against the various opportunistic pathogenic yeasts (*Candida* species), the filamentous molds (*Aspergillus* and *Alternaria* species). When testing susceptibility to the various Candida species in a broth microdilution, TTO was found to inhibit *C. albicans, Candida glabrata, Candida krusei*, and *Candida tropicalis* strains at the MIC of 0.06-0.5% v/v (26.32--29.85 \mu L/mL) [21,22].

Of importance, the fungicidal activity of TTO against a fluconazole-resistant strain of *C. albicans* was upheld. TTO inhibited Aspergillus flavus strains with an MIC of 0.31-0.7% v/v and MFC of 2.0-4.0% v/v; however, the Alternaria species were highly susceptible, with an MIC of 0.016-0.12% v/v [23].

Table 2: Comparative in vitro antifungal spectrum, Minimum Inhibitory Concentrations (MIC), and Minimum Fungicidal Concentrations (MFC) of tea tree oil

Pathogens	Species	Indication	MIC (% v/v)	MFC / MBC (% v/v)	Mechanism
Dermatophytes	*Trichophyton rubrum*	Onychomycosis, Tinea pedis	0.03–0.12	0.03–0.25	Cell wall/membrane disruption; high sensitivity
Dermatophytes	*Trichophyton mentagrophytes*	Interdigital tinea pedis	0.02–0.07	0.07	Complete hyphal growth suppression
Dermatophytes	*Trichophyton schoenleinii*	Favus, Chronic tinea capitis	0.40	0.40	Resistant dermatophyte chemotype
Dermatophytes	*Trichophyton tonsurans*	Tinea capitis	0.10–0.20	0.20	Germination tube inhibition

Yeasts	*Candida albicans*	Vaginal/Oral candidiasis	0.06–0.50	0.25–1.00	Biofilm disruption; membrane permeabilizatio n
Yeasts	*Candida glabrata*	Azole-resistant candidiasis	0.25–0.50	0.50–1.00	Fungicidal against fluconazole-res istant yeast
Yeasts	*Candida krusei*	Refractory candidemia	0.25–0.50	0.50	Dissipation of membrane potential
Molds	*Aspergillus flavus*	Otomycosis, Keratitis	0.31–0.70	2.00–4.00	Mycelial extension arrest
Molds	*Alternaria spp.*	Cutaneous phaeohyphomycosi s	0.016–0.12	0.06–2.00	High sensitivity to monoterpene fraction

IV. Mechanism of Antifungal Action

Membrane Permeabilization and Lysis

In summary, the main antifungal mechanism of TTO to fungal membranes consists in plasma membrane destruction and permeabilization, and involves the partitioning of the monoterpenes into the lipophilic membrane phase (log K_{OW} > 3) [24]. Their insertion between the fatty acyl chain groups, such as terpinen-4-ol, α-terpineol and γ-terpinene, perturbs the integrity and causes increases in membrane fluidity and permeability, triggering the following characteristic cascade: leakage of intracellular K+ and H+; disruption of the electrochemical potential and proton motive force; loss of active conformation of the membrane-bound enzymes (e.g., the ergosterol synthesizing machinery); influx of excessive amounts of water (osmotic lysis); cytoplasmic lysis; cell death. Since the target is the fundamental structural elements of the membrane lipids and not one protein component of a metabolic pathway, there is a high obstacle for acquiring resistance mechanisms based on targeted-site mutation [25,26,27].

Biofilm Disruption and Inhibition

Fungal biofilms (colonies of cells encased within an extracellular polymeric substance pose a significant clinical threat due to antifungal resistance. Fluconazole susceptibility can be reduced 1,000-fold for *Candida* strains in biofilms when compared to planktonic counterparts. Tea tree oil exhibits significant activity against fungal communities at both forming and mature stages of biofilm development [28]. MTT metabolic assays indicate that 0.2-0.8% v/v concentrations of TTO inhibit Candida biofilm biomass growth by 30-70%. Lipophilic monoterpenes effectively penetrate the thick EPS matrix which disassembles cell-substrate and cell-cell anchoring. Inhibition of cell growth at sub-inhibitory concentrations of terpinen-4-ol blocks the yeast-to-hyphae dimorphic transition in *C. albicans* and in turn, inhibits tissue invasion and subsequent biofilm maturation [29].

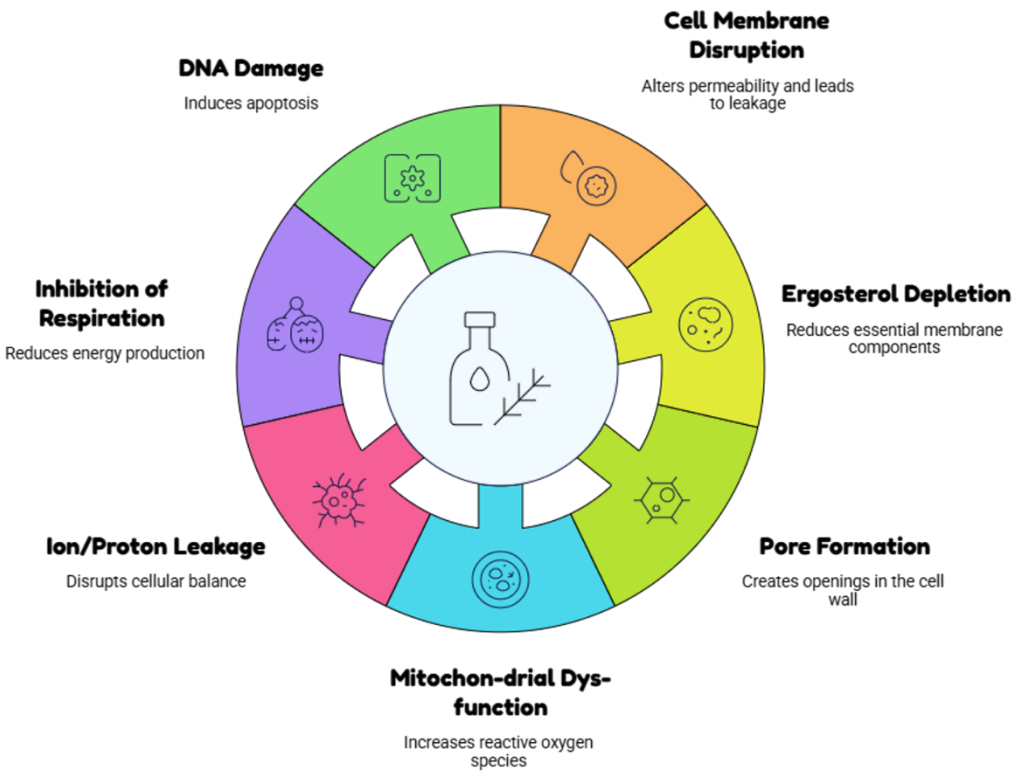


Figure 1: Mechanism of pathways

Secondary Intracellular and Oxidative Targets

In addition to membrane damage itself, TTO causes damage through secondary intracellular processes. Once intracellular TTO components traverse dysfunctional membranes, they affect the integrity of the mitochondrial membrane. Compromised mitochondrial membranes reduce electron transport, leading to intracellular build-up of reactive oxygen species (ROS), which, through overwhelming oxidative stress, leads to lipid peroxidation of organelle membranes, damage to fungal DNA, and triggering of the apoptotic cascade [30,31]. Furthermore, the terpenes interact with cell heat-shock proteins, paralyzing the heat-shock stress response.

V. Topical Applications of Tea Tree Oil

Tinea Pedis

Tinea pedis (athlete's foot) is a common superficial dermatophytosis caused mainly *by T. rubrum* and *T. mentagrophytes*. Clinical assessments with topical TTO show concentration-related therapeutic value. A randomized double blind trial comparing 10% w/w TTO cream, 1% tolnaftate cream, and placebo has shown that 10% TTO cream provided symptom control similar to 1% tolnaftate (redness, scaling, burning, itching) but with a significantly lower conversion to negative culture-positive mycological cure rate of only 30% (vs placebo 21%, vs tolnaftate 85%) [32] .

To achieve actual mycological clearance much higher concentrations of TTO would have to be employed. Another trial then examined the double-blind approach using 25% TTO formulation, 50% TTO formulation and placebo applied bd for four weeks.50% TTO formulation recorded a

mycological cure rate of 64% over placebo (31%) (p <0.001$). Clinical results were also noted in 72% of the 25% group and 68% of the 50% group. It is thus understood that while TTO in smaller concentrations (~ 10 %) may act more like an anti-inflammatory in symptom control, at greater than 25 %-50 % concentrations its effect can be used to achieve a fungicidal cure [33].

Onychomycosis

Poor Drug penetration in the keratinized structures associated with Onychomycosis, which represents a fungal infection of the nail plate and underlying bed, provides a challenge in treatment modalities. A randomized double blind clinical trial has studied and compared 117 patients suffering from subungual distal onychomycosis and treated with twice-a-day application of 100% pure TTO or 1% clotrimazole solution combined with mechanical nail debridement for six months [34]. After 6 months, clinical and mycologic outcomes are similar in both groups (culture-positive response with TTO and clotrimazole: 18 and 11%, clinically partial or full response 60% and 61%). Follow-up results (9 months) revealed about 56% of TTO patients with stabilized clinical improvement.

The combined therapies of TTO and synthetically created antifungals have an improved level of effect. A clinical study comparing the efficacy of a topical cream with 2% butenafine hydrochloride and 5% TTO versus a placebo cream with only TTO achieved an 80% cure rate (negative culture, healthy nail regrowth and clinical clearance of infection) at nine months for the active study, versus no cure rate in the placebo study ($p < 0.001$) [35]. Terpenes within TTO enhance chemical penetration and can actually affect nail keratin morphology to increase drug penetration.

Mucosal and Cutaneous Candidiasis

TTO appears to be clinically effective at treating the fungal infections of mucous membranes. It has been found to be effective at treating oral and vulvovaginal candidiasis (VVC). Oral rinse containing TTO was effective at killing axole-resistant *Candida glabrata* and *Candida albicans* in oral candidiasis. It was also found to eradicate Candida in the vagina in VVC by using vaginal suppositories but not inhibiting normal Lactobacillus species or disrupting vaginal pH [36] .

VI. Tea Tree Oil-Based Novel Drug Delivery Systems

Solid Lipid Nanoparticles (SLNs) and Nanolipogels

For all issues with crude TTO (volatility, oxidation and skin irritation), a nano-encapsulation platform has been designed. The SLNs have essential oil as core and solid lipid (as glyceryl monostearate at 2% w/w) as the core and non-ionic surfactants (as Poloxamer 188 at 5% w/w) as stabilized materials [37]. Optimum TTO-SLNs show a spherically layered core-shell structure, with mean particle diameter (Z-average) 135.90±6.65 nm, PDI value 0.19±0.04 and Z-potential -31.07 ± 1.05 mV, and entrapment efficiency 92.74% ± 0.01 %. The stable dispersing medium showed that non-volatile terpenes are protected by lipids, thus protected from photo-oxidation and remain dispersed for more than 120 days[38,39].

Using nanolipogels based on hydrogels or sugarcane bagasse nanofiber cellulose, it showed shear-thinning flow properties after TTO-SLN was incorporated. It presents 70.52% permeation rate across the porcine oral mucosa barrier after 160min incubation (in vitro mucosal) and delivers into tissue more effectively. There was low cytotoxicity to human keratinocytes [40,23].

Nanoemulsions and Nanoemulgels

Nanoemulsions are oil-in-water microemulsions with thermodynamic stability, with droplets that are less than 100nm. Droplets 51nm (PDI=0.236± 0.021) were achieved using ultrasound in the presence of emulsifiers like the biocompatible surfactants Tween 80 & carboxymethyl chitosan. By incorporating the TTO nanoemulsions into hydrogels, nanoemulgels can be formed [41]. Nanoemulgels have various functional aspects:

Small (below 100nm) sized droplets increase the surface area, causing effective permeation into and across the stratum corneum intercellular lipid pathway.

Controlled release network enhances delivery of the terpene to beyond 24hrs.

Nanoemulgels act as a co-delivery network for synthetic drugs, such as azithromycin or neomycin, thereby providing additive antimicrobial effect and reduced drug dose [42].

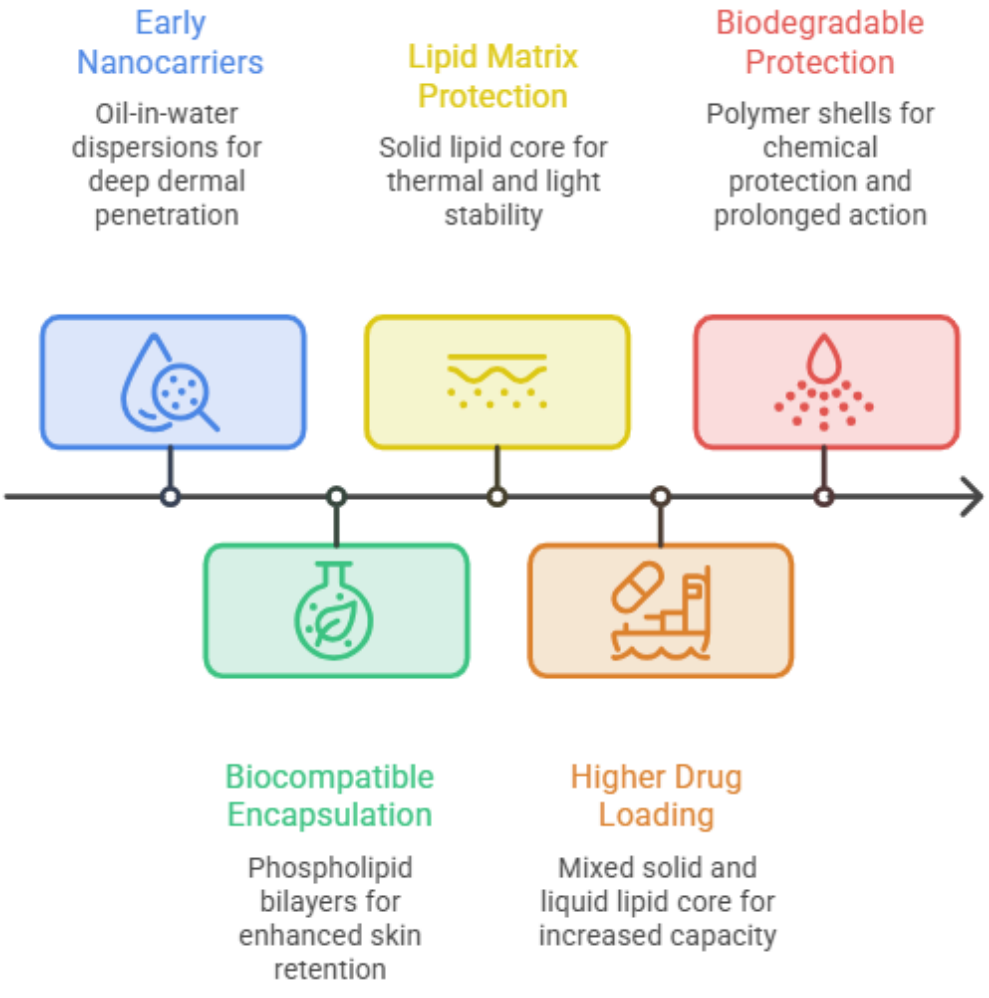


Figure 2: Novel nanocarrier delivery systems

Microencapsulation and Polymeric Delivery Carriers

Spray-drying microencapsulation is an effective method for application of industrial packaging, textiles, and medical use as well as topical dusting powders, resulting in lasting effect. The spherical polymeric TTO microcapsules of regular morphology are developed with particle diameters between 6μm and 12μm. The system exhibits high encapsulation yield (80-85%) and microencapsulation efficiency (90-95%), and high active loading amount (~ 40%). Protected by polymeric matrix from evaporation, it could be sustained-release controlled by friction and moisture [43,44].

VII. Future Research Directions

To develop TTO from an adjuvant topical extract to a routine mainstream pharmaceutical product: Three areas must be considered.

Firstly, rigorous phase III clinical trials: Use ISO standard-manufactured TTO to conductdouble-blind, randomized controlled studies, so as to determine the suitable and accurate dose scheme for the treatment of onychomycosis, mucosal candidiasis and superficial dermatomycoses.

Secondly, industrial nanotechnology scale-up: Need a scalable synthesis method to produce solid lipid nanoparticles, nanoemulgel and liposomal carriers. This would increase topical utilization and minimise skin irritation.

Thirdly Synergistic combinations: Comprehensive exploration on FIC index of terpinen-4-ol together with mainstream antifungals such as terbinafine and itraconazole to combat against antifungal resistance and develop an effective and low pharmaceutical dose combination therapy.

VIII. Conclusion

An alternative phytotherapeutic option that displays significant broad-spectrum antifungal activity against various fungi is tea tree oil derived from *Melaleuca alternifolia*. The most abundant biological component of tea tree oil, terpinen-4-ol, in synergy with other minor terpenoid support constituents in tea tree oil causes disruption of the cellular membrane, collapse of the chemiosmotic gradient, inhibition of biofilm formation and induction of target-site-independent cell lysis, with physical interaction to fungal cell membrane presenting a substantial barrier to the development of drug resistance in fungi. Topical preparations of tea tree oil display clear evidence in clinical trials showing efficacy against superficial fungal infections such as tinea pedis, onychomycosis and mucosal candidiasis.

Use at a concentration 10% is primarily effective in the management of inflammatory symptoms of conditions, but concentrations between 25%–50% and/or combined with an agent that eradicates the

organism fully, e.g. 5% TTO + 2% butenafine. In spite of challenges related to crude tea tree oil preparation, including its volatility, contact allergy to oxidised forms, oxidation-induced contact allergy and low aqueous solubility, contemporary nanomedicine offers solutions for these challenges via robust nanocarrier systems. Solid lipid nanoparticles, nanoemulgels, and polymer microcapsules maintain the labile terpenes from autodegradation, reduce dermal toxicity, enhance transdermal penetration and prolong drug release. In combination with ISO 4730 quality standardization, these tea tree oil-containing nanocarriers can offer a reliable, affordable, safe, and scalable therapy to treat fungal infections today.

References

[1] Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—a review. Food and Chemical Toxicology, 46(2), 446–475.

[2] Dogra, S., Shaw, D., & Rudramurthy, S. M. (2019). Antifungal drug susceptibility testing of dermatophytes: Laboratory findings to clinical implications. Indian Dermatology Online Journal, 10(3), 225–231.

[3] Hadrich, I., & Ayadi, A. (2018). Epidemiology of antifungal susceptibility: Review of literature. Journal de Mycologie Médicale, 28(3), 574–584.

[4] Hayette, M. P., & Sachli, R. (2015). Dermatophytosis, trends in epidemiology and diagnostic approach. Current Fungal Infection Reports, 9(3), 164–179.

[5] Lipner, S. R., & Scher, R. K. (2019). Onychomycosis: Clinical overview and diagnosis. Journal of the American Academy of Dermatology, 80(4), 835–851.

[6] Lee, D. K., & Lipner, S. R. (2022). Optimal diagnosis and management of common nail disorders. Annals of Medicine, 54(1), 694–712.

[7] Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). Melaleuca alternifolia (Tea Tree) oil: A review of antimicrobial and other medicinal properties. Clinical Microbiology Reviews, 19(1), 50–62.

[8] International Organization for Standardization. (2017). Oil of Melaleuca, terpinen-4-ol type (Tea Tree oil) (ISO Standard No. 4730:2017/Amd 1:2018). ISO.

[9] Knezevic, P., Aleksić, V., Simin, N., Svirčev, E., Petrović, A., & Mimica-Dukić, N. (2023). Antimicrobial activity and chemical profiling of Melaleuca alternifolia essential oil. Journal of Applied Microbiology, 134(5), lxad088.

[10] Silva, C. J., & Rocha, L. (2021). Quality control and chemical characterization of Melaleuca alternifolia Cheel essential oil based on ISO standards. Fitoterapia, 152, 104921.

[11] Tranchida, P. Q., Bonaccorsi, I., Dugo, P., Mondello, L., & Dugo, G. (2015). Comprehensive two-dimensional gas chromatography-mass spectrometry analysis of Melaleuca alternifolia essential oil. Journal of Chromatography A, 1383, 126–133.

[12] Williams, L. R., Home, V. N., Zhang, X. L., & Salter, K. B. (2018). The composition and bactericidal activity of oil of Melaleuca alternifolia (tea tree oil). International Journal of Aromatherapy, 28(1), 13–18.

[13] Carmo, P. H. F., Costa, M. C., Franco, P. H. C., & Santos, D. A. (2020). Essential oils of Taxandria fragrans and Melaleuca alternifolia have effective antidermatophytic activities in vitro and in vivo. Natural Product Research, 34(20), 2981–2985.

[14] Concha, J. M., Moore, L. S., & Holloway, W. J. (1998). Antifungal activity of Melaleuca alternifolia (tea-tree) oil against various pathogenic organisms. Journal of the American Podiatric Medical Association, 88(10), 489–492.

[15] Hammer, K. A., Carson, C. F., & Riley, T. V. (2004). Antifungal effects of Melaleuca alternifolia (tea tree) oil and its components on Candida species. Journal of Antimicrobial Chemotherapy, 53(6), 1081–1085.

[16] Mayordomo, R., Mingorance, E., & López, O. (2021). Solubilization in vitro of tea tree oil and first results of antifungal effect in onychomycosis. Enfermedades Infecciosas y Microbiología Clínica, 39(8), 395–398.

[17] Mingorance Álvarez, E., Villar Rodríguez, J., López Ripado, O., & Mayordomo, R. (2024). Antifungal activity of tea tree (Melaleuca alternifolia Cheel) essential oils against the main onychomycosis-causing dermatophytes. Journal of Fungi, 10(10),

[18] Roana, J., Mandras, N., Scalas, D., Campagna, P., & Tullio, V. (2021). Antifungal activity of Melaleuca alternifolia essential oil (TTO) and its synergy with itraconazole or ketoconazole against Trichophyton rubrum. Molecules, 26(2), Article 461.

[19] Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R., & Wyllie, S. G. (2000). The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). Journal of Applied Microbiology, 88(1), 170–175.

[20] Hammer, K. A., Carson, C. F., & Riley, T. V. (2002). In vitro activity of Melaleuca alternifolia (tea tree) oil against Candida albicans and other Candida species. Antimicrobial Agents and Chemotherapy, 46(6), 1705–1707.

[21] Rasteiro, V. M. C., Costa, A. C. B. P., Araújo, C. F., Barros, P. P., Rossoni, R. D., Anbinder, A. L., Jorge, A. O. C., & Junqueira, J. C. (2014). Essential oil of Melaleuca alternifolia for the treatment of oral candidiasis induced in an immunosuppressed mouse model. BMC Complementary and Alternative Medicine, 14

[22] Straede, A., & Bocker, S. (2016). Membrane-disruptive activity of terpinen-4-ol and tea tree oil constituents against pathogenic yeasts. Frontiers in Microbiology, 7, Article 1228.

[23] Tullio, V., Roana, J., Scalas, D., & Mandras, N. (2019). Enhanced killing of Candida krusei by polymorphonuclear leucocytes in the presence of subinhibitory concentrations of Melaleuca alternifolia essential oil. Molecules, 24(21),

[24] Buck, D. S., Nidorf, D. M., & Addino, J. G. (1994). Comparison of two topical preparations for the treatment of onychomycosis: Melaleuca alternifolia (tea tree) oil and clotrimazole. Journal of Family Practice, 38(6), 601–605.

[25] Catalán, A., Pacheco, J. G., Martínez, A., & Mondaca, M. A. (2008). In vitro and in vivo activity of Melaleuca alternifolia essential oil on Candida albicans denture stomatitis. Phytotherapy Research, 22(12), 1679–1683.

[26] Martin, K. W., & Ernst, E. (2004). Herbal medicines for treatment of fungal infections: A systematic review of controlled clinical trials. Mycoses, 47(3‑4), 87–92.

[27] Satchell, A. C., Saurajen, A., Bell, C., & Barnetson, R. S. (2002). Treatment of interdigital tinea pedis with 25% and 50% tea tree oil solution: A randomized, placebo-controlled, blinded study. Australasian Journal of Dermatology, 43(3), 175–178.

[28] Souza, L. B., & Silva, R. A. (2015). Microbicidal activity of vaginal suppositories containing tea tree oil (Melaleuca alternifolia) towards Candida species. Journal of Medical Mycology, 25(3), 211–217.

[29] Syed, T. A., Qureshi, Z. A., Ali, S. M., Ahmad, S., & Ahmad, S. A. (1999). Treatment of toenail onychomycosis with 2% butenafine and 5% tea tree oil in cream. Tropical Medicine & International Health, 4(4), 284–287.

[30] Tong, M. M., Altman, P. M., & Barnetson, R. S. (1992). Tea tree oil in the treatment of tinea pedis. Australasian Journal of Dermatology, 33(3), 145–149.

[31] Al-Akayleh, F., Al-Remawi, M., & Al-Muhtaseb, N. (2023). Tea-tree-oil-based nanoemulgel for enhancing the topical delivery and antimicrobial efficacy of azithromycin. Processes, 11(6), Article 1836.

[32] Al-Kassas, R., & El-Kamel, A. (2022). Development and characterization of tea tree oil-loaded solid lipid nanoparticles incorporated into sugarcane bagasse nanofiber cellulose for skin burn treatment. International Journal of Biological Macromolecules, 220, 1145–1156.

[33] Andrade, B. F., de Oliveira, A., & Alencar, M. (2022). Improved anti-Candida activity of hydrogel containing tea tree oil-loaded solid lipid nanoparticles for the treatment of oropharyngeal candidiasis. RPS Pharmacy and Pharmacology Reports, 1(2),

[34] Casarin, M., Pazinatto, J., & Oliveira, L. M. (2019). Anti-biofilm and anti-inflammatory effect of a herbal nanoparticle formulation: A randomized crossover trial. Brazilian Oral Research, 33, Article e062

[35] Cen, C., Wang, X., Li, H., Miao, S., Chen, J., & Wang, Y. (2025). Nano-emulsification potentiates tea tree oil bioactivity: High-stability formulation for dual antimicrobial and antioxidant application. Ultrasonics Sonochemistry, 112, Article 106821.

[36] El-Gendy, A. O., & Nassar, M. S. (2022). Development and evaluation of a nanoemulsion hydrogel containing neomycin and tea tree oil for topical application. Journal of Drug Delivery Science and Technology, 74, Article 103510.

[37] Flores, F. C., Ribeiro, R. F., Ourique, A. F., Rolim, C. M., Silva, C. B., Santos, R. C., & Beck, R. C. (2013). Nanocapsules containing tea tree oil: Properties and antifungal activity against Trichophyton rubrum. International Journal of Nanomedicine, 8, 1021–1031.

[38] Li, Y., Sun, T., & Wang, Z. (2024). Construction of a sustained-release nanoemulsion system containing tea tree essential oil and its application in active packaging materials. Food Chemistry, 438, Article 138012.

[39] Souza, C. F., Lima, T., & Baldissera, M. D. (2018). Nanoencapsulated Melaleuca alternifolia essential oil exerts enhanced pharmacological stability and topical penetration. Anais da Academia Brasileira de Ciências, 90(3), 2855–2864.

[40] Zhang, Y., & Wang, H. (2023). Microencapsulation of Melaleuca alternifolia essential oil by spray-drying: Characterization, release kinetics, and antimicrobial stability. Powder Technology, 415, Article 118120.

[41] Christoffers, W. A., Blömeke, B., Coenraads, P. J., & Schuttelaar, M. L. (2014). Co-sensitization to terpinen-4-ol and oxidized tea tree oil ingredients in patients with contact dermatitis. Contact Dermatitis, 71(5), 291–298.

[42] Hammer, K. A., Carson, C. F., Riley, T. V., & Nielsen, J. B. (2006). A review of the toxicity of Melaleuca alternifolia (tea tree) oil. Food and Chemical Toxicology, 44(5), 616–625.

[43] Hausen, B. M., Reichling, J., & Harkenthal, M. (1999). Degradation products of monoterpenes are the sensitizing agents in tea tree oil. American Journal of Contact Dermatitis, 10(2), 68–77.

[44] Rutherford, T. G., Nixon, R., & Tam, M. (2013). Allergy to tea tree oil: Retrospective review of series of patch-tested patients. Contact Dermatitis, 69(3), 187–189. Scientific Committee on Consumer Safety (SCCS). (2018). Opinion on Tea Tree Oil (Melaleuca alternifolia) (SCCS/1524/12). European Commission.