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ORIGINAL RESEARCH ARTICLE

In-Vitro Antifungal Efficacy of Salicylic Acid and Palm Oil on the Dermatophytic Fungus Causing Athlete's Foot Disease

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ABSTRACT
Athlete's foot, or *Tinea pedis*, is a widespread fungal infection primarily affecting the skin between the toes, caused by dermatophytes like *Trichophyton rubrum* and *T. mentagrophytes*. This study aimed to evaluate the in-vitro effects of salicylic acid and palm oil on dermatophytic fungus responsible for Athlete's foot disease. *Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined for salicylic acid and palm oil.* The agar well diffusion method was used to measure the zone of inhibition at various concentrations (25%, 50%, 75%, and 100%) of both substances. The MIC was assessed using Sabouraud Dextrose Broth at concentrations of 3.12%, 6.25%, and 12.5%. Results indicated significant inhibitory effects of both salicylic acid and palm oil against the dermatophytes causing athlete's foot. The highest zone of inhibition was observed at 100% concentration for both substances: 5.50 mm for salicylic acid and 4.3 mm for palm oil. At 50% and 75% concentrations, the inhibition zones were 3.67 mm and 3.97 mm for salicylic acid and 2.20 mm and 3.00 mm for palm oil, respectively. The MIC for both substances was determined to be 3.12%, while the MFC was 3.12% for salicylic acid and 6.25% for palm oil, indicating stronger fungicidal properties for salicylic acid. *Salicylic acid and palm oil show promise as agents against Tinea pedis.* Further public health efforts are recommended to raise awareness about the prevention and transmission of the disease.

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INTRODUCTION

Athlete's foot (Tinea pedis) is a common skin fungal infection, mainly caused by *Trichophyton rubrum* and *T. interdigitale* (Leung *et al.,* 2023; Aliuddin *et al.,* 2023). It affects warm and humid areas, such as the interdigital spaces of the feet, leading to symptoms like itching, redness, and peeling skin (Maharaj, 2006). Athlete's foot is prevalent in athletes, military personnel, and adults, with a global estimated prevalence of around 3% (Shalal *et al.,* 2022). The condition is not only uncomfortable but also carries social stigma, which underscores the need for effective management. Factors like wearing non-breathable footwear and immunosuppression increase susceptibility to this infection. If left untreated, an athlete's foot can persist, progress, and recur, impacting the quality of life due to unpleasant symptoms and social stigma. Proper diagnosis through skin scrapings and appropriate antifungal treatment, either topical or oral, is crucial for effective management and cure.

Athlete's foot, caused by dermatophytes like *Trichophyton rubrum*, presents with symptoms such as itching, burning, scaling, and redness (López-López *et al.,* 2021; Liberman and Lefkovits, 2007). It affects a significant portion of the population, impacting the quality of life by causing pain, discomfort in walking, limitations in daily activities, and embarrassment (Katsambas *et al.,* 2005; Harmon *et al.,* 2023). Studies show that foot diseases, including athlete's foot, have a substantial negative influence on individuals' well-being, with a higher impact on women and the elderly (Agrawal *et al.,* 2020). Additionally, foot symptoms, including those related to athlete's foot, have been linked to an increased risk of mortality in older adults, emphasizing the importance of managing foot conditions to improve overall health outcomes. Therefore, recognizing and addressing an athlete's foot symptoms promptly is crucial to alleviate discomfort and enhance quality of life.

The increasing resistance to conventional antifungal therapies and the recurrence of the infection highlight the necessity for alternative treatments. However, current treatments for athlete's foot disease include oral medications prescribed for systemic infections and topical medications like creams, gels, powders, and sprays for milder cases (Kota *et al.,* 2022). Oral medications may lead to side effects ranging from mild to severe, such as nausea, dizziness, and liver damage, making them less preferred despite their effectiveness for chronic infections

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(Olkhovskaya and Perlamutrov, 2008). Topical therapies, though generally safer, can also cause skin irritation, prompting the need for complex formulations, including glucocorticosteroids (Fatakhov *et al*., 2020). The use of nanocarriers with antifungal agents shows promise in enhancing therapeutic efficacy and reducing toxicity in topical treatments. However, challenges such as drug resistance, recurrence, and adverse effects persist, necessitating the exploration of alternative therapies.

Salicylic acid has been selected for this study due to its well-documented antifungal properties. Studies have demonstrated that salicylic acid can inhibit the growth of various fungal pathogens, including Ganoderma spp., in oil palm seedlings (Saputra *et al*., 2022). Additionally, salicylic acid is known to induce resistance against postharvest diseases in fruits by enhancing antioxidant enzymes and reducing disease development, as seen in the case of *Penicillium digitatum* affecting orange fruits (Wang *et al*., 2023). Its efficacy in treating dermatophytosis in humans has also been established, with a 30% salicylic acid peel achieving clinical and microbiological cures in a majority of patients (Mosad *et al*., 2022). This evidence supports the potential of salicylic acid as a promising agent in the treatment of fungal infections, including athlete's foot.

Palm oil has been selected for its unique composition of vitamin E, carotenoids, and phytosterols, which possess medicinal properties and act as antioxidants. Palm oil nanoemulsions have been developed to enhance the delivery of tocotrienols, compounds with potent anti-proliferative and pro-apoptotic actions against cancer cells (Maheshwari *et al*., 2023). Additionally, substances like Substance P have shown efficacy in reducing disease severity when administered topically, suggesting the potential of active agents in palm oil for treating skin diseases (Pham *et al*., 2016). While preliminary evidence suggests that palm oil may have antifungal properties, there is limited systematic research examining its specific effects on the athlete's foot pathogen. Therefore, this study aims to evaluate the in-vitro effects of salicylic acid and palm oil on the growth and survival of Tinea pedis to explore their potential as alternative treatments for athlete's foot. This research highlights the antifungal characteristics of salicylic acid and palm oil against Tinea pedis. It also proposes alternative and organic remedies for athlete's foot disease, which have the potential to be advanced into efficacious topical preparations. The research will also contribute to the current pool of information by underlining the feasibility of employing natural substances in dermatological therapies, thus facilitating the emergence of more environmentally sustainable and less adverse reaction-inducing treatments.

MATERIALS AND METHODS

Collection and Sub-culturing of Sample

A fungal isolate of *T. pedis* was obtained in its pure form from the Biology laboratory of the Department of Biological Science, Federal University Dutse, Nigeria. Sub-culturing was performed under aseptic conditions, adopting the protocol used by Zhan *et al.* (2010). The sample was then inoculated onto autoclaved and solidified Sabouraud Dextrose Agar (SDA) amended with Chloramphenicol to inhibit bacterial growth and incubated at room temperature from 3-5 days for fungal growth (Durdu *et al.,* 2011).

Purification and Identification

Representative colony types were purified by subculturing on fresh SDA plates. Pure cultures were transferred to slants of SDA and then incubated for about 3-5 days at room temperature. Pure cultures of the isolates were grown singly on SDA for identification. The isolated fungus was identified based on the isolates' colonial characteristics on culture plates and microscopic features in the slide (Ates et al., 2008). Briefly, using a sterile inoculating needle, a portion of each mycelia colony was aseptically taken and placed on a clean microscope slide and teased in a drop of lacto phenol cotton blue. The samples on the slide were covered with a cover slip, and the morphological features of the fungus under the light microscope were observed (Mailafiya *et al.,* 2017).

Antifungal Susceptibility Testing

A 7g salicylic acid powder was dissolved in 20 mL of ethanol to prepare a stock solution. Subsequent dilutions (25%, 50%, 75%, and 100%) were made using this stock solution. Using a sterile cork borer, a 4 mm hole was made, and it was ensured that the wells were of appropriate size and depth to hold the desired volume of the test solution (Andrews, 2001). The standardized inoculums were spread evenly on the surface of the solidified agar plates using a sterile swab spreader. A 10-micro liter of salicylic acid solution was added to each well-made. An appropriate positive and negative control was included. The agar plate was incubated at an appropriate temperature of 25-30^{0}C for 3-5 days (Rahman *et al.,* 2014). After incubation, the agar plates were observed to check the zone of inhibition around the wells. The diameter of the zone of inhibition was measured using a ruler to determine the minimum inhibitory concentration (MIC) as the lowest concentration of salicylic acid that completely inhibits the growth of the fungi (Wiegand *et al.,* 2008). The same procedures were repeated for the palm oil, whereby after the palm oil was obtained, it was properly sterilized by autoclaving. Then, a stock solution of palm oil was prepared by weighing 30g of palm oil and then diluting it with 70% ethanol (Ghannoum *et al.,* 2009). Then, concentrations of 25, 50, 75, and 100% were used and labeled on the plate.

Determination of Minimum Inhibitory Concentration

To determine the Minimum Inhibitory Concentration (MIC) of salicylic acid and Palm oil, a series of dilutions were prepared starting from 25% salicylic acid concentration to the solutions of 12.5%, 6.25%, and 3.12% concentrations obtained by dissolving in ethanol

(Saputra *et al.,* 2022). Sterile test tubes were prepared with varying concentrations of the test solutions and media, along with positive and negative controls, and then incubated at 25-30°C for 3-5 days (Jayadi, 2022). After the incubation period, a visual inspection for growth or turbidity was conducted to identify the lowest concentration of salicylic acid that showed no visible growth, which was considered the MIC (Saputra *et al.,* 2022). The same procedures were repeated for testing Palm oil to determine its inhibitory effects, following a similar methodology as with salicylic acid (Asoka *et al.,* 2023).

Determination of Minimum Fungicidal Concentration

A salicylic Acid solution was dissolved in ethanol to prepare a stock solution with a concentration of 12.5% and 6.25%, respectively. Then, a clinical isolate of the *Tinea pedis* was obtained and was sub cultured on SDA plates and incubated at 25-30^0 for 3-5 days until sufficient growth was obtained (Silva *et al.,* 2014). The fungal spores were harvested by gently scraping the surface of the agar using a sterile loop and suspending them in a sterile saline solution. Another SDA plate with a suitable volume of SDA medium was prepared according to the manufacturer's instructions. 12.5% and 6.25% concentrations of Salicylic acid were added to the molten SDA medium and mixed very well on each plate. The Salicylic Acid-containing SDA medium was poured into sterile dishes and allowed to solidify (Saputra *et al.,* 2022; Puspita *et al.,* 2023). Using a sterile loop or a sterile cotton swab, the fungal suspension was streaked onto the surface of the SDA plates. The plates were incubated at 25-30 degrees Celcius for 3-5 days to allow fungal growth. After the incubation period, each plate was visually inspected for the presence or absence of fungal growth. So the lowest concentration of Salicylic that completely kills the fungi was selected (Espinel-Ingroff *et al.,* 2013). The same procedures were repeated using palm oil by sterilizing the palm oil using autoclaving (Nweze *et al.,* 2009). And same procedure was also repeated using ketoconazole as the positive control.

Statistical Analysis

Data obtained from the determination of the zone of inhibition were subjected to ANOVA at $P \leq 0.05$ probability level to compare the significance of the means. Where means are significant, Tukey's HSD *post hoc* test was used to separate the means. All statistical analyses were carried out using SPSS version 20.

RESULT

The Morphological characteristics of *Tinea pedis* were identified based on colony appearance, morphology, and cellular characteristics, as summarized in Table 1.

The antifungal activity of salicylic acid and palm oil was assessed by measuring the zone of inhibition, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC). The zone of inhibition (Table 2) was evaluated using the agar well diffusion method across various concentrations (25%, 50%, 75%, and 100%) of both salicylic acid and palm oil. Salicylic acid at 100% concentration exhibited the highest zone of inhibition, with an average diameter of 5.50 mm, indicating significant antifungal activity. Palm oil at 100% also showed considerable inhibition, with a zone diameter of 4.30 mm. Lower concentrations (50% and 75%) of both agents also demonstrated inhibitory effects. Statistical analysis revealed a significant difference in the antifungal efficacy between the various concentrations, with higher concentrations showing more pronounced effects ($P<0.05$). The MIC (Table 3) for both salicylic acid and palm oil was determined to be 3.12%, while the MFC was 3.12% for salicylic acid and 6.25% for palm oil.

Table 1: Morphological Characteristics of the Test Organism (*Trichophyton rubrum*) Causing Athlete's Foot Disease

Macromorphology	Micromorphology	Organism
White cottony hyphae appearing yellowish underside	A small oval-shaped and clustered chains or grape-like clusters. The conidia are often smooth-walled	*Trichophyton rubrum*

Table 2: Effect of Different Concentrations of Salicylic Acid and Palm Oil on the Zone of Inhibition on *T. pedis* (mm)

	Zone of Inhibition (MM)	
Concentrations (%)	Salicylic Acid	Palm Oil
Control	0.00 ± 0.00^a	0.00 ± 0.00^a
25	2.50 ± 0.10^b	2.20 ± 0.20^b
50	3.67 ± 0.15^c	3.00 ± 0.10^c
75	3.97 ± 0.06^d	3.40 ± 0.20^d
100	5.50 ± 0.10^e	4.30 ± 0.30^e

Key: Mean± S.D for the zone of inhibition of both salicylic acid and palm oil

Table 3: Determination of Minimum Inhibitory Concentrations (MIC) of Salicylic Acid and Palm Oil on the Dermatophyte Causing *T. Pedis*

	MIC	
Concentrations (%)	Salicylic Acid	Palm Oil
3.12	++	++
6.25	+	+
12.5	+	+
Negative Control	-	-
Positive Control	+	+

Key: + = MIC Positive (no fungal growth observed)
++= MIC Value
- = MIC Negative (Fungal Growth observed).

The determination of the minimum fungicidal concentration (MFC) of salicylic acid and palm oil against the *Tinea pedis* pathogen yielded remarkable results (Table

4). On Sabouraud Dextrose Agar (SDA) plates, the MFC was identified at a concentration of 6.25% for both test solutions, meaning that this was the lowest concentration at which visible fungal growth was completely inhibited. This finding is a crucial indicator of the strong antifungal activity of these substances. Notably, a similar MFC result was obtained when the positive control, ketoconazole, was tested, further validating the efficacy of salicylic acid and palm oil as potential antifungal agents.

Table 4: Determination of Minimum Fungicidal Concentrations of Salicylic Acid and Palm Oil on the *T. pedis*

	MIC	
Concentrations (%)	Salicylic Acid	Palm Oil
3.12	++	-
6.25	+	++
12.5	+	+
Negative Control	-	-
Positive Control	+	+

Key: + = MFC Positive (no fungal growth observed)
++= MFC Value
- = MFC Negative (Fungal Growth observed).

DISCUSSION

Effect of Salicylic Acid and Palm Oil on Zone of Inhibition of *T. pedis*

The significant inhibitory effects observed at higher concentrations of salicylic acid and palm oil suggest their potential as effective antifungal agents against the dermatophytes causing athlete's foot. This study demonstrates that both salicylic acid and palm oil possess significant antifungal properties against *T. pedis*. Salicylic acid, known for its anti-inflammatory and keratolytic properties, has previously been reported to exhibit antifungal activity against various fungal pathogens (Wang *et al.,* 2023; Hawar *et al.,* 2023). The ability of salicylic acid to disrupt fungal cell walls and inhibit enzyme activity might explain its strong antifungal properties (Al-Surhanee, 2022). Additionally, palm oil's rich composition of fatty acids and antioxidants, such as tocotrienols, likely contributes to its antifungal action by disrupting the cell membrane integrity of the fungi (Hamid *et al.,* 2022). The presence of fatty acids and other bioactive constituents in palm oil likely contributes to its antifungal properties by potentially disrupting the cell membrane integrity of *T. pedis*, leading to the inhibition of mycelial growth and sporulation. The effect of palm oil could also be due to increased permeability and leakage of cell contents as a result of the fungal cell wall destruction.

Our findings align with previous studies demonstrating the antifungal properties of salicylic acid. For instance, Yan *et al.* (2023) reported similar inhibitory effects of salicylic acid on fungal pathogens. Additionally, the antifungal activity of palm oil has been attributed to its bioactive components, such as phenolic compounds. The findings of this study support the potential of palm oil as a natural antifungal agent against *T. pedis* (Zang *et al.,* 2023; Saputra *et al.,* 2022). These findings are further supported by Dhoot (2020), who found that a combination of luliconazole and salicylic acid was effective in treating hyperkeratotic tinea pedis with fewer adverse effects.

Additionally, Saputra *et al.* (2022) demonstrated that salicylic acid at concentrations of 50-200 ppm inhibited *Ganoderma* spp. growth *in vitro* and improved oil palm seedling growth. In another study, Alamir *et al.* (2020) observed synergistic antifungal effects of miconazole, salicylic acid, and benzoic acid against *T. mentagrophytes*, with the largest inhibition zone produced by a lanette cream formulation containing all three compounds.

Minimum Inhibitory Concentration of Salicylic Acid and Palm Oil on *T. pedis*

In this study, the minimum inhibitory concentrations (MIC) of salicylic acid and palm oil were determined using concentrations of 3.12%, 6.25%, and 12.5%. The findings indicate that at a concentration of 3.12% for both salicylic acid and palm oil, a significant reduction in fungal growth was observed. As the concentrations increased to 6.25% and 12.5%, a dose-dependent decrease in fungal growth was consistently noted. These results align with previous studies, such as those by Khurana et al. (2019), which have demonstrated the antifungal properties of salicylic acid and palm oil. The MICs identified in this study suggest the effective concentrations required to inhibit fungal growth. In addition, Saputra *et al.* (2022) found that salicylic acid effectively inhibited *Ganoderma* spp. growth in oil palm seedlings, supporting the antifungal efficacy observed in this study. In the same vein, Dhoot (2020) reported clinical improvement in over 60% of patients with hyperkeratotic tinea pedis after two weeks of treatment with a fixed-dose combination of luliconazole and salicylic acid, highlighting the potential of salicylic acid in combination therapies.

Minimum Fungicidal Concentration of Salicylic Acid and Palm Oil on *T. pedis*

The research findings from various studies indicate that salicylic acid and palm oil exhibit significant antifungal properties against dermatophytes. Salicylic acid was shown to have a 100% inhibitory effect on test fungi, aligning with its efficacy as a topical treatment for superficial fungal infections caused by various fungi. In another study, Barchiesi *et al.* (2009) compared fluconazole, a lipidated peptide, and tea tree oil component against dermatophytes, with the peptide showing promising results. Yang *et al.* (2024) also developed pH- and MMP-responsive microneedles loaded with salicylic acid and FK13-a1, demonstrating efficacy against various fungi in a guinea pig model of *T. pedis*. These studies highlight the potential of salicylic acid in combination with other antifungal agents for treating fungal infections, particularly in cases where thick scales or barriers impede traditional treatments.

Additionally, palm oil demonstrated high antidermatophytic activity at concentrations of 6.25% and

12.5%, leading to complete inhibition of fungal growth. These results are consistent with prior research highlighting the antifungal effects of salicylic acid and palm oil. Moreover, ketoconazole, a commonly used antifungal agent, was also found to be highly effective against the tested fungal species, supporting its use in treating superficial fungal infections of the skin (Ogba *et al.,* 2023; Sunandar *et al.,* 2023; Jayadi, 2022; Alolofi, 2022).

In a nutshell, the observed MIC and MFC values indicate that salicylic acid has a stronger fungicidal effect than palm oil, which might be due to its ability to penetrate the fungal cell wall more effectively. These results are consistent with Saputra et al. (2022), who found that salicylic acid effectively inhibits the growth of various fungal pathogens. In contrast, palm oil's antifungal activity, though significant, appears less potent, which could be attributed to its primarily protective rather than fungicidal properties. However, the limitations of this study include the in-vitro nature of the experiments, which may not fully replicate in-vivo conditions. Therefore, further research is needed to evaluate the efficacy of salicylic acid and palm oil in clinical settings.

CONCLUSION

In conclusion, salicylic acid and palm oil demonstrated significant antifungal activity against Tinea pedis *in vitro*, suggesting their potential as effective topical treatments for athlete's feet. Salicylic and palm oil inhibit fungal growth at 3.12 % concentration. Also, salicylic acid has a stronger fungicidal effect than palm oil. Further *in-vivo* studies and clinical trials are recommended to validate these findings.

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