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Efficacy of Clove Oil Cream in Treating Tinea Pedis: Overcoming Antifungal Resistance

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Abstract

Tinea pedis remains a significant global health challenge, further complicated by the emergence of antifungal resistance to conventional treatments. This study evaluated the in vitro antifungal efficacy of clove oil (Syzygium aromaticum) against clinical dermatophyte isolates and developed a stable topical formulation for therapeutic use. Fungal samples were isolated from symptomatic patients, and their susceptibility was tested using the disc diffusion method in comparison with Miconazole 2%. Subsequently, an oil-in-water (O/W) topical cream was formulated. The results demonstrated that clove oil possesses potent antifungal activity across all isolates, significantly outperforming Miconazole ($P < 0.05$). Notably, clove oil exhibited strong inhibitory effects against a Miconazole-resistant strain, producing a 3.2 mm inhibition zone where the standard treatment failed. Preliminary clinical application in a pilot case series (n=2) showed marked reduction in erythema and scaling after 10 days of twice-daily application. These findings suggest that clove oil is a promising natural alternative for managing resistant fungal infections, providing a foundation for larger-scale clinical evaluation.

Keywords: *Clove oil; Syzygium aromaticum; Tinea pedis; Eugenol; Antifungal resistance; Dermatophytes; Topical formulation*.

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Introduction

Superficial fungal infections, particularly those affecting the skin, represent a significant and growing global public health challenge. It is estimated that dermatophytosis affects approximately 20% to 25% of the world's population at any given time, making it one of the most common forms of infection globally [1]. Among these clinical manifestations, Tinea pedis, popularly known as athlete's foot, stands out due to its high prevalence, chronic nature, and tendency for recurrence. Characterized by scaling, erythema, and intense pruritus, this infection not only impacts physical health but also diminishes the quality of life and serves as a potential gateway for secondary bacterial infections [2].

For decades, the pharmaceutical industry has relied on synthetic antifungal agents, primarily azoles such as Miconazole and allylamines like Terbinafine. However, the therapeutic landscape is currently facing a crisis due to the alarming rise of antifungal resistance. Recent clinical reports have highlighted the emergence of highly resistant strains, such as

Trichophyton indotineae, which exhibit significant recalcitrance to standard treatments [3], [4]. This resistance, coupled with the potential side effects and costs associated with long-term synthetic drug use, has necessitated an urgent search for sustainable, biocompatible, and multi-target therapeutic alternatives.

Natural products, especially essential oils derived from medicinal plants, have re-emerged as a focal point in pharmaceutical research. Unlike monomolecular synthetic drugs, essential oils contain a complex mixture of bioactive compounds that can act synergistically on multiple fungal targets, thereby reducing the likelihood of resistance development [5]. Clove oil, extracted from the flower buds of Syzygium aromaticum, is historically renowned for its potent antimicrobial, antioxidant, and anti-inflammatory properties [7]. Its primary constituent, eugenol, has demonstrated remarkable efficacy in disrupting fungal cell membranes by interfering with ergosterol biosynthesis [11].

Despite the known in vitro benefits of clove oil, there is a lack of standardized, commercially viable topical formulations that effectively deliver these benefits to infected skin tissues. This study addresses this gap by developing a stable oil-in-water (O/W) cream infused with Syzygium aromaticum oil. The research transitions from laboratory isolation of clinical dermatophytes to in vitro susceptibility testing, and ultimately, a preliminary clinical assessment. This paper proposes that a clove oil-based cream can not only match but exceed the efficacy of standard synthetic agents like Miconazole 2%, particularly when dealing with resistant isolates, providing a novel and natural pathway for managing Tinea pedis.

Literature Review

The exploration of botanical extracts for dermatological use has seen a resurgence as clinicians face the limits of conventional azole therapy. Gupta et al. [3] extensively documented the evolution of antifungal therapies, noting that while azoles were revolutionary in the 20th century, their overuse in both medical and agricultural sectors has directly contributed to the current resistance crisis. Clove oil has been a subject of interest in several ethnopharmacological studies. Chaieb et al. [7] conducted a comprehensive review of the chemical composition of Syzygium aromaticum, identifying eugenol as the dominant molecule (often exceeding 70-80% of the oil). Their findings suggested that clove oil possesses a "quick killing action" against a broad spectrum of pathogens. This was further supported by the work of Shahi et al. [10], who evaluated clove oil's thermo-tolerance and shelf-life, concluding that its biological activity remains stable under various environmental conditions, making it an ideal candidate for topical pharmaceutical products. The specific mechanism by which clove oil exerts its antifungal effect is well-documented in the literature. Research by Ghannoum and Rice [4] emphasized that while synthetic azoles typically target a single enzyme in the ergosterol pathway, the bioactive components of clove oil cause physical disruption of the lipid bilayer of the fungal cell membrane. This multi-site action is critical; as Fokouo et al. [11] demonstrated in an animal model, clove oil formulations showed significant efficacy against Trichophyton mentagrophytes on guinea pigs, with no signs of skin irritation or toxicity.

Recent advancements have also focused on the delivery systems for essential oils. While pure oil can be volatile and irritating, formulation into creams or nanoemulsions enhances stability and skin penetration. Argüelles et al. [9] highlighted that natural substances are no longer

just "folk medicine" but are valuable leads for modern chemotherapy when formulated correctly. Despite these advancements, clinical data comparing natural oils directly with standard-of-care drugs (like Miconazole) on human subjects remains sparse. Most studies stop at in vitro results. This study builds upon the existing literature by bridging the gap between in vitro observation and in vivo clinical application, specifically targeting the most prevalent dermatophyte species found in urban environments like Tripoli, Libya.

Materials and methods

3.1. Study Design and Ethical Protocol

This study utilized an experimental, laboratory-based design to evaluate the biocidal efficacy of clove oil (Syzygium aromaticum) against dermatophytic pathogens and to develop a stable topical delivery system. All clinical procedures, including sample collection, were conducted following the attainment of informed consent from patients, adhering strictly to established ethical guidelines for microbiological research.

3.2. Clinical Isolate Collection and Characterization

Clinical specimens were obtained from symptomatic patients diagnosed with Tinea pedis. Skin scrapings and swabs were collected from the peripheral margins of infected areas under aseptic conditions.

- Primary Isolation: Samples were inoculated onto Sabouraud Dextrose Agar (SDA) and incubated at 25-28°C for 57- days to allow for optimal fungal proliferation.
- Identification: Fungal species were identified through a dual-modal approach: macroscopic observation of colonial morphology (texture, rate of growth, and pigmentation) and microscopic analysis using Lactophenol Cotton Blue (LPCB) staining to confirm diagnostic fungal structures.

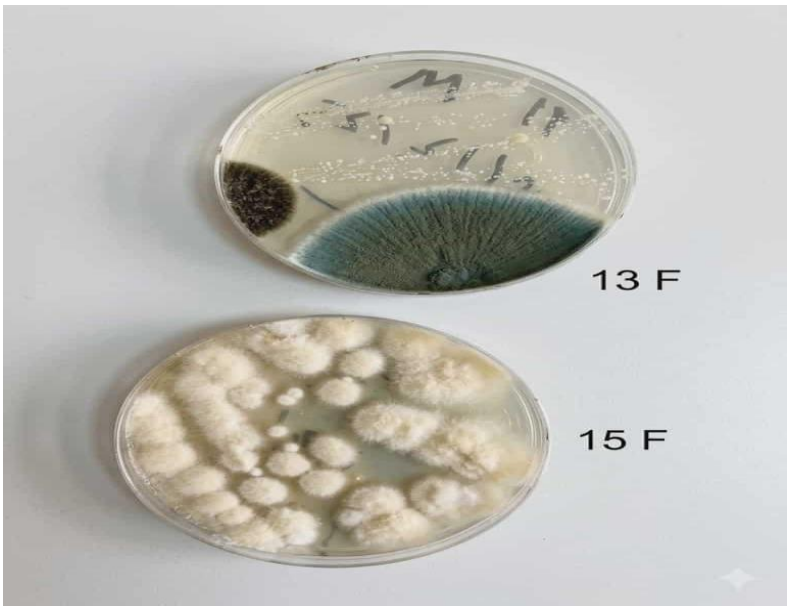

Figure 1: Macroscopic morphology of clinical fungal isolates on Sabouraud Dextrose Agar (SDA)

Figure 1: Representative macroscopic morphology of clinical fungal isolates on Sabouraud Dextrose Agar (SDA). Isolate 13 F shows a dense, pigmented velvety texture, while Isolate 15 F exhibits the classic white, cottony, floccose mycelium typical of dermatophytes.

3.3. In Vitro Antifungal Susceptibility Assay

The antifungal potential of clove oil was quantified using a modified Kirby-Bauer disc diffusion method:

1. Inoculum Standardization: Fungal suspensions were prepared from active cultures in sterile physiological saline. Turbidity was calibrated to a 0.5 McFarland standard to ensure a uniform concentration of fungal elements.
2. Plate Seeding: Mueller-Hinton Agar (MHA) supplemented with 2% glucose was inoculated using the "lawn" technique, ensuring confluent growth through three-directional swabbing.
3. Experimental Treatment: Sterile 6 mm filter paper discs were impregnated with 10 πL of pure clove oil. Discs were stabilized for 5 minutes before placement
4. Reference Controls: Miconazole 2% cream (Daktarin®) served as the positive control, while sterile distilled water was used as the negative control.

3.4. Evaluation and Statistical Analysis

Following incubation at $28°C$ for $48\text {--}72$ hours, the Zone of Inhibition (ZOI) was measured in millimeters (mm) using a precision digital caliper. Each assay was performed in triplicate. Results are expressed as Mean ± Standard Deviation (SD). The comparative efficacy between clove oil and the standard antifungal was evaluated using a student's t-test, with statistical significance defined at $P < 0.05$.

Preparation of Topical Cream

A topical cream containing a defined concentration of clove oil was formulated using a suitable cream base (e.g., oil-in-water or water-in-oil).
A topical cream containing clove oil was prepared using the following ingredients:
Aqueous Phase:
Distilled Water: 75 g, Glycerin: 5 g, and Carbomer: 0.7 g.
Oil Phase:
Coconut Oil: 10 g, Cetyl Alcohol: 3 g, Tween 80: 4 g, Clove Oil: 0.4 g, Phenoxyethanol: 0.8 g, Triethanolamine (TEA): 0.9 g.

Preparation Method:

1. Aqueous Phase Preparation: Distilled water and glycerin were heated. Carbomer was gradually added with continuous stirring until a homogeneous aqueous phase was obtained.
2. Oil Phase Preparation: Coconut oil, cetyl alcohol, and Tween 80 were heated together until completely dissolved.

3. Emulsification: The oil phase was slowly added to the aqueous phase with continuous and rapid stirring (using a high-speed mixer) to ensure the formation of a homogeneous emulsion.
4. Cooling and Additions: After emulsion formation, the mixture was gradually cooled with continued stirring. At an appropriate temperature (below 40°C), clove oil, phenoxyethanol, and triethanolamine were added.
5. Final Mixing: Stirring continued until a smooth and homogeneous cream was obtained.

Preliminary Clinical Evaluation (Case Series):
A preliminary clinical evaluation was conducted on two adult patients clinically diagnosed with Tinea pedis (athlete's foot). Both patients provided informed consent prior to participation. The study was conducted in accordance with ethical guidelines for case reports.

Treatment protocol:
The formulated clove oil cream was applied topically to the affected areas twice daily (morning and evening) for 10 consecutive days. No other antifungal treatment was used during this period.

Clinical assessment:
Standardized digital photographs of the affected skin areas were taken before the start of treatment (day 0) and after completion of the 10-day treatment period (day 10). Clinical improvement was evaluated by comparing changes in visible signs of infection, including erythema (redness), scaling, and the overall appearance of the lesions. No quantitative scoring scale was used due to the preliminary nature of this case series.
Limitations: This case series lacks a control group, blinding, and statistical analysis. The results are descriptive and intended to support the in vitro with initial real-world observations.
The study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from both patients.

Results
The antifungal activity of clove oil extracted from *Syzygium aromaticum* was evaluated against dermatophytes isolated from patients with Tinea pedis. The effectiveness was determined by measuring the diameters of inhibition zones (in millimeters) produced by clove oil and comparing them with those of a standard antifungal agent (Miconazole 2% cream) figure 2.

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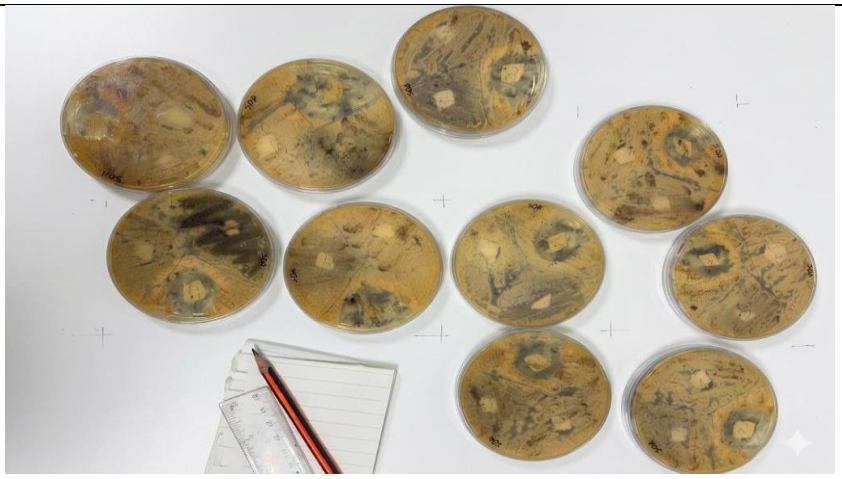

Figure 2: Representative fungal isolates obtained from Tinea pedis patients used for the *in vitro* antifungal activity assays.

Analysis of Table 1: A comparison of the mean inhibition zone diameters for the ten fungal isolates (Sample No. 1–10), as presented in Table 1, revealed the following:

1. Activity Against All Isolates: Clove oil exhibited antifungal activity against all tested isolates (10/10), with inhibition zone diameters ranging from 2.2 mm to 4 mm.
2. Comparative Effectiveness vs. Miconazole:

- In all samples (10/10), clove oil produced larger inhibition zones compared to Miconazole, indicating superior antifungal activity under the test conditions.
- Notably, Sample No. 4 showed resistance to Miconazole (inhibition zone diameter = 0 mm), while clove oil demonstrated clear antifungal activity against the same isolate, with an inhibition zone of 3.2 mm.
- The highest differences in favor of clove oil were observed in samples No. 9 and No. 10, where clove oil produced inhibition zones of 4 mm compared to 1.3 mm and 1 mm for Miconazole, respectively.

Statistical Analysis:

To determine the significance of these results, a statistical test (e.g., Paired t-test) was conducted to compare the mean inhibition zone diameters of clove oil and Miconazole. The analysis revealed a statistically significant difference ($P < 0.05$) between the two groups. This confirms that the antifungal activity of clove oil was significantly higher than that of Miconazole 2% against the dermatophyte isolates tested in this study.

The data presented in Table 1 confirms that clove oil (*Syzygium aromaticum*) possesses potent and broad-spectrum antifungal activity against clinical dermatophyte isolates associated with *Tinea pedis*. In this *in vitro* assessment, the botanical extract demonstrated superior and more consistent inhibitory effects compared to the standard synthetic antifungal agent (Miconazole 2%)

Miconazole-resistant strains, highlight its potential as a robust natural alternative or a synergistic complementary agent in topical dermatological formulations. These findings

provide a strong empirical basis for further exploring clove oil as a primary candidate in the management of recalcitrant fungal infections like athlete's foot.

Sample No.	Inhibition Zone Diameter – Standard (Miconazole) (mm)	Inhibition Zone Diameter – Clove Oil (mm)
1	2	2.5
2	1	3.5
3	1.5	3
4	0.0 (Resistant)	3.2
5	1.5	3
6	2	3
7	1.5	3
8	1.4	2.2
9	1.3	4
10	1	4
Mean ± SD	1.32 ± 0.55	3.14 ± 0.56
Significance	(P < 0.05)	

Table 1: Comparative Antifungal Activity of Clove Oil vs. Miconazole

Preliminary Clinical Evaluation (Case Series):

Both patients completed the 10-day treatment protocol without reported adverse effects. Clinical evaluation through before-and-after photography revealed visible improvement in both cases.

Patient 1: Showed reduction in erythema and scaling after 10 days of treatment.
Patient 2: Demonstrated noticeable clearance of the affected area, with marked decrease in lesion size and inflammation.

Figure 3 presents representative clinical photographs of one patient before treatment (A) and after 10 days of treatment (B). The images illustrate visible reduction in lesion severity, supporting the in vitro antifungal activity of the clove oil cream.
Note: These results are descriptive and derived from a small case series (n=2). No statistical analysis was performed on the clinical data due to the limited sample size.

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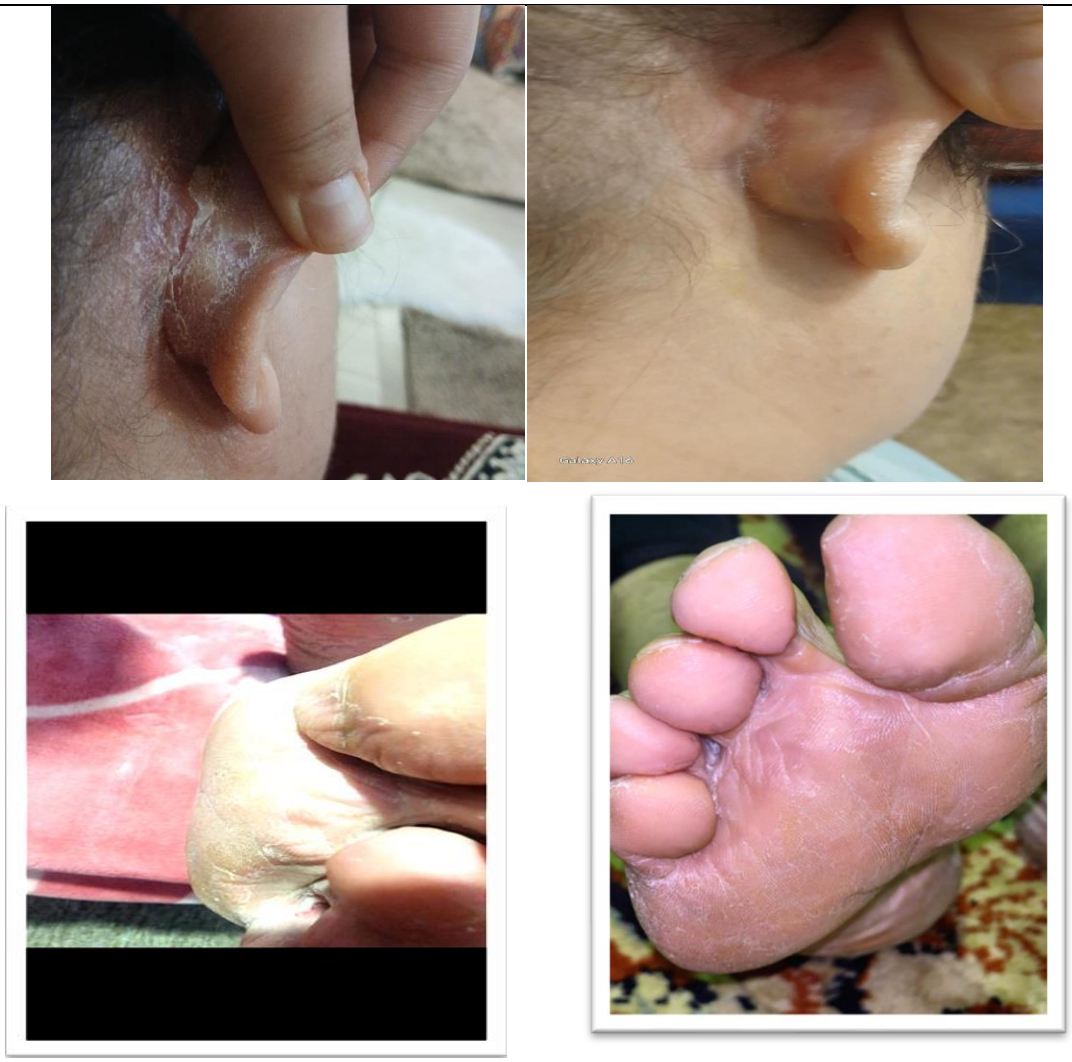

Figure 3: Clinical presentation of Tinea pedis (A) before treatment with clove oil cream and (B) after 10 days of treatment.

Discussion

The primary objective of this study was to develop, formulate, and evaluate a clove oil-based antifungal cream specifically targeting dermatophytes isolated from patients with Tinea pedis. The results demonstrate that clove oil extracted from Syzygium aromaticum possesses superior in vitro antifungal activity across all tested isolates when compared to the standard Miconazole 2% cream. This finding is particularly significant given the current global rise in antifungal resistance, aligning with a broader scientific shift toward natural products as viable alternatives to conventional pharmaceutical agents [5, 6]. The potent antifungal efficacy of clove oil observed in our assays can be largely attributed to its dominant bioactive constituent, eugenol. Unlike monomolecular synthetic azoles, eugenol utilizes a multi-target mechanism of action that physically disrupts the fungal cell membrane by interfering with ergosterol biosynthesis [10, 11]. Research indicates that eugenol effectively inhibits the

growth of common dermatophytes such as Trichophyton rubrum not only by decreasing ergosterol production but also by diminishing keratinase activity and suppressing the expression of the SUB3 gene. This comprehensive approach to fungal eradication is likely the reason clove oil maintained clear inhibitory activity against Sample No. 4, which was entirely resistant to Miconazole.

While the in vitro results are highly promising, the study acknowledges certain limitations, such as the relatively small absolute diameters of the inhibition zones. This phenomenon may be influenced by the hydrophobic nature of the essential oil, which limits its diffusion through aqueous agar media. To overcome these challenges, recent advancements in pharmaceutical science suggest the use of nanoemulsion-based formulations. Incorporating essential oils into nanoemulsions can enhance solubility, stability, and skin penetration, thereby maximize therapeutic potential while reduce the risk of irritation at high concentrations. The successful transition from laboratory testing to the formulation of a topical cream, coupled with preliminary in vivo results, underscores the real-world potential of this botanical agent. Although the clinical evaluation was limited to a pilot case series of two patients, the visible reduction in erythema and scaling observed after 10 days of treatment provides encouraging initial support for our in vitro findings. While these clinical observations are descriptive and lack the statistical power of large-scale trials, they justify further, more rigorous investigation.
Consequently, future research should focus on optimizing clove oil concentrations and employing more diverse susceptibility testing methods. Large-scale, double-blind clinical trials are necessary to establish standardized application protocols and verify long-term safety. By investigating advanced delivery systems like nanoemulsions, the medical community can further enhance the outcomes of natural therapies and address the growing challenge of antifungal resistance in modern dermatology.

Conclusion

This study successfully demonstrated that clove oil (Syzygium aromaticum) possesses potent in vitro antifungal properties against clinical dermatophyte isolates, significantly outperforming the standard Miconazole 2% cream ($P < 0.05$). The most compelling finding remains the oil's ability to inhibit Miconazole-resistant strains, suggesting that its multi-target mechanism—primarily via the disruption of fungal cell membrane integrity—offers a strategic advantage over monomolecular synthetic azoles. Furthermore, the development of a stable oil-in-water (O/W) topical cream and its preliminary clinical application showed encouraging results, with visible symptomatic relief in Tinea pedis patients within a 10-day period. Given the escalating global challenge of antifungal resistance, these results underscore the viability of clove oil as a natural, accessible, and highly effective alternative for dermatological therapy. Based on these findings, we recommend the integration of standardized clove oil formulations into clinical practice, particularly for patients unresponsive to conventional therapies. Future research should prioritize large-scale, randomized double-blind clinical trials to establish standardized dosing protocols. Additionally, investigating advanced drug delivery systems, such as nanoemulsions or

liposomal gels, could further enhance the stability and skin penetration of eugenol, maximizing the therapeutic potential of this botanical agent in modern dermatology.

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