

Antifungal Potential of Pineapple Rind and Foliage Crown (*Ananas comosus* L. Merr) Waste Extracts Against Dermatophyte and Non-Dermatophyte Fungi

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Abstract

Background: Fungal infections (mycoses) are common health problems in Indonesia, where tropical climatic conditions favor fungal growth. These infections are generally classified into dermatophytosis and non-dermatophytosis and are commonly treated using synthetic antifungal agents such as ketoconazole, which may cause adverse effects. Therefore, safer natural alternatives are needed. Pineapple rind and foliage crown (*Ananas comosus* L. Merr), which are typically discarded as agricultural waste, contain bioactive compounds with potential antifungal activity, including flavonoids, alkaloids, saponins, and tannins. **Purpose:** This study aimed to evaluate the antifungal activity of ethanol extracts from pineapple rind and foliage crown waste against *Trichophyton rubrum* and *Aspergillus niger*. **Methods:** An experimental study was conducted using the Kirby–Bauer disc diffusion method with extract concentrations of 25%, 50%, and 75%, along with negative (0%) and positive (ketoconazole) controls. **Results:** Both pineapple rind and foliage crown extracts inhibited the growth of *T. rubrum* and *A. niger*. The 75% concentration exhibited the largest inhibition zones, indicating the highest antifungal effectiveness. **Conclusion:** These findings suggest that pineapple rind and foliage crown waste have potential as natural antifungal agents. **Keywords:** *Ananas comosus* L. Merr; *Aspergillus niger*; Dermatophyte; Mycosis; non-dermatophyte; *Trichophyton rubrum*.

1. INTRODUCTION

Indonesia is a country with a tropical climate, and tropical climates are known for high humidity. With the moisture, the fungus can easily spread and infect. The skin is a place that is easily infected by fungi. Fungal infections that cause skin diseases are often found in tropical countries because of the humid air that supports the development of skin fungi (1). Fungal skin infections are classified into two types: dermatophytosis and non-dermatophytosis.

Dermatophytosis is an infection caused by the colonisation of dermatophyte fungi that invade keratinised tissues, including the stratum corneum of the skin, nails, and hair in humans. *Microsporum*, *Epidermophyton*, and *Trichophyton* are the three genera of dermatophytosis. Throughout 2016, there were 6,776 total cases of dermatomycosis with 5,772 cases of dermatophytosis, based on research conducted in Japan (2). Dermatophytosis infection in Indonesia ranks second after pityriasis versicolor (3). Of all these prevalences, the most common in workers is usually caused by *Trichophyton rubrum*, which causes Tinea pedis disease. (4).

Trichophyton rubrum is an anthropophilic dermatophyte species that utilizes keratin as a nutrient source. Its colony growth is generally slow to moderate. Macroscopically, the colonies appear yellowish-white, brass-colored, or reddish-violet, with a soft texture (5). *Trichophyton rubrum* is one of the major causes of human

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dermatophytosis, including tinea capitis, tinea corporis, tinea cruris, tinea pedis, tinea unguium, and tinea barbae (6).

Several studies have reported *Trichophyton rubrum* infections among various occupational groups. Supriyatin (2017) reported that six automotive cleaning workers in the rural community of Arjawinangun, Cirebon Regency, were diagnosed with tinea pedis. This condition was associated with prolonged use of closed footwear, which created a moist environment conducive to fungal growth. Similarly, Hardika (5) reported cases of tinea pedis caused by *Trichophyton rubrum* in four farmers from Barong Sawahan Hamlet, Jombang Regency, who experienced prolonged itching and discomfort due to the continuous use of enclosed footwear.

In addition to tinea pedis, *Trichophyton rubrum* is also a common cause of tinea corporis. Riani (7) It was reported that 94 inhabitants of the coastal settlement, within the Jambu Health Centre area of Rokan Hilir Regency, were affected by tinea corporis in 2016, primarily due to poor personal hygiene and other contributing factors.

Non-dermatophytosis is a fungal infection of the outer skin caused by fungi that are unable to secrete enzymes capable of digesting skin keratin. One example of non-dermatophytic infection is otomycosis. Otomycosis is a fungal ear infection characterised as a superficial infection of the external auditory canal. These ear infections can be acute or subacute, with typical signs of inflammation, itching, and discomfort. This mycosis causes swelling, sloughing of the superficial epithelium, accumulation of debris in the form of hyphae, accompanied by suppuration and pain. In 80% of cases of otomycosis, *Aspergillus* is the cause, followed by *Candida* as the second most common cause of otomycosis. *Aspergillus niger* is the most commonly identified species, while other fungi frequently associated with otomycosis include *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida albicans*, and *Candida parapsilosis* (8).

Currently, fungal infections are commonly treated using antifungal agents, which are medications designed to manage infections caused by fungi. One antifungal drug widely available on the market is griseofulvin; however, cases of fungal resistance to this drug have been reported. In instances of griseofulvin resistance, ketoconazole is often used as an alternative therapy. Nevertheless, prolonged use of ketoconazole, particularly for more than 10 days, poses a risk of hepatotoxicity. Due to the limitations and potential adverse effects of synthetic antifungal drugs, there is increasing public interest in the use of herbal medicines, which are perceived to be safer alternatives derived from medicinal plants (9).

Natural ingredients, also known as traditional medicines, have more advantages than chemicals because natural ingredients have fewer side effects and risks than chemicals. The price of natural ingredients can also be said to be relatively cheap (10). In fact, many plant-derived secondary metabolites represent important sources of fungicides, bactericides, pesticides, and numerous pharmaceutical drugs (11). Research by Yusuf et al. (12), showed that the ethanol extract of pineapple rind (*Ananas comosus* L. Merr) exhibits antifungal activity against *Pityrosporum ovale* and *Candida albicans*, which are associated with dandruff. His activity is attributed to bioactive compounds in pineapple rind, including flavonoids, alkaloids, tannins, and saponins (13). Furthermore, pineapple foliage crowns also contain alkaloids, tannins, and flavonoids with potential antifungal properties. (14).

Indonesia is a major producer of pineapple (*Ananas comosus* L. Merr), a horticultural commodity widely cultivated across the country (15) and it ranks third in global revenue among tropical fruit commodities worldwide (16). His plant is widely distributed throughout Indonesia (17). From 2017 to 2019, there was an increase in pineapple production in Indonesia (18). It can be said that many people are interested in consuming pineapple (*Ananas comosus* L. Merr). Increased pineapple (*Ananas comosus* L. Merr) production has led to high levels of trade in both fresh and processed products, resulting in substantial amounts of rind and foliage crown waste. Several studies have shown that Pineapple rind and foliage crown (*Ananas comosus* L. Merr) contain reusable bioactive compounds. Therefore, these by-products have the potential to provide economic value while simultaneously reducing environmental waste. (19) Accordingly, this study aimed to evaluate the antifungal activity of ethanol extracts from Pineapple rind and foliage crown (*Ananas comosus* L. Merr) waste against dermatophyte and non-dermatophyte fungi.

2. METHOD

Sample Preparation

The materials used in this study were Saboroud Dextrose Agar (SDA) media (grade : Microbiological, Brand : Merck, Company : Merck KGaA, Made in Germany), chloramphenicol (Grade : Pharmaceutical, brand : Kimia Farma, company : PT Kimia Farma Tbk., country of manufacturer : Indonesia), *Trichophyton rubrum* isolate (Grade : Research, brand : ATCC (ATCC® 28188™), company : American Type Culture Collection, country of manufacturer : USA), *Aspergillus niger* isolate (Grade : Research, brand : ATCC (ATCC® 16404™), company : American Type Culture Collection, country of manufacturer : USA), ketoconazole (Grade : Pharmaceutical, brand : Kimia Farma, company : PT Kimia Farma Tbk., country of manufacturer : Indonesia), Barium chloride solution (BaCl₂) (Grade : Analytical, brand : Merck, company : Merck KGaA, country of manufacturer : Germany), Sulfuric acid solution (H₂SO₄) (Grade : Analytical, brand : Merck, company : Merck KGaA, country of manufacturer : Germany), Pineapple rind and foliage crown (*Ananas comosus* L. Merr) extract were obtained from various markets in Surabaya, East Java, and identified at the UPT Herbal Materia Medica Laboratory, Batu City, East Java, NaCl 0.9% (Grade : Pharmaceutical, brand : Kimia Farma, company : PT Kimia Farma Tbk., country of manufacturer : Indonesia), paper discs (Grade : Microbiological, brand : Oxoid, company : Thermo Fisher Scientific, country of manufacturer : UK), petridishes (Grade : Microbiological, brand : SPL Life Sciences, company : SPL Life Sciences Co., Ltd., country of manufacturer : South Korea).

Soxhlet Extraction of Pineapple rind and foliage crown (*Ananas comosus* L. Merr)

In this study, pineapple rind and crown foliage (*Ananas comosus* L. Merr.) were extracted using the Soxhlet extraction method. Soxhlet extraction is considered a more effective technique because it requires a relatively short extraction time, uses less solvent, allows for thorough extraction of the sample, and operates as a hot extraction method that can yield higher extract recovery. In addition, this method helps preserve biological activity during heating, making it suitable for the identification and screening of potential bioactive compounds for drug discovery (20). The following procedure was carried out.

Powdered simplicia of pineapple (*Ananas comosus* L. Merr.) rind and crown leaves, weighing 50 g, was prepared, wrapped in filter paper, and placed inside the Soxhlet chamber, while a distillation flask was positioned below the apparatus. Approximately 300 mL of 96% ethanol was used as the solvent and circulated for two cycles, and a condenser was mounted on top of the apparatus. The distillation flask was then heated to initiate the Soxhlet extraction process, which was continued until the solvent in the extraction chamber became clear. After completion, the extract collected in the distillation flask was retrieved and subsequently evaporated to remove the remaining 96% ethanol.

Preparation of Standard McFarland Turbidity

A 1% barium chloride (BaCl₂) solution and a 1% sulfuric acid (H₂SO₄) solution were prepared. To obtain a 0.5 McFarland standard, 9.95 mL of H₂SO₄ was mixed with 0.05 mL of BaCl₂, with H₂SO₄ added first. The mixture was tightly sealed and stored at room temperature in a dark place.

Preparation of *Trichophyton rubrum* Suspension

All tools and materials were prepared in advance, and the equipment was sterilized by autoclaving. A loopful of *Trichophyton rubrum* colonies was suspended in 10 mL of 0.9% saline, and the inoculum density was adjusted to match the 0.5 McFarland turbidity standard.

Preparation of *Aspergillus niger* Suspension

All tools and materials were prepared in advance, and the equipment was sterilized by autoclaving. A loopful of *Aspergillus niger* colonies was suspended in 10 mL of 0.9% saline, and the inoculum density was adjusted to match the 0.5 McFarland turbidity standard.

Preparation of Sabouraud Dextrose Agar (SDA) Medium

All tools and materials were prepared in advance. Sabouraud Dextrose Agar (SDA) powder (32.5 g) was dissolved in 500 mL of distilled water and heated until completely dissolved, with the pH adjusted to 5.8. The Erlenmeyer flask containing the SDA medium was covered with cotton gauze, while empty Petri dishes were wrapped in

newspaper; both were sterilized in an autoclave at 121 °C and 1.5 atm for 15 minutes. After sterilization, 1 mL of chloramphenicol was added to the medium. The SDA medium was then poured aseptically into Petri dishes, gently rotated to ensure even distribution, and allowed to solidify.

Preparation of Positive and Negative Controls

For the positive control, 0.1 g of ketoconazole was weighed and dissolved in 5 mL of sterile distilled water. The negative control consisted of 5 mL of sterile distilled water without any additives.

Preparation of Extract Dilutions

For the 25% concentration, 1.25 g of pineapple rind and crown foliage (*Ananas comosus* L. Merr.) extract was dissolved in 5 mL of sterile distilled water. For the 50% and 75% concentrations, 2.5 g and 3.7 g of the extract, respectively, were each dissolved in 5 mL of sterile distilled water (21).

Antifungal Test of Pineapple Extract

A 0.1 mL suspension of *Trichophyton rubrum* was inoculated onto the surface of solidified Sabouraud Dextrose Agar (SDA) in each Petri dish using a sterile cotton swab and evenly spread across the medium. The same procedure was applied to the *Aspergillus niger* suspension. Paper discs were then immersed in each treatment solution and allowed to stand for 15 minutes. After immersion, the discs were placed onto the surface of each inoculated plate using sterile tweezers. All treated Petri dishes were labeled and incubated at 25 °C for 7 days. *Following incubation, the inhibition zones formed around each paper disc were measured in millimeters (mm).*

Statistical analysis to compare the significant differences

Data analysis was performed after all experimental results were obtained. Data normality and homogeneity of variance were assessed using the Shapiro–Wilk test and a homogeneity of variance test. If the data were normally distributed and homogeneous, a one-way analysis of variance (ANOVA) with a 95% confidence level was performed to evaluate the effect of different concentrations of methanolic extracts of pineapple rind and crown foliage (*Ananas comosus* L. Merr.) on the inhibition zone diameters of *Trichophyton rubrum* and *Aspergillus niger*. When the ANOVA results showed statistical significance ($p < 0.05$), post hoc Least Significant Difference (LSD) testing was conducted to determine differences between treatment groups. If the data were not normally distributed, a non-parametric Kruskal–Wallis test followed by post hoc Mann–Whitney analysis was applied.

3. RESULT

Examination of the inhibition test on *Saboroud Dextrose Agar* (SDA) media that had been planted with *Trichophyton rubrum* and *Aspergillus niger* mushrooms were given disc paper that had been soaked at each concentration of extracts of the skin and crown of pineapple leaves (*Ananas comosus* L. Merr) where the concentration was 0% as negative control, ketoconazole as a positive control, 25%, 50% and 75%. The area measured was a clear area around the disc paper which was not overgrown with *Trichophyton rubrum* and *Aspergillus niger* fungi. The following table 1 shows the results of the inhibition zones for each fungus:

Table 1. Data tabulation of inhibition zones produced by pineapple rind and foliage crown extracts (*Ananas comosus* L. Merr) against the growth of *Trichophyton rubrum*

No	Treatment	Inhibition zone diameter on each iteration (mm)					Mean \pm SD
		I	II	III	IV	V	
		1	Negative control (0%)	0	0	0	
2	Positive control (+)	37	38	35	38	40	37.6 \pm 1.81
3	25%	7	8	7	8	10	8 \pm 1.22
4	50%	12	10	10	11	10	10.6 \pm 0.89
5	75%	14	15	20	11	12	14.4 \pm 3.50

Based on Table 1, pineapple rind and foliage crown waste extracts (*Ananas comosus* L. Merr) showed concentration-dependent inhibition of *Trichophyton rubrum*, with mean inhibition zones of 8.0, 10.6, and 14.4 mm at concentrations of 25%, 50%, and 75%, respectively. The 50% concentration group showed a non-normal data distribution; therefore, the Kruskal–Wallis test was applied. The results showed a significant difference in inhibition zone diameters among treatment groups ($p = 0.000$; $p < 0.05$), indicating concentration-dependent inhibition of *Trichophyton rubrum* by pineapple rind and foliage crown extracts. Post hoc Mann–Whitney analysis confirmed significant differences between all treatment groups ($p < 0.05$).

Table 2. Data tabulation of inhibition zones produced by pineapple rind and foliage crown extracts (*Ananas comosus* L. Merr) against the growth of *Aspergillus niger*

No	Treatment	Inhibition zone diameter on each iteration (mm)					Mean \pm SD
		I	II	III	IV	V	
1	Negative control (0%)	0	0	0	0	0	0
2	Positive control (+)	32	35	30	32	30	31,8 \pm 1.83
3	25%	6	7	6	8	8	7 \pm 0.89
4	50%	9	6	8	8	8	7,8 \pm 0.98
5	75%	10	8	9	9	9	9 \pm 0.63

Based on Table 2, pineapple rind and foliage crown extracts (*Ananas comosus* L. Merr) showed concentration-dependent inhibition of *Aspergillus niger*, with mean inhibition zones of 7.0, 7.8, and 9.0 mm at concentrations of 25%, 50%, and 75%, respectively. Statistical analyses were performed to assess data normality and homogeneity of variance. All groups showed a normal distribution; therefore, a one-way ANOVA was applied. The ANOVA results revealed a highly significant difference in the inhibition zone diameters of *Aspergillus niger* among different concentrations of pineapple rind and foliage crown extracts (*Ananas comosus* L. Merr), with a high F value ($F = 528.812$) and a significance level of $p = 0.000$ ($p < 0.05$). These findings indicate that extract concentration significantly affects the inhibition of *A. niger*, with higher concentrations producing larger inhibition zones. Post hoc LSD analysis showed no significant differences between the 25% vs. 50% and 50% vs. 75% treatment groups ($p > 0.05$), while all other pairwise comparisons were statistically significant.

Based on Figure 1, pineapple rind and foliage crown extracts (*Ananas comosus* L. Merr) showed greater antifungal activity against *Trichophyton rubrum* (dermatophyte) than against *Aspergillus niger* (non-dermatophyte). The 75% extract concentration produced the largest inhibition zones for both fungi.

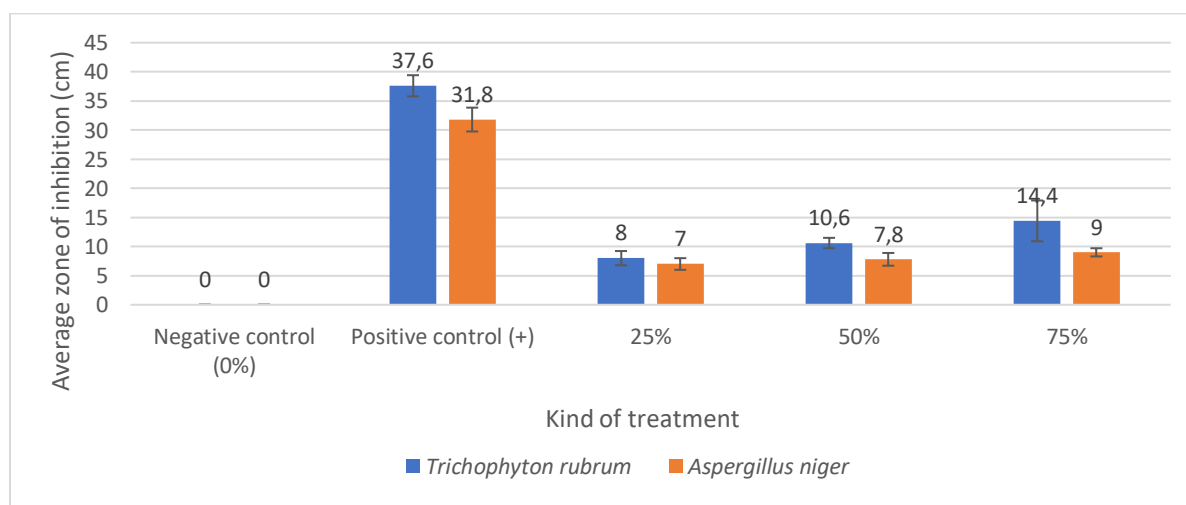


Figure 1. Effectiveness Test of Pineapple (*Ananas comosus* L. Merr) Waste Extracts against *Trichophyton rubrum* and *Aspergillus niger*. The 75% concentration produced the largest inhibition zones compared with the other concentrations.

After the effectiveness test was carried out, the results obtained from the study showed that the diameter of the inhibition zone in the treatment of waste extract of the skin and crown of pineapple leaves (*Ananas comosus* L. Merr) against *Trichophyton rubrum* at concentrations of 25%, 50% and 75% obtained an inhibition zone with a size of an average of 8 mm, 10.6 mm, 14.4 mm and the positive control resulted in an average of 37.6 mm. This indicates that there is an inhibition zone growth response. While the administration of extracts of waste skin and crown of pineapple leaves (*Ananas comosus* L. Merr) to *Aspergillus niger* at concentrations of 25%, 50% and 75% obtained inhibition zones with an average size of 7mm, 7.8mm, and 9mm.

Statistical analysis using SPSS confirmed significant differences among treatments. Post hoc Mann–Whitney analysis showed significant differences for *Trichophyton rubrum* ($p < 0.05$), whereas post hoc LSD analysis for *Aspergillus niger* showed no significant differences between the 25%–50% and 50%–75% groups ($p > 0.05$), with all other comparisons being significant.

4. DISCUSSION

Based on the study results, pineapple rind and crown foliage (*Ananas comosus* L. Merr.) extracts exhibited antifungal activity, as indicated by the formation of inhibition zones at concentrations of 25%, 50%, and 75%. Increasing extract concentrations resulted in larger inhibition zones, suggesting enhanced antifungal activity due to higher levels of bioactive compounds. These findings are consistent with the study by Juariah, which demonstrated that increasing concentrations of pineapple peel ethanol extract enhanced its inhibitory effect on the growth of *Trichophyton mentagrophytes* (14,22).

The antifungal activity may be attributed to the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which are known to possess antifungal properties and are present in pineapple rind (23). In addition, the crown leaves of pineapple also contain similar bioactive components, including alkaloids, tannins, and flavonoids, which may contribute to the observed antifungal effects.

Among these bioactive compounds, flavonoids exert antifungal effects by disrupting nutrient diffusion into fungal cells, leading to growth inhibition or cell death. Saponins act as polar surfactants that damage lipid components of the cell membrane, increasing membrane permeability and causing fungal cells to swell and eventually lyse (24). Tannins inhibit chitin synthesis, an essential component of fungal cell wall formation, and also disrupt cell membranes, thereby suppressing fungal growth. Alkaloids exhibit antifungal activity by intercalating between the cell wall and DNA, inhibiting DNA replication and ultimately interfering with fungal proliferation (24).

The extraction method used in this study was Soxhlet extraction with 96% ethanol as the solvent. Soxhlet extraction is a hot extraction technique that allows continuous solvent circulation, facilitating efficient extraction of active compounds. In addition, the use of 96% ethanol contributed to the formation of inhibition zones, as this solvent is effective in extracting bioactive compounds from pineapple rind and crown foliage (*Ananas comosus* L. Merr.). According to Hakim (25), 96% ethanol offers advantages due to its ability to extract a wide range of active compounds while maintaining relatively low toxicity.

Differences in inhibition zones observed at concentrations of 25%, 50%, and 75% may have been influenced by the disc immersion process, where extract absorption may not have been optimal due to variations in solution viscosity at different concentrations. Furthermore, during fungal suspension preparation, the number of inoculum loops must comply with the predetermined McFarland standard to ensure consistency. As reported by Nadziroh et al. (26), antimicrobial activity can be influenced by several factors, including microbial load, environmental pH, the concentration and potency of antimicrobial agents, and microbial susceptibility to antifungal compounds.

To ensure even fungal distribution, the suspensions were standardized against the 0.5 McFarland standard, which corresponds to approximately 1.5×10^8 CFU/mL (27).

This study has several limitations. Although pineapple rind and crown foliage are available in large quantities as agricultural waste, the extraction yield obtained was relatively low, with a thick texture and brownish color. Future studies are recommended to optimize the extraction process to obtain a more stable extract, such as a powdered formulation.

5. CONCLUSION

Based on the research findings and statistical analyses, pineapple (*Ananas comosus* L. Merr.) rind and crown foliage extracts demonstrated antifungal activity against *Trichophyton rubrum* and *Aspergillus niger*, as indicated by the formation of inhibition zones at concentrations of 25%, 50%, and 75%. Among the tested concentrations, the 75% extract produced the largest inhibition zones against both fungi, indicating the highest antifungal effectiveness under the conditions of this study.

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