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Original Research Paper

In-vitro interaction of *lemongrass* essential oil alone and in combination with antifungal drugs against *Trichophyton rubrum*

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Abstract

Dermatophytosis continues to be one of the most common superficial mycoses, affecting an estimated Twenty-five percent of the global population. Trichophyton rubrum is the leading causative fungus . Recently, interest has surged in combining standard antifungal drugs with plant-derived oils to improve treatment outcomes. This study looked at how lemongrass (Cymbopogon citratus) oil against T. rubrum in vitro individually and in combination with two antifungal agents . The oil alone clearly inhibited the fungal growth, clear zones of 7.6–20.5 mm were observed across the concentrations tested. Clotrimazole and terbinafine were also inhibitory with terbinafine producing larger clear zones, 10.8–39.1 mm. Terbinafine and the oil interacted in an additive not synergistic manner. Overall, the data suggest lemongrass oil as a useful adjunct to terbinafine for developing new topical treatments for infections caused by T. rubrum.

Keywords: Cymbopogon citratus; lemongrass oil; antifungal; terbinafine; dermatophyte; combination therapy.

1. Introduction

Fungal skin disease is a longstanding problem in hot, humid climates where dermatophytes and Candida spp. make up a large proportion of infections of the skin and mucosal surfaces. Genera including Microsporum, Trichophyton and Epidermophyton can infect the skin, hair and subcutaneous layers of both humans and animals (Majid and Ali, 2008). Of these, T. rubrum is noted as the organism most often linked to persistent, difficult to clear infections (Sousa et al., 2015). Standard antifungal drugs, while effective, come with drawbacks: allergic reactions, adverse effects, and microbes that gradually stop responding to them. Plant-derived remedies sidestep many of these problems, and their comparatively mild safety profile has pushed herbal antifungal research forward in recent years (Lewis et al., 2022). Lemongrass oil has also proven active against several bacteria reports describe strong effects against Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, and Klebsiella pneumoniae (Man et al., 2019) and separate work credits it with antifungal, anti-yeast, insecticidal, and antiparasitic action (Faheem et al., 2022). Much of this fungicidal power is credited to citral together with related Monoterpenes, including α-terpineol and myrcene , which appear to work best in combination rather than singly. A commonly proposed explanation is that these molecules compromise the target cell's membrane, setting off structural breakdown, escape of cytoplasmic content, and eventual cell death (Khosakueng et al., 2024). Rhimi and colleagues (2022) separately tested C. citratus oil against a panel of fungal organisms, Malassezia furfur among them, and confirmed measurable antifungal action. Pairing established antifungal drugs with plant-derived compounds has been put forward as a route toward overcoming resistance and improving outcomes in dermatophytosis treatment (Lopes et al., 2017; Brescini et al., 2021). Combinations of this kind can sharpen the action of standard drugs and work against multidrug-resistant organisms by hitting more than one target at once. They may also allow the conventional drug to be given at a reduced dose, which in turn lessens its toxicity and adverse consequences (Van Vuuren and Viljoen, 2011; Zuzarte et al., 2021). Given lemongrass oil has shown quantifiable efficacy against dermatophytes and that conventional drugs remain the clinical mainstay for their treatment, the combination of the two is a logical next step. To study this concept, the present work was performed by screening of lemongrass oil alone and in combination with clotrimazole and terbinafine for antifungal activity against T. rubrum.

2. Materials and Methods

2-1. Antifungal drugs used

The two antifungal drugs used in this study were Clotrimazole and Terbinafine acquired from a drugstore in Wasit Province.

Lemongrass oil source

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Lemongrass oil used in the present work was obtained from Scientific Research Commission, Baghdad, Iraq.

2-2. Fungal isolate

The strain of T. rubrum used in this work was obtained from the biology department, College of Science, Wasit University, Iraq and was cultured on Sabouraud Dextrose Agar (SDA) medium to obtain a pure culture for subsequent assays.

2-3. Disc diffusion assay of lemongrass oil against T. rubrum

T. rubrum's response to lemongrass oil was measured through a disc diffusion assay. Concentrations of Solutions of 100%, 50%, and 25% were prepared by dissolving the oil in dimethyl. sulfoxide (DMSO). Sterile 5-mm discs were dipped in each concentration and set onto SDA plates already seeded with the fungus. The plates sat undisturbed for two hours at ambient temperature so the solution could spread through the agar, after which they went into an incubator held at 25±2°C for the set duration; clear-zone diameters were then read off in millimeters (Asumani et al., 2026).

2-4. Poisoned food assay of lemongrass oil against T. rubrum

For the Assay of Contaminated food use 10 ml of each oil concentration (100%, 50%, and 25%). went into 90 ml of freshly autoclaved, still-molten SDA before being poured into 9-cm dishes; dishes prepared with plain, oil-free medium acted as controls. A 10-mm mycelial plug cut from the test fungus was set at the centre of every dish, which was then held at 25±2°C for the designated interval. Colony diameters were measured once growth was complete, and percentage growth inhibition (GI%) was worked out from GI% = (dc − dt)/dc × 100, dt being the average colony diameter in treated dishes and dc the average in controls. Three replications were performed in a Completely Randomised Design (CRD) (Premathilake et al., 2018).

2-5. Impact of antifungal agents (clotrimazole, terbinafine) on the proliferation of T. rubrum by disc diffusion methodology

The antifungal activity of clotrimazole and terbinafine (Lamicol) against T. rubrum was assayed by three different concentrations of each drug. The concentrations were as follows: stock solution (S), S/2 and S/4. 5-mm sterile paper discs were soaked in each dilution and placed in the centre of SDA plates pre-inoculated with the target fungus. The plates were incubated for 2 h at room temperature for diffusion and at 25±2°C for the required time. The diameter of clear zone was measured and the diameter of disc was subtracted to get the inhibitory zone in mm (Zarei et al., 2015).

2-6. Impact of terbinafine in conjunction with essential oil on the proliferation of T. rubrum using the disc diffusion technique

The antifungal efficacy of terbinafine and lemongrass essential oil in conjunction against T. rubrum was assessed using disc diffusion. Equal amounts (5 ml) of essential oil at each concentration (100%, 50%, and 25%) were mixed with 5 ml of terbinafine at the S/2 concentration. Sterile 5-mm discs were soaked in the resulting mixtures and placed on SDA plates pre-inoculated with the fungus; discs saturated in distilled water served Discs containing terbinafine alone functioned as a positive control, whereas a negative control was used. Plates were maintained at room temperature for two hours to facilitate diffusion before being incubated at 25±2°C for the specified time, following which inhibitory zones were quantified in millimetres (Mukherjee et al., 2003). The type of interaction between the essential oil and terbinafine was determined by comparing the calculated χ^2 value against the tabulated critical value for 1 degree of freedom (3.84). An interaction was classified as synergistic when the calculated χ^2 exceeded the tabulated value, and as additive when the tabulated value exceeded the calculated χ^2, using The equation proposed by Goyal et al. (2020):

$ZE = ZT + ZO (1 - ZT / 100)$

$\chi^2 = (ZTO - ZE)^2 / ZE$

ZE = anticipated inhibition zone

ZT = inhibitory zone produced by terbinafine exclusively

ZO = inhibitory zone produced only by lemongrass essential oil

ZTO = inhibitory zone resulting from the combination

$\chi 2$ = Computed Chi-squared

2-7. Statistical Analysis

Statistical analysis of the data was conducted using SPSS software, using one-way analysis of variance (ANOVA), with mean differences across treatments assessed by the Least Significant Difference (LSD) test at $p < 0.05$.

3. Results and Discussion

3-1. Impact of lemongrass essential oil on the proliferation of T. rubrum using the disc diffusion technique

As shown in Table (1), lemongrass essential oil displayed clear antifungal activity against T. rubrum, inhibiting fungal growth on the culture plates, with inhibition zones of 20.5, 15.5, and 7.6 mm recorded at concentrations of 100, 50, and 25%, respectively.

Table (1) Impact of lemongrass essential oil on T. rubrum by disc diffusion technique

Treatment	Concentration	Inhibition zones (mm)
Essential oil	100	20.5
	50	15.5
	25	7.6
Control		0
LSD (0.05)		0.200

Each value is a mean of three replicates

Figure (1) Effect of essential oil on T. rubrum by disc diffusion method

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(A- 25%, B- 50%, C- 100%, D- control)

This is in line with the rich phytochemical profile of lemongrass essential oil (LGEO). The wide bioactivity of LGEO is attributed to citral isomers and other related terpene compounds. These constituents are particularly important in food-related applications, where LGEO has been investigated for its capacity to mitigate oxidative degradation , reduce growth of pathogenic and spoilage-causing microorganisms, and inhibit biofilm formation. In turn, the increasing resistance of fungal pathogens to synthetic antifungals has resulted in a growing interest in plant-derived antifungal alternatives (Ahmad et al., 2026).

Species reported Organisms susceptible to C. citratus essential oil include Candida albicans, Candida pseudotropicalis, Microsporum gypseum, Botrytis cinerea, Aspergillus niger, and Beauveria bassiana. The oil has shown effectiveness against dermatophyte species such as Aspergillus fumigatus, Trichophyton rubrum, Microsporum gypseum, Cladosporium trichoides, Trichophyton mentagrophytes, Epidermophyton floccosum, Botrytis cinerea, and Aspergillus nidulans. (He et al., 2023; Sahal et al., 2020).

These results are in line with the work of Lucia and Eva (2023) who tested the oil of C. Citratus on T. mentagrophytes at concentrations ranging from 100% to 10% and concluded that some essential oils can be superior to commercial antifungal drugs in the treatment of dermatophytoses.

Similar results were obtained by Khan and Ahmad (2013) when they tested C. citratus oil in vitro against azole-resistant Aspergillus and Trichophyton strains; they observed promising antifungal activity with inhibition zones ranging from 24.66 to 42.00 mm and fungicidal action against T. rubrum.

Wannissorn et al. (1996) similarly identified lemongrass oil as one of the most potent agents against human dermatophytes, showing strong activity against T. mentagrophytes, T. rubrum, Epidermophyton floccosum, and Microsporum gypseum, while other authors have documented its efficacy against 32 keratinophilic fungi responsible for ringworm and food spoilage (Kishore et al., 1993; Abe et al., 2003).

3-2. Impact of lemongrass essential oil on the proliferation of T. rubrum using the poisoned food methodology

Table (2) shows that lemongrass essential oil suppressed T. rubrum development in a concentration-dependent manner: radial mycelial growth measured 3.33, 4.6, and 6.5 cm at concentrations of 100, 50, and 25%, respectively, compared with 8.2 cm in the untreated control.

Correspondingly, growth inhibition reached 59.39%, 43.90%, and 20.73% at the 100%, 50%, and 25% concentrations, respectively.

Table (2) Effect of lemongrass essential oil on T. rubrum by poisoned food technique

Treatment	Concentration (mg/ml)	Radial growth (cm)	Inhibition %
Essential oil	100	3.33	59.39
	50	4.6	43.90
	25	6.5	20.73
Control		8.2	
LSD (0.05)		0.178	

Each value is a mean of three replicates

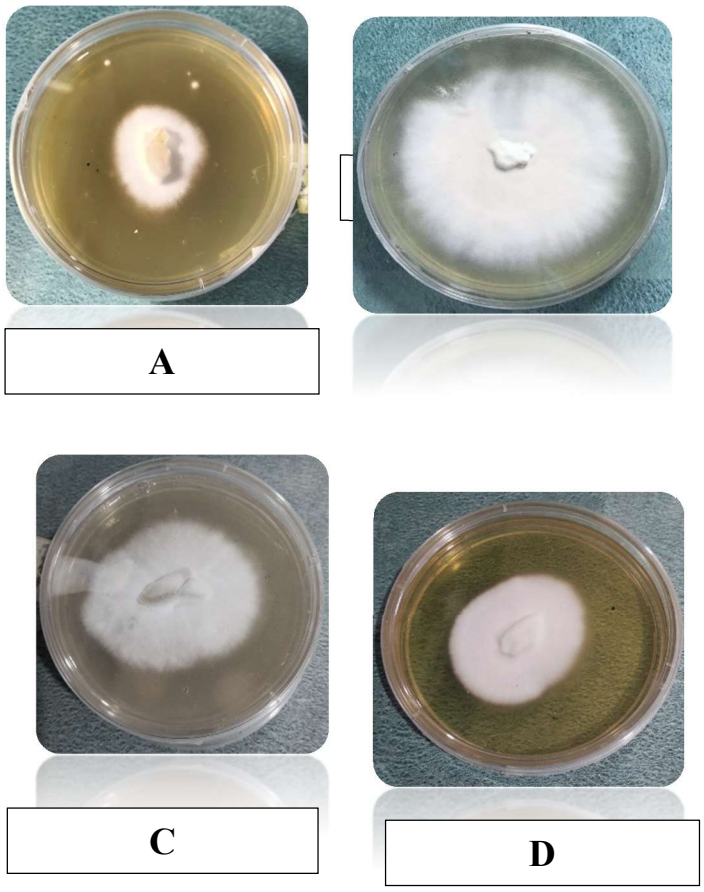

Figure (2) Effect of essential oil on T. rubrum by poisoned food technique
(A- 100%, B- 50%, C- 25%, D- control)

These findings confirm the strong antifungal potential of lemongrass essential oil against T. rubrum, Consistent with previous research by Capoci et al. (2015), which demonstrated significant fungistatic and fungicidal properties of Cymbopogon nardus against M. canis isolates from animals and domestic environments supporting its potential use as a household preventive measure against dermatophytosis. Earlier work also showed that C. citratus oil completely suppressed both growth and sporulation of Aspergillus flavus (Paranagama et al., 2003).

A 10% lemongrass oil ointment has likewise been shown effective against Malassezia furfur, with several independent studies confirming that this concentration inhibits its growth and could serve as an alternative topical treatment (Abd Rashed et al., 2021; Bismarck et al., 2020; Sahal et al., 2020).

Zeng et al. (2015) evaluated the fennel seed essential oil against T. rubrum, T. tonsurans, M. gypseum, and T. mentagrophytes for parameters such as mycelial growth, spore germination, MIC, minimum fungicidal concentration, and biomass. The oil was found to be more effective than the standard antifungals amphotericin B and fluconazoleUddandapu et al. (2016) screened twenty South Indian medicinal plant extracts against T. mentagrophytes, Epidermophyton floccosum and Candida albicans by agar well diffusion method and found broad spectrum of antifungal activity in the extracts.

The antifungal mechanism of citral, the main active ingredient in lemongrass, is thought to be through the alteration of genes related to sporulation and growth that are involved in ergosterol biosynthesis, thereby weakening the fungal cell membrane (Tang et al., 2018; Zheng et al., 2021). Due to the growing concern about antifungal resistance, plant-derived antifungals are increasingly being considered as a means of limiting the spread of resistant fungal strains (Herman et al., 2022; Walusansa et al., 2023).

3-3. Impact of antifungal agents clotrimazole and terbinafine on the development of T. rubrum assessed by disc diffusion methodology

The results of this experiment are shown in Figure (3) and Table (3). Inhibition of T. rubrum with Terbinafine was concentration dependent and the maximum inhibition zone was obtained for the stock solution (S) (39.1 mm), and the minimum inhibition zone was obtained for the S/4 dilution of clotrimazole (10.5 mm).

Terbinafine was more effective than clotrimazole against T. rubrum with inhibition zones of 39.1, 20.9 and 10.8 mm at S, S/2 and S/4 concentrations, respectively.

The mean zone of inhibition was 23.6 mm for terbinafine and 23.13 mm for clotrimazole in all concentrations, which shows that terbinafine was more potent overall.

Table (3) Efficacy of antifungal agents against T. rubrum by disc diffusion methodology

Drug	Concentration (%)	Inhibition zone (mm)	Mean (mm)
Clotrimazole	Stock (S)	38.5	23.13
	S/2	20.6	
	S/4	10.5	
Terbinafine	Stock (S)	39.1	23.6
	S/2	20.9	
	S/4	10.8	
Control		0	
LSD (0.05)		0.230	

Each value is a mean of three replicates

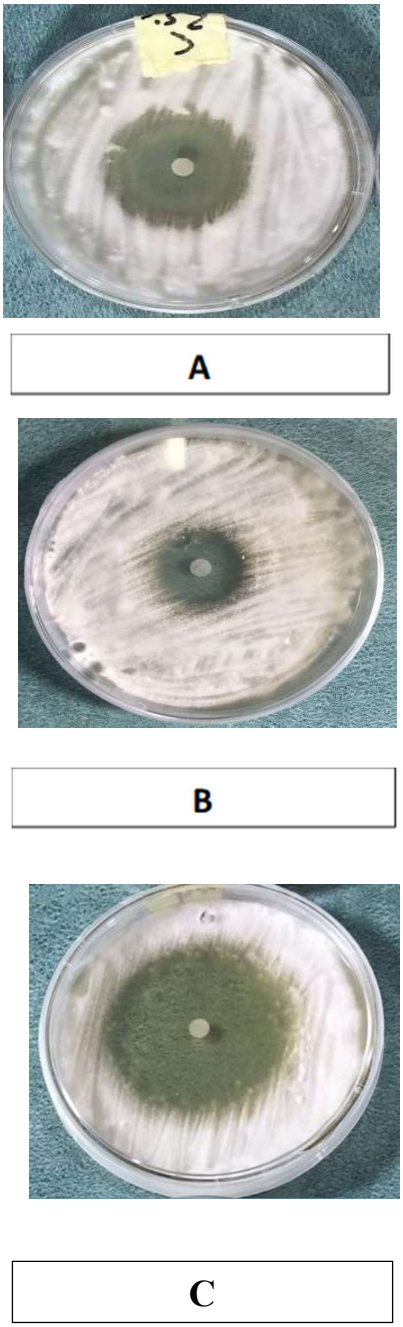

Figure (3) Effect of terbinafine on T. rubrum by disc diffusion method
(a- Stock (S), b- S/2, c- S/4)

Terbinafine (TRB) is considered as a first-line agent against Trichophyton infections due to its fungicidal effect. It inhibits squalene epoxidase (SQLE), an important enzyme in the biosynthesis of fungal ergosterol. Inhibition of ergosterol biosynthesis leads to destabilisation of the fungal membrane leading to cell lysis (Shen et al., 2022). Keyvane et al. (2009) tested six antifungal agents on clinically important dermatophytes and found terbinafine and clotrimazole to be the most effective agents and fluconazole to have the least activity.

Diogo et al. (2010) tested the effect of terbinafine at different concentrations using the disc diffusion method against T. mentagrophytes and obtained an inhibition zone of 40 mm. Zarei et al. (2015) also reported that terbinafine (Lamisil) showed higher activity against A. niger, A. flavus and A. terreus than clotrimazole with inhibition zones 18–43 mm. Rizwan et al (2025) compared topical clotrimazole and terbinafine in the treatment of fungal otitis externa, and concluded that terbinafine had significantly better mycological and clinical outcomes and a better safety profile.

According to Mayank (2020), terbinafine has become the drug of choice due to its pharmacologic profile, favourable microbiologic activity, and effective mechanism of action. Terbinafine can be given both topically and systemically depending on the clinical indication. The fungicidal effect is due to the inhibition of squalene epoxidase, an enzyme essential to the integrity of the fungal membrane leading to the breakdown of fungal cells. The same source also states that the combination of terbinafine with other antifungals may increase its therapeutic value and help to limit the development of resistance. A 4-week course of terbinafine was found to be equivalent to an 8-week course of griseofulvin in comparative clinical trials with clinical cure rates of 64–93% and mycological cure rates of 70–93% for terbinafine and 67–80% and 72–88% for griseofulvin respectively (Fuller et al., 2001).

3-4. Impact of essential oil in conjunction with antifungal agents on the development of T. rubrum assessed by disc diffusion methodology

Combining essential oil (concentration S) with terbinafine (concentration S/2) produced greater anti-Trichophyton activity than either agent alone: the combined treatment yielded an inhibition zone of 24.6

mm, compared with 20.9 mm for terbinafine (S/2) alone and 20.5 mm for the essential oil (S) alone.

Table (4) Efficacy of Combined Treatments Against T. rubrum by Disc Diffusion Method

Treatment	Inhibition zone (mm)
Terbinafine (S/2) + essential oil (S)	24.6
Terbinafine (S/2) alone	20.9
Essential oil (S) alone	20.5
Essential oil (S/2) alone	15.5
Essential oil (S/4) alone	7.6
Control	0
LSD (0.05)	0.223

Each value is a mean of three replicates

Since the calculated χ^2 value (1.96) was lower than the tabulated critical value (3.84), the interaction between terbinafine and lemongrass essential oil was classified as additive (Table 5).

Table (5) Chi-squared (χ2) test for the type of interaction between terbinafine and essential oil against T. rubrum

Essential oil (S) alone	Terbinafine (S/2) alone	Expected	Observed (combination)	Calculated χ2	Type of interaction
20.5	20.9	32.6	24.6	1.96	Additive

Each result is the average of three replicates.

The higher activity of the combined treatment compared to the individual agents may be due to the disruptive action of the essential oil on the fungal cell membrane, which probably allows greater penetration of terbinafine into the fungal cells. Thus, the ability of lemongrass to damage membranes and organelles seems to complement the drug mechanism, resulting in a greater overall effect than when each component is used alone (Dias et al. 2017). This is consistent with the findings of Khan and Ahmad (2013) who also found that the combination of lemongrass essential oil and fluconazole was more effective than either treatment alone.

The use of essential oils with traditional antibacterial agents has been documented as a method to attain optimal therapeutic effectiveness via additive or synergistic interactions (Wagner and Ulrich-Merzenich, 2009).

Trifan et al. (2021) shown that the combination of terbinafine and essential oils is a potential treatment strategy for dermatophytosis caused by T. rubrum and T. mentagrophytes.

Orchard et al. (2019) similarly reported that fungal pathogens such as T. mentagrophytes were highly susceptible to combined essential-oil formulations, which also appeared to limit the emergence of resistant strains.

Noor (2016) examined the interaction between a methanolic lemongrass extract and several antibiotics against clinical bacterial isolates and reported only synergistic or additive effects, with no antagonism observed, concluding that lemongrass extract could be useful for treating bacterial infections either alone or combined with antibiotics.

In a comparable example, Pillai et al. (2013) reported a synergistic interaction between ampicillin and fresh garlic extract against various S. aureus isolates.

Hoque et al. (2024) found that combining terbinafine with itraconazole achieved a higher clinical cure rate than either systemic antifungal used alone.

A wide range of essential oils has been screened for antimycotic activity in vivo and in vitro, with several shown to be effective antifungal candidates. Their action is thought to target primarily the fungal cell membrane — causing structural disruption, leakage, and cell death — while also interfering with membrane synthesis, spore germination, fungal proliferation, and cellular respiration (Harris, 2002).

Irfan et al. (2023) shown that clove and eucalyptus oils increased the antifungal effectiveness of ketoconazole against Candida albicans in comparison to ketoconazole used in isolation. Arun et al. (2024) demonstrated that Eucalyptus globulus oil enhanced the antifungal effectiveness of an azole medication against Aspergillus and Mucor species.

4. Conclusion

In conclusion, lemongrass essential oil suppressed the proliferation of T. rubrum and
terbinafine was better than clotrimazole on this fungus. In combination, lemongrass essential oil and terbinafine had an additive antifungal effect on T. rubrum allows for a reduced effective concentration of the medicine compared to each agent individually.

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