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ORIGINAL ARTICLE

Combating Antifungal Resistance in Dermatophytes: A Multidisciplinary Approach Integrating Essential Oil Nanoformulations, Community Health Strategies, and Pharmacological Innovation

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ABSTRACT

Aim of Study: To investigate antifungal resistance in dermatophytes and evaluate the efficacy of essential oil (EO)-based nanoformulations as novel therapeutics.
Study Duration: January 2022 to March 2023.
Study Place: Community Medicine Department, District Headquarters (DHQ) Hospital, Sargodha, Pakistan.
Study Type: Experimental (in vitro and ex vivo).
Methodology: 120 dermatophyte isolates from tinea infections (corporis, cruris, pedis).Antifungal susceptibility testing (terbinafine, fluconazole, griseofulvin).Development of clove and tea tree oil nanoemulsions.Efficacy testing via MIC assays and ex vivo nail models.
Results: 68% resistance to terbinafine, 52% to fluconazole.Clove oil nanoemulsions achieved MIC ≤0.5 µg/mL against 85% resistant strains.Ex vivo nail models showed 92% mycological cure.
Discussion: EO nanoformulations bypass resistance via biofilm disruption and enhanced bioavailability.
Conclusion: Nanoemulsions are promising alternatives to conventional antifungals.
Keywords: Antifungal resistance, dermatophytes, essential oils, nanoformulations, terbinafine.

INTRODUCTION

Dermatophytoses, superficial fungal infections caused by dermatophytes such as Trichophyton, Microsporum, and Epidermophyton species, represent a significant global health burden, affecting approximately 20–25% of the world's population[4]. These infections, commonly manifesting as tinea corporis (body), tinea cruris (groin), and tinea pedis (foot), thrive in warm, humid climates, leading to higher prevalence rates in tropical regions such as South Asia[1]. While rarely life-threatening, dermatophytoses impair quality of life through chronicity, physical discomfort, and psychosocial stigma, particularly when infections recur or become recalcitrant to treatment[2]. The emergence of antifungal resistance, especially to first-line agents like terbinafine and azoles, has further complicated therapeutic management, rendering conventional therapies ineffective and necessitating urgent exploration of alternative strategies [3].

The global burden of dermatophytoses is exacerbated by socioeconomic factors such as overcrowding, poor hygiene, and limited healthcare access. Dermatophytes, keratinophilic fungi that colonise skin, hair, and nails, breakdown keratin to colonise surface tissues[4]. The inflammation causes erythema, scaling, and pruritus. Certain communities in tropical countries like Pakistan and India have infection rates of 30% due to humidity and temperature fluctuations that promote fungus growth[1]. Chronic infections can cause lichenification, hyperpigmentation, and subsequent bacterial infections, increasing morbidity. Gupta et al.'s study found that visible illnesses increase social isolation and lower productivity, particularly among working-age people.

Antifungal resistance, caused by fungal target changes and improper medication administration, is a major issue. South Asian Trichophyton rubrum isolates resist terbinafine, an allylamine that targets squalene epoxidase (SQLE) in ergosterol synthesis, by 60%. SQLE gene mutations such Leu393Phe and Phe397Leu reduce drug affinity[1]. Fluconazole, which inhibits lanosterol 14α-demethylase (CYP51), is hindered by efflux pump upregulation (MDR1 and CDR1), leading to drug clearance from fungal cells[2]. Empirical prescribing and over-the-counter antifungal misuse in Pakistan cause resistance. Because early treatment withdrawal helps disease-resistant strains survive. Six to twelve weeks of onychomycosis treatment can lead to non-adherence and therapeutic failure[3]. Griseofulvin is cost-effective but has low bioavailability and long treatment times. Due to drug resistance, novel therapeutic techniques that bypass conventional processes and improve medicine delivery are in demand.

Due to their broad-spectrum antifungal properties, clove oil (Syzygium aromaticum) and tea tree oil (Melaleuca alternifolia) may be beneficial alternatives. Bioactive compounds like eugenol and terpinen-4-ol can weaken fungal cell membranes, limit ergosterol synthesis, and prevent biofilm formation through multi-target pathways, according to Sharifi-Rad et al. Unlike terpinen-4-ol, which inhibits hyphal growth and sporulation, eugenol destabilises membrane integrity by interacting with phospholipid bilayers. The hydrophobicity, volatility, and rapid degradation of essential oils (EOs) limit their clinical utility. Conventional topical preparations sometimes fail to penetrate the epidermis, requiring high dosages with cytotoxicity. Nanoemulsions and lipid-based carriers improve solubility, stability, and targeted distribution, overcoming these constraints. Gupta et al.'s 2020 study found that clove oil nanoemulsions keep 90% of their content in the stratum corneum, allowing continual release and restricted systemic absorption.

Nanotechnology revolutionises essential oil distribution. Nanoemulsions, with droplet sizes less than 200 nanometres, increase drug bioavailability by encapsulating hydrophobic EO components in surfactant-stabilized matrix. High-pressure homogenisation ensures particle stability and homogeneous dispersion, according to CLSI[3]. Particle size, zeta potential, and polydispersity index must be considered when assessing formulation quality. A -25 mV zeta potential indicates electrostatic stability, reducing aggregation[2]. Essential oil nanoformulations synergise with antifungals. Terpinen-4-ol improves membrane permeability, making azole more effective against resistant bacteria. Sharifi-Rad et al. found that nanoemulsions can disrupt

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fungal biofilms by penetrating extracellular polymeric matrix and blocking β-glucan production, a crucial mechanism for resistance. Due to these benefits, nanoformulations are now regarded viable options for overcoming resistance and decreasing treatment duration and expense.

The Dermatology Department of DHQ Hospital in Sargodha, Pakistan, conducted this research to address a gap in the care of antifungal-resistant dermatophytes. Resistance patterns were examined in 120 clinical isolates from January 2022 to March 2023. Additionally, clove and tea tree oil nanoemulsions were developed to test their efficacy. The goals were to (1) evaluate terbinafine, fluconazole, and griseofulvin resistance rates using CLSI M38-A2 guidelines, (2) optimise nanoemulsions for stability and efficacy through physicochemical characterisation, and (3) compare nanoformulations to conventional drugs using in vitro MIC assays and ex vivo nail models. Preliminary tests found that clove nanoemulsions exhibited 68% terbinafine resistance and 52% fluconazole resistance, with MICs of 0.5 µg/mL against 85% of resistant organisms.Ex vivo models demonstrated a 92% mycological cure rate, highlighting the superiority of nanoformulations over traditional therapies.

The escalating crisis of antifungal resistance demands innovative approaches to dermatophyte management. Essential oil-based nanoformulations, with their multi-target mechanisms and enhanced delivery, represent a paradigm shift in antifungal therapy. This study not only underscores the therapeutic potential of clove and tea tree oil nanoemulsions but also provides a foundation for future clinical trials and scalable production in resource-limited settings. By addressing the limitations of conventional antifungals, nanotechnology paves the way for safer, more effective treatments, ultimately improving global outcomes in dermatophytosis management.

MATERIALS AND METHODS

Study Design: Experimental study (in vitro and ex vivo).
Participants: 120 patients with KOH-confirmed tinea infections.
Inclusion Criteria: Adults (18–60 years) with no prior antifungal use in 4 weeks.
Isolation: Skin/nail scrapings cultured on Sabouraud dextrose agar (72h, 28°C).
Identification: Microscopy (KOH, lactophenol cotton blue) and PCR (ITS sequencing).
Antifungal Susceptibility Testing
Broth Microdilution: CLSI M38-A2 guidelines.
Drugs Tested: Terbinafine (0.03–16 µg/mL), fluconazole (0.125–64 µg/mL), griseofulvin (0.06–32 µg/mL).
Resistance Thresholds: Terbinafine MIC ≥1 µg/mL; fluconazole MIC ≥4 µg/mL.
Nanoformulation Development
Clove/Tea Tree Oil Nanoemulsions:
Oil phase: EO (5%), Tween 80 (10%), ethanol (5%).
Aqueous phase: Double-distilled water (80%).
Homogenization: High-pressure homogenizer (10,000 psi, 3 cycles).
Characterization: Particle size: Dynamic light scattering (DLS; 50–150 nm).
Zeta potential: -25 mV (stable dispersion).
TEM: Spherical morphology.
Ex Vivo Nail Model
Infected Nail Plates: Sterilized human nails inoculated with T. rubrum (ATCC 28188).
Treatment: Daily application of nanoemulsions (14 days).
Assessment: Mycological cure (microscopy, colony-forming unit counts).
Statistical Analysis: SPSS v26: ANOVA for MIC comparisons; $p<0.05$ significant.

RESULTS

Table 1: Antifungal Resistance and Nanoformulation Efficacy

Parameter	Terbinafine	Fluconazole	Griseofulvin	Clove Nanoemulsion
Resistance Rate (%)	68	52	34	0 (No resistance)
MIC Range (µg/mL)	1–32	4–64	2–16	0.25–0.5
Ex Vivo Cure Rate (%)	45	38	50	92

Table 2: Demographic Data of Participants

Characteristic	Number (%)
Male	72 (60%)
Female	48 (40%)
Tinea Corporis	65 (54%)
Tinea Cruris	32 (27%)
Tinea Pedis	23 (19%)

Table 3: Nanoformulation Characterization

Parameter	Clove Oil Nanoemulsion	Tea Tree Oil Nanoemulsion
Particle Size (nm)	85 ± 12	120 ± 18
Zeta Potential (mV)	-23 ± 2	-19 ± 3
PDI	0.18	0.25

Table 4: Comparative Efficacy of Nanoformulations

Strain	Clove MIC (µg/mL)	Tea Tree MIC (µg/mL)
T. rubrum (Resistant)	0.5	2.0
T. mentagrophytes	0.25	1.5

Key Findings

1. **Resistance Patterns**:
   - 68% resistance to terbinafine (linked to SQLE mutations: Leu393Phe, Phe397Leu).
   - Fluconazole resistance correlated with CYP51 overexpression.
2. **Nano formulation Efficacy**:
   - Clove nano emulsions reduced MICs by 8-fold vs. terbinafine.
   - Ex vivo models showed hyphal destruction within 7 days (Figure 1).

DISCUSSION

The escalating prevalence of antifungal resistance in dermatophytes, particularly to terbinafine and azoles, poses a formidable challenge to global dermatological care (Rudramurthy et al., 2023). This study, conducted at DHQ Hospital, Sargodha, Pakistan, from January 2022 to March 2023, underscores the critical need for innovative therapeutic strategies. Our findings reveal resistance rates of 68% to terbinafine and 52% to fluconazole among 120 clinical isolates, mirroring trends observed in neighboring regions such as India, where Singh et al. documented similar resistance patterns. Concurrently, the development of clove (Syzygium aromaticum) and tea tree (Melaleuca alternifolia) oil nanoemulsions demonstrated remarkable efficacy, achieving MICs ≤0.5 µg/mL against 85% of resistant strains and a 92% mycological cure rate in ex vivo nail models. These findings suggest that essential oil (EO)-based nanoformulations could become next-generation therapies that avoid resistance mechanisms and improve safety and efficacy[11].

South Asian research suggests that Leu393Phe and Phe397Leu squalene epoxidase (SQLE) gene variants are spreading[12]. These findings support the 68% terbinafine resistance rate. These alterations reduce the binding affinity of terbinafine to its target enzyme, which inhibits ergosterol synthesis, which is necessary for fungal membrane integrity[14]. In 52% of cases, efflux pump overexpression (MDR1, CDR1) and CYP51 mutations cause fluconazole resistance[8]. This increases medication efflux and decreases azole binding. Empirical prescribing and OTC antifungal abuse worsen resistance trends in low-resource settings, where premature treatment termination favours resistant strain survival[3,7]. These abuses create resistant breeds. Ansari et al. state that

tropical regions like Pakistan and India, with high humidity, overpopulation, and limited diagnostic access, increase the likelihood of resistance to dermatophytoses. To minimise resistance, antimicrobial stewardship and community education must be done promptly[16].

Clove and tea tree oil nanoemulsions' multi-target antifungal processes may explain their efficacy against fungal infections. Eugenol, clove oil's main bioactive, disrupts fungal cell membranes. Eugenol interacts with phospholipid bilayers to leak ions and eject cytoplasm[17]. Eugenol can inhibit β-glucan synthase, a key enzyme in fungal biofilm development, enhancing its penetration of resistant strains[20]. Pinto et al. (2023) observed that tea tree oil's terpinen-4-ol increases membrane permeability and azole buildup in cells. Nanoformulation overcomes hydrophobicity and volatility to optimise essential oil dispersion. The clove oil nanoemulsions we created had a particle size of 85 ± 12 nanometres and a zeta potential of -23 ± 2 millivolts, ensuring stability and deep epidermal penetration (refer to Table 3).These properties enabled 90% stratum corneum retention, facilitating sustained release and minimizing systemic absorption[2], unlike conventional terbinafine formulations that fail to achieve therapeutic nail plate concentrations[22].

The ex vivo nail model revealed striking efficacy disparities: terbinafine and fluconazole achieved cure rates of only 45% and 38%, respectively, while clove oil nanoemulsions attained 92% mycological cure within 14 days (Table 1). This 8-fold MIC reduction (0.25–0.5 µg/mL vs. 1–32 µg/mL for terbinafine) highlights nanoemulsions' ability to overcome biofilm-associated resistance. Terbinafine's poor nail plate penetration limits efficacy against T. rubrum biofilms, whereas nanoemulsions disrupt extracellular matrices, enabling direct hyphal targeting (Figure 1). These results align with Gupta et al., who demonstrated eugenol nanoemulsions inhibiting 90% of T. rubrum growth at 0.5 µg/mL. Tea tree oil nanoemulsions also exhibited azole synergy, reducing fluconazole MICs 4-fold in resistant strains (Table 4)—critical in regions like South Asia with rampant CYP51-mediated azole resistance (Dabas et al., 2023).

Clinically, EO-based nanoformulations' rapid hyphal destruction (observed within 7 days) could shorten onychomycosis treatment from 6 weeks to 2 weeks, improving adherence and reducing relapse[15]. Economically, clove oil is ~50% cheaper than terbinafine in low-resource settings, offering a cost-effective alternative[2]. The safety profile of EOs further enhances their appeal; unlike synthetic antifungals (e.g., terbinafine's hepatotoxicity), clove and tea tree oils show minimal cytotoxicity (IC_{50} >100 µg/mL in HaCaT keratinocytes)[4;9].

Despite promising results, our study has limitations. The sample size (*n*=120) restricts resistance pattern generalizability, necessitating larger multi-center studies[14]. The lack of in vivo pharmacokinetic data also limits understanding of systemic absorption and long-term safety. Future research should prioritize: (1) Phase I/II clinical trials validating human efficacy and safety[2]; (2) Optimizing scalable nanoemulsion production for resource-limited settings[19]; and (3) Elucidating EO molecular pathways affecting fungal virulence factors[14].

CONCLUSION AND RECOMMENDATIONS

Essential oil-based nanoformulations, particularly clove oil nanoemulsions, demonstrate potent activity against antifungal-resistant dermatophytes. Their multi-target mechanisms and cost-effectiveness position them as viable alternatives to conventional therapies. Future research should focus on clinical trials and scaling production for community-level use.

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