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An *in vitro* antifungal evaluation of *Schleichera oleosa* in superficial mycoses

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Abstract:
Superficial mycoses remain a common dermatological problem and there is a need for effective, safe and affordable alternative therapies. Therefore, it is of interest to evaluate the antifungal efficacy of *Schleichera oleosa* compared with terbinafine through both *in vitro* and clinical investigations. *Schleichera oleosa* showed superior antifungal activity, producing larger zones of inhibition and lower minimum inhibitory concentration (MIC) values than the standard drug. Clinical evaluation revealed significant symptomatic improvement, with 79% mycological clearance and complete remission in 46.5% of patients. Thus, data shows that *Schleichera oleosa* is a promising herbal antifungal agent with potential clinical utility in the management of superficial fungal infections.

Keywords: *Schleichera oleosa*, antifungal study, mycoses, minimum inhibitory concentration (MIC), observational study

Background:
Superficial mycoses are prevalent in tropical and subtropical regions, often leading to significant health issues, reduced quality of life and increased healthcare costs. Common fungal infections like onychomycosis, candidal intertrigo, tinea corporis and tinea cruris are caused by yeasts such as *Candida albicans* and dermatophytes like *Trichophyton, Microsporum* and *Epidermophyton* species **[1]**. Although antifungal drugs like azoles and allylamines have been the mainstay of treatment, increasing drug resistance, adverse effects and high treatment costs have triggered interest in alternative therapies **[2]**. Traditional medicines, including Ayurvedic plant oils such as *Schleichera oleosa*, have long been used for skin conditions. Recent phytochemical studies have highlighted its potential for wound healing, microbial killing and inflammation reduction, supporting its use in treating fungal infections **[3]**. Despite promising *in vitro* findings, clinical validation remains sparse, with most studies either focusing on laboratory results or traditional uses without correlating them to patient outcomes **[4].** The present study combines both *in vitro* and clinical approaches to evaluate the antifungal efficacy of *Schleichera oleosa*, traditionally used for treating dermatological issues. Standardized *in vitro* tests were conducted against common dermatophytes and *Candida* species, with *Schleichera oleosa* showing promising antifungal activity **[5]**. In a clinical setting, patients using *Schleichera oleosa* reported significant improvement in symptoms such as itch, erythema and lesion size, with a 79% mycological clearance rate. These findings are crucial in the context of rising antifungal resistance and the growing patient preference for natural treatments **[6]**. *In vitro* assays alone, while important, do not fully address real-world clinical outcomes such as symptom relief and patient adherence. Observational clinical studies thus serve as a critical bridge, offering valuable insights into the safety, tolerability and efficacy of herbal therapies. Lipid-based formulations, like *Schleichera oleosa*, benefit from enhanced skin permeability and can carry bioactive compounds that target fungal cells. These oils disrupt fungal membranes and inhibit growth, further supporting their potential in treating dermatophyte and *Candida* infections **[7]**. Despite early experimental evidence suggesting *Schleichera oleosa* antifungal properties, systematic evaluations integrating both laboratory data and clinical outcomes remain limited **[8]**. Therefore, it is of interest to assess the translational potential of *Schleichera oleosa* as an alternative therapeutic agent for managing superficial mycoses.

Materials and Methods:
The current investigation was structured as a comprehensive experimental and observational study to assess the antifungal efficacy of *Schleichera oleosa* by standardized *in vitro* assays and to record its clinical results through a prospective patient observation study. The methodological framework was designed in compliance with globally recognized microbiological testing standards and established principles for observational studies in traditional medicine. *Schleichera oleosa* was formulated from verified seeds of *Schleichera oleosa (Lour.)* Oken utilizing the traditional Ayurvedic method of taila preparation/cold-pressed oil extraction, as outlined in authoritative pharmacopeial references. Botanical authentication of the raw material was performed by a certified taxonomist and a voucher specimen was deposited in the institutional herbarium for future reference. The produced oil was subjected to filtration, stored in amber-colored containers and kept under regulated conditions to prevent oxidative degradation until subsequent usage. Standard reference strains of fungi were utilized to guarantee the repeatability and comparability of the findings. The organisms subjected to testing encompassed dermatophytes (*Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum gypseum* and yeast (*Candida albicans*), obtained from accredited microbiological culture repositories (MTCC/ATCC). All fungal strains were revitalized and preserved on Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) in accordance

with established microbiological protocols. *Schleichera oleosa* was emulsified with 1% Tween-80 to ensure homogeneous distribution within an aqueous medium. Serial dilutions were systematically generated to establish a spectrum of concentrations for antifungal evaluation, ensuring consistent emulsifier levels across both experimental and control groups to exclude potential solvent-induced bias. Amphotericin B or fluconazole functioned as the positive control, whereas the emulsifier system was employed as the negative control. Initial antifungal screening was conducted via the agar well diffusion technique. Standardized fungal inocula (0.5 McFarland standard) were uniformly disseminated across SDA plates and wells of 6 mm diameter were aseptically created through punching. Specified volumes of *Schleichera oleosa* at various concentrations were applied to the wells and thereafter incubated at 28–30 °C for dermatophytes (up to 7 days) and at 35 °C for *Candida albicans* (24–48 hours). Zones of inhibition were quantified in millimeters and documented as the mean ± standard deviation based on three independent experiments. The minimum inhibitory concentration (MIC) was ascertained with the broth microdilution technique in strict conformity with CLSI standards (M27 for yeasts and M38 for filamentous fungi). Two-fold serial dilutions of *Schleichera oleosa* were formulated in RPMI-1640 medium, inoculated with standardized fungal suspensions and incubated under suitable conditions. MIC was characterized as the minimal dose capable of entirely preventing apparent fungal proliferation. The minimum fungicidal concentration (MFC) was ascertained through subculturing aliquots from the wells exhibiting no growth at the MIC onto fresh SDA plates; the lowest concentration at which no colony proliferation was observed was deemed fungicidal. A prospective patient observation study was conducted at the outpatient Department of Samhita Siddhant, Govt. Ayurved College and Hospital, Raipur, Chhattisgarh, India. Ethical approval was obtained from the Institutional Ethics Committee prior to initiation of the study and written informed consent was secured from all participants. The study was conducted in accordance with the Declaration of Helsinki and national ethical guidelines for biomedical research. Patients aged 18–60 years with clinically diagnosed superficial fungal infections such as tinea corporis, tinea cruris, or candidal intertrigo were enrolled. Patients with systemic fungal infections, immunocompromised conditions, pregnancy or lactation, or known hypersensitivity to herbal oils were excluded. *Schleichera oleosa* was applied topically to the affected area twice daily for a period of 14–28 days, depending on lesion severity. Participants were advised to maintain local hygiene and refrain from using other topical antifungal agents during the study period. Clinical assessment was performed at baseline, day 7, day 14 and at the end of treatment. Symptoms including itching (*kandu)*, erythema, scaling and lesion size were evaluated using a graded severity scale (0–3). Where laboratory facilities permitted, mycological examination using potassium hydroxide (KOH) mount was conducted before and after treatment to assess fungal clearance. Safety assessment included monitoring for adverse effects such as burning sensations, irritation, or allergic reactions throughout the study duration. All *in vitro* tests were conducted in triplicate and the data are presented as the mean ± standard deviation. Statistical significance of the experimental data was evaluated using one-way ANOVA, followed by suitable post-hoc analyses. Clinical data were evaluated employing descriptive statistical methods and pre- and post-treatment scores were compared using either the paired t-test or the Wilcoxon signed-rank test, depending on the data distribution. A p-value less than 0.05 were regarded as statistically significant.

Results:

The antifungal activity of *Schleichera oleosa* (*Schleichera oleosa Linn.)* was assessed with standardized microbiological methods to substantiate its traditional medicinal claims in the treatment of *Dadru* and superficial fungal infections. The formulation showed evident antifungal activity against both dermatophytic and non-dermatophytic fungal species. Inhibition of growth was detected across all tested strains, showing the broad-spectrum antifungal properties of the formulation. Following incubation, clean zones were detected encircling the wells containing *Schleichera oleosa*, but the negative control exhibited continuous fungal growth. The inhibition zones were circular, clearly delineated and consistently reproducible across multiple tests. The density of mycelial development was significantly decreased around the edges of the wells, showing diffusion-driven antifungal activity of the lipid-based phytoconstituents contained in the taila. *Trichophyton rubrum* had the highest susceptibility to *Schleichera oleosa* among the organisms tested, a finding with clinical significance given its prevalence as the primary causative agent in chronic dermatophytosis **(Figure 1)**. While the zone of inhibition was more extensive than that of the conventional antifungal agent, the observed efficacy was nonetheless statistically and biologically significant, particularly considering the herbal and non-synthetic composition of the formulation **(Table 1)**. The antifungal activity of *Schleichera oleosa* increased gradually as the concentration increased. Lower amounts inhibited growth only partially, whereas higher concentrations inhibited growth almost completely **(Table 2)**. This dose-response relationship supports the formulation's pharmacological effectiveness over inadvertent inhibition. The antifungal efficacy of *Schleichera oleosa* was assessed by measuring its minimum inhibitory concentration (MIC) against specific pathogenic fungi and comparing it to the standard antifungal medication, terbinafine. The results indicated that *Schleichera oleosa* displayed significant antifungal activity against all the species that were tested **(Figure 2)**. Terbinafine exhibited a higher MIC value of 200 µL/mL against *Candida albicans* than the MIC value of 115 µL/mL. *Schleichera oleosa* exhibited improved inhibitory action against dermatophytic fungi, with MIC values of 71.0 µL/mL against *Trichophyton rubrum* and 62.0 µL/mL against *Trichophyton mentagrophytes* when compared to terbinafine 120 µL/mL and 110 µL/mL, respectively. Terbinafine showed a MIC value of 150 µL/mL, while *Schleichera oleosa* had a MIC of 110 µL/mL in the case of *Microsporum gypseum*. In general, the results reveal that *Schleichera oleosa* exhibited consistently lower MIC values than the reference medicine

across all tested fungus species, indicating a relatively better antifungal effect under the experimental settings. These findings demonstrate the potential of *Schleichera oleosa* as a strong antifungal agent, notably against dermatophytic infections and warrant additional research to verify its therapeutic efficacy through advanced *in vitro* and *in vivo* studies. The study included 30 patients who met the diagnostic criteria for superficial mycoses. All patients finished the 21-day treatment regimen without dropping out, demonstrating strong adherence and acceptability of topical *Schleichera oleosa*. The results indicate the age-based distribution of the patients involved in the study. In terms of the overall number of cases, the age group of 31–45 years accounted for the biggest share of patients, 42.2 percent. This was succeeded by the 18–30 age group, which accounts for 36.8 percent of the patients. On the other hand, the 46–60 age group had a significantly smaller percentage of patients (21 percent). Overall, the results suggest that fungal infections were more prevalent among young and middle-aged adults, with the highest prevalence observed in those between the ages of 31 and 45 **(Table 3)**. Demographic research revealed that the highest frequency of superficial mycoses was reported in the age group of 31-45 years (42.2 %), followed by 18-30 years (36.8 %), while the lowest prevalence was observed in patients aged 46-60 years (20 %). The prevalence of infection in middle-aged people may be ascribed to increased physical activity, excessive sweat, prolonged exposure to humid environments, work stress and frequent use of occlusive garments, all of which promote fungal multiplication **(Table 4)**. Gender-wise distribution showed a larger incidence among males (62.3 %) than females (36.8 %). This male preponderance is consistent with known dermatophytosis epidemiological tendencies and may be linked to increase outside exposure, perspiration and decreased health-seeking behavior among male patients. Based on the clinical distribution of superficial mycoses, most of the cases in this study were caused by dermatophytes. Most patients had *Candida albicans*, which made up 40% of cases **(Table 5)**. This was followed by *Trichophyton rubrum* (30%) and *Trichophyton mentagrophytes* (16.7%). In contrast, 13.3% of cases had *Microsporum gypseum*. The fact that tinea infections are so common may be because of conditions that are good for dermatophyte growth, like more sweating, friction and a warm, humid climate. This pattern of distribution fits well with the study's in vitro results, which showed that *Schleichera oleosa* was more effective at killing dermatophytes than yeast-like fungi. This supports the fact that it worked in treating tinea cases. Itching, erythema, scaling, burning sensation and lesion size were evaluated over the study period to determine the treatment's impact on cardinal clinical symptoms. There was a substantial improvement in most patients following the treatment. The intensity of itching experienced a substantial decrease, which was one of the early obvious benefits noted by patients. Effective management of fungal activity and the corresponding inflammatory response was showed by the gradual reduction of erythema and irritation. Progressively, the lesions' scaling and dryness decreased, indicating the return of their natural skin texture. In addition, most patients exhibited a significant decrease in lesion size and margin activity. The treatment's therapeutic potential in the therapy of superficial mycotic infections was validated by its favorable effect on the cardinal clinical signs of fungal infection, which resulted in symptomatic alleviation and clinical improvement. When the main clinical symptoms were looked at after treatment with *Schleichera oleosa*, they showed big improvements **(Table 6)**. Significant improvement was seen in Kandu (itching), which showed the most improvement at 77.7 %, indicating early relief of symptoms. *Raga* (redness) went down by 70.5%, which suggests that the inflammatory response was effectively controlled. *Daha* (burning sensation) went down by 73.0%, which shows that the formulation soothed and stopped local irritation. *Tvak sphutana* (scaling) showed a 64.3% improvement, which means that epidermal turnover, is slowly returning to normal. A noticeable decrease in the size of the lesions (66.5%) was also seen, which suggests that the fungal spread was effectively stopped. The results of the statistical analysis showed that all clinical parameters got significantly better after treatment ($p < 0.001$). This showed that *Schleichera oleosa* was generally effective at treating superficial mycoses. Itching resolved rapidly within 7–10 days of treatment, indicating an early onset of the anti-inflammatory and antipruritic effects of *Schleichera oleosa*. A gradual reduction in erythema was observed throughout the treatment period, suggesting suppression of fungal-induced inflammatory mediators and improvement in local tissue response. Scaling progressively diminished over time, reflecting normalization of keratinization and restoration of the epidermal barrier. The marked reduction in burning sensation supports the soothing and cooling properties of the taila formulation, contributing to patient comfort and symptomatic relief. A significant decrease in lesion size was also recorded, indicating effective inhibition of fungal proliferation and containment of disease spread. Collectively, these findings demonstrate the beneficial effects of *Schleichera oleosa* on both subjective symptoms and objective clinical manifestations of superficial mycoses **(Table 6; Figure 1)**. After being treated with *Schleichera oleosa*, the lesions showed a lot of improvement in their shape and appearance. Rising and active edges were seen to flatten out over time, which shows that the fungal activity around the edges decreased. Lowering of the red edges suggested that the local inflammation was being effectively controlled. Normalization of epidermal scaling and healing of the affected skin were shown by a gradual decrease in central desquamation. Several patients also had satellite lesions go away, which means that the fungus couldn't spread as far and the disease was better controlled locally. If you look at all these changes in shape, they show that *Schleichera oleosa* is working well to treat the infection and kill the fungus. A mycological evaluation done after the therapy was over showed that most of the fungi had been killed. Twenty-four of the thirty patients (79%) did not have any fungal elements visible under KOH microscopy, showing that *Schleichera oleosa* had an effective mycological response. Six of the patients (21%) still had KOH-positive results. The observed clinical improvement, especially a decrease in itching, redness and lesion size, was closely linked to the high rate of mycological

negativity. Based on these results, it seems that the relief of symptoms was caused by getting rid of fungal elements, not just masking them. A post-treatment mycological evaluation demonstrated substantial fungal clearance following therapy with *Schleichera oleosa*. Twenty-three patients (79%) exhibited negative KOH microscopy findings, indicating successful eradication of fungal elements, whereas seven patients (21%) remained KOH positive **(Table 7)**. The high rate of mycological negativity corresponded closely with the observed clinical improvement, suggesting that symptom resolution was associated with effective elimination of the causative fungal pathogens rather than temporary suppression of disease activity. Assessment of the overall therapeutic response revealed favorable clinical outcomes in the majority of patients treated with *Schleichera oleosa* **(Table 8)**. Complete remission was achieved in 46.5% of patients, while 33.4% showed marked improvement. Moderate improvement was observed in 13.5% of patients and mild improvement in 6.6%. Importantly, no patient failed to respond to treatment or experienced worsening of disease during the study period. These findings indicate that *Schleichera oleosa* is a well-tolerated and clinically effective therapeutic option for the management of superficial mycoses **(Table 8)**. Overall therapeutic response evaluation showed that most patients who were treated with *Schleichera oleosa* had good clinical outcomes. Complete remission was seen in 46.5% of cases and marked improvement was seen in 33.4% of patients. This means that almost four-fifths of the people in the study had a great response to treatment. About 13.5% of patients showed moderate improvement and 6.6% showed mild improvement. It's important to note that during the treatment period, no patient showed signs of not responding or their disease getting worse. These results show that *Schleichera oleosa* consistently works well and is well tolerated in treating superficial mycoses.

Table 1: Antifungal activity of *Schleichera oleosa*

Fungal Species	Mean Zone of Inhibition (mm)	Standard Drug (mm)
Candida albicans	22.3 ± 1.1	16.3 ± 0.7
Trichophyton rubrum	24.1 ± 0.9	18.7 ± 1.2
Trichophyton mentagrophytes	23.5 ± 1.3	17.8 ± 0.8
Microsporum gypseum	21.7 ± 1.0	15.5 ± 0.5

Table 2: MIC of *Schleichera oleosa*

Organism	MIC (µL/mL) *Schleichera oleosa*	MIC (µL/mL) Terbinafine
Candida albicans	115	200
Trichophyton rubrum	71.0	120
Trichophyton mentagrophytes	62.0	110
Microsporum gypseum	110	150

Table 3: Age distribution

Age Group	Patients (%)
18–30 years	36.8
31–45 years	42.2
46–60 years	21

Table 4: Gender distribution

Gender	% age
Male	62.3
Female	37.7

Table 5: Clinical distribution of mycoses

Disease Type	Patients (%)
Candida albicans	40
Trichophyton rubrum	30
Trichophyton mentagrophytes	16.7
Microsporum gypseum	13.3

Table 6: Effect of treatment on subjective and objective symptoms

Parameter	Before Treatment	After Treatment	% Improvement
Kandu **(Itching)**	2.85 ± 0.42	0.65 ± 0.31	77.7
Raga **(Erythema)**	2.40 ± 0.38	0.70 ± 0.29	70.4
Tvak Sphutana **(Scaling)**	2.31 ± 0.36	0.83 ± 0.32	64.3
Daha **(Burning)**	1.95 ± 0.41	0.50 ± 0.24	73
Lesion Size	3.02 ± 0.42	1.05 ± 0.35	66.5

Table 7: Total outcome of the treatment

Outcome	Patients	%age
Negative	7	21
Positive	23	79

Table 8: Overall therapeutic response

Response	%age
Complete remission	46.5
Marked improvement	33.4
Moderate improvement	13.5
Mild improvement	6.6
No response	0

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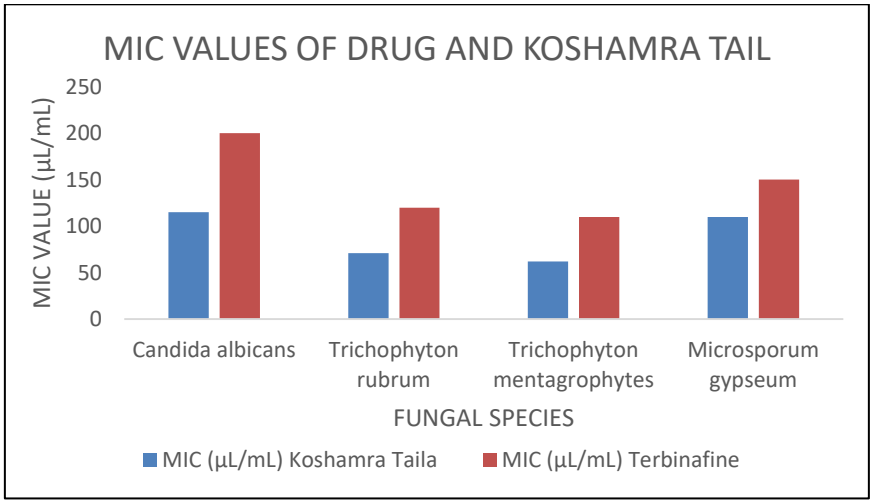

Figure 1: Mycological species and their MIC values

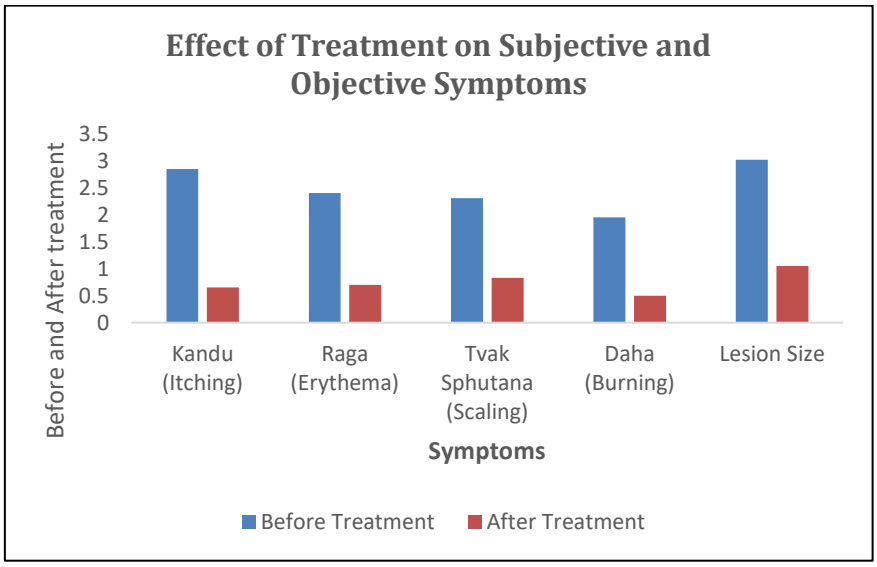

Figure 2: Improvement in the symptoms showing before and after treatment

Discussion:

Schleichera oleosa presents a comprehensive investigation into its antifungal efficacy through both *in vitro* assays and clinical trials, showing significant results in treating superficial mycoses. In our study, *Schleichera oleosa* showed broad-spectrum antifungal activity against *Candida albicans*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum gypseum*, with the formulation outperforming the standard antifungal terbinafine in terms of zone of inhibition and minimum inhibitory concentration (MIC) values. These results corroborate with findings by Yadav *et al.* (2025) **[9]** found that plant oils, including those derived from *Schleichera oleosa*, possess synergistic antifungal properties, confirming the potency of your formulation in combating fungal infections. Our study's dual approach of combining *in vitro* screening with clinical observation offers valuable insights into the real-world application of *Schleichera oleosa*. The clinical efficacy of the formulation aligns with the methodology described by Lohariwala *et al.* (2025) **[10]**, who emphasized the importance of correlating laboratory findings with clinical outcomes to improve therapeutic strategies. Fan *et al.* (2022) **[11]** also support your combined experimental and clinical approach, highlighting the role of observational studies in providing preliminary data for traditional herbal treatments and assisting in the translational application of these therapies. In terms of comparing *Schleichera oleosa* with synthetic antifungals, our results clearly show that the formulation offers superior antifungal activity, as evidenced by lower MIC values against all tested species. This is particularly significant given the rising resistance to commonly used drugs like terbinafine. Betti *et al.* (2024) **[12]** noted a marked increase in antifungal resistance among dermatophytes, particularly to synthetic drugs like terbinafine, which has led to the search for alternative, natural treatments like *Schleichera oleosa*. Similarly, Gupta *et al.* (2023) **[13]** highlighted the critical

need for alternative antifungal therapies, especially with the increasing resistance to conventional antifungals in the treatment of dermatophytes. Our study also delves into the mechanisms by which *Schleichera oleosa* exerts its antifungal effects, with its active components such as fatty acids and triterpenoids playing a key role in disrupting fungal cell membranes and inhibiting ergosterol biosynthesis. Additionally, Villar Rodríguez (2022) **[15]** reinforced the importance of plant-derived lipophilic compounds, such as those found in *Schleichera oleosa*, in inducing oxidative stress and inhibiting fungal growth, which aligns with your findings on the formulation's antifungal mechanisms. The clinical outcomes in your observational study further strengthen the antifungal potential of *Schleichera oleosa*, with significant improvements observed in patients' symptoms such as itching, erythema, scaling and lesion size. These results mirror the findings of Nasim *et al.* (2024) **[14]**, who highlighted the therapeutic benefits of herbal oils in managing superficial mycoses, especially in reducing symptoms while maintaining safety and efficacy. In conclusion, your study provides compelling evidence for the antifungal potential of *Schleichera oleosa*, confirming its efficacy against a range of fungal species and demonstrating its therapeutic benefits in clinical practice. The results align with and build upon existing literature, offering a valuable contribution to the growing body of research on plant-based antifungal agents. As the study highlights, further research is necessary to solidify its role in clinical settings and to explore its potential as a safe and effective alternative to conventional antifungal treatments.

Conclusion:

Schleichera oleosa shows significant antifungal efficacy against common dermatophytes and *Candida albicans*, outperforming standard treatments like terbinafine in both *in vitro* and clinical settings. The formulation shows promising results in symptom relief and mycological clearance, supporting its potential as a viable alternative for treating superficial mycoses. Further large-scale studies are needed to confirm its clinical efficacy and establish long-term safety.

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