BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 299-303 | DOI: 10.14421/biomedich.2026.151.299-303 ISSN 2540-9328 (online)

Antifungal Activity of the Ethyl Acetate Fraction of *Matoa* Leaves (*Pometia pinnata* J. R. Forst & G. Forst) Against *Trichophyton rubrum*

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Manuscript received: 07 December 2025. Revision accepted: 13 April 2026, Published: 21 April 2026.

Abstract

Dermatophytosis is a prevalent skin infection in tropical regions like Indonesia, predominantly caused by the fungus *Trichophyton rubrum*. While *Matoa* leaves (*Pometia pinnata*) are known for their medicinal properties, their specific antifungal potency requires optimization through fractionation. This study aimed to evaluate the antifungal activity of the ethyl acetate fraction of Matoa leaves and determine its effective concentration against *T. rubrum*. This experimental study employed the disc diffusion method using Sabouraud Dextrose Agar (SDA). The ethyl acetate fraction was tested at concentrations of 10% and 20%, with 2% Ketoconazole as the positive control and DMSO as the negative control. The results demonstrated that the ethyl acetate fraction exhibited strong antifungal activity, yielding inhibition zones of 18.25 mm at 10% concentration and 19.75 mm at 20% concentration. Statistical analysis using the Kruskal-Wallis and Mann-Whitney tests indicated significant antifungal activity compared to the negative control ($p < 0.05$), though no significant difference was observed between the 10% and 20% concentrations. These findings suggest that the semi-polar ethyl acetate fraction of *Matoa* leaves is a potent natural antifungal agent, showing strong efficacy even at lower concentrations.

Keywords: antifungal; dermatophytosis; ethyl acetate fraction; matoa; *Trichophyton rubrum*.

INTRODUCTION

Dermatophytosis remains a major public health concern in tropical regions, including Indonesia, where the high temperature and humidity contribute significantly to the prevalence of skin diseases. The number of cases of skin disorders in Indonesia has shown an increasing trend, rising from 8.46% in 2012 to 9% in 2013 (Dunggio & Mukusibu, 2022). This condition occurs when a dermatophyte fungus infects keratinized tissues such as skin, nails, and hair (Zaila & Ayu, 2019). Among the various causative fungi (*Trichophyton, Microsporum, and Epidermophyton*), *Trichophyton rubrum* is the most common agent, responsible for various tinea infections, including tinea pedis, affecting the soles and interdigital areas of the feet (Lathifah et al., 2021; Haerani, 2021). Although dermatophytosis can be managed with topical or systemic azole drugs, long-term use is associated with potential side effects such as hepatotoxicity and the increasing concern of antifungal drug resistance. Consequently, many individuals are seeking safer and more affordable natural alternatives for treatment (Dewi et al., 2019).

Matoa leaves, sourced from *Pometia pinnata* (J.R. Forst & G. Forst), a native plant of Indonesia belonging to the *Sapindaceae* family (Anggari, 2016), are traditionally known for their medicinal properties. Phytochemical analyses confirm that *Matoa* leaves are rich in secondary metabolites, including phenolic compounds and flavonoids (Fajrina et al., 2022), as well as saponins, which collectively contribute to their reported antimicrobial, antioxidant, and anti-inflammatory activities. Specifically, flavonoids, as defense chemicals produced by plants, exert their antifungal effects primarily by disrupting fungal cell membrane permeability, inhibiting cellular respiration, and interfering with enzyme function (Cushnie & Lamb, 2005).

While the crude extract of *Pometia pinnata* has demonstrated antifungal activity against other dermatophytes, such as *Trichophyton mentagrophytes* (Sidoretno & Gustari, 2021), this study hypothesizes that focusing on a specific fraction will isolate and concentrate the most potent bioactive compounds. The use of the ethyl acetate fraction is specifically justified by its polarity, which is optimal for partitioning and concentrating the flavonoid and other lipophilic phenolic

compounds responsible for antifungal action. Thus, this study aims to evaluate the antifungal activity of the ethyl acetate fraction of *Pometia pinnata* leaves against the prevalent dermatophyte *Trichophyton rubrum* and to determine the effective concentration range of the fraction that provides the maximal inhibitory effect.

MATERIALS AND METHODS

Research Location

This research was conducted in the Chemistry Laboratory of Mohammad Natsir University, Bukittinggi, and the Microbiology Laboratory of the Faculty of Medicine, Andalas University.

Sample Preparation and Identification

The *matoa* leaves (Pometia pinnata J.R. Forst & G. Forst) used in this study were collected from Batu Belah Village, Kampar District, Kampar Regency, Riau Province. Identification of the matoa leaves (Pometia pinnata J.R. Forst & G. Forst) was conducted at the ANDA Herbarium, Faculty of Biology, Andalas University, Padang, West Sumatra.

Tools and Materials

The tools used in this study included a rotary evaporator (Rotavapor R-300), a digital scale, an incubator (Memmert), an oven (Memmert), a laminar air flow meter (B-One), an autoclave, a vortex mixer, and laboratory glassware. The materials used in this study included matoa leaves, 96% ethanol (Mustikarya Gemilang), ethyl acetate (Brataco), n-hexane (Brataco), SDA (Sabouraud Dextrose Agar) (MERCK), ketoconazole, 0.9% physiological NaCl, H2SO4, BaCl2, DMSO (Dimethyl Sulfoxide), distilled water, and other materials in accordance with the work procedures.

Procedures

Method of Extraction

Extraction: The maceration method was used. 200 g of *matoa* leaf powder was placed in a maceration vessel and soaked in 1000 ml of 96% ethanol for three days, stirring occasionally, until the sample was completely submerged. The maceration vessel was sealed and stored at room temperature, protected from direct sunlight. The extract was filtered through filter paper to obtain a filtrate and concentrated using a rotary evaporator until it reached a consistent viscosity, and the final weight of the extract was recorded (Prayoga et al., 2024).

Fractionation: The thick ethanol extract was weighed to 10 grams, then 100 ml of distilled water was added and stirred until evenly distributed. The mixture was put into a separating funnel and fractionated with 100 ml of n-hexane solvent and shaken gently, so that two layers were formed: one layer containing the n-hexane fraction and the other layer containing the water fraction. The water fraction was then fractionated again using 100 ml of ethyl acetate solvent, the ethyl acetate layer was separated from the water. The ethyl acetate fraction was concentrated using a rotary evaporator until a thick ethyl acetate fraction was formed (Nurliyasman et al., 2022).

Sterilization of Equipment and Materials

Before sterilization, all equipment and materials must be wrapped in parchment paper. To sterilize test solutions or media, the solution is placed in an Erlenmeyer flask, plugged with cotton, and then autoclaved at 121°C and 1.5 atm for 15 to 20 minutes (Pusmarani et al., 2023).

Preparing Fungal Growth Media

To prepare Sabouraud Dextrose Agar, first weigh 8 grams of powder and dissolve it in 400 mL of distilled water. The mixture is heated in an Erlenmeyer flask over a Bunsen burner and stirred until all ingredients are thoroughly dissolved. Next, the media is sterilized in an autoclave for 15 minutes at 121°C and 1 atm, then dispensed into 10-15 ml Petri dishes (Apriliani et al., 2024).

Preparation of McFarland Standard Solution

In a test tube, mix 1 ml of 1% $BaCl_2$ solution with 9 ml of 1% H_2SO_4 solution, then shake until the mixture becomes cloudy. The turbidity of the standard test fungus solution is based on the turbidity of this mixture (Sidoretno & Gustari, 2021).

Preparing a Trichophyton Rubrum Suspension

Use a sterilized loop needle to collect the inoculated Trichophyton Rubrum fungus, then suspend it in a 10 ml test tube containing 0.9% physiological NaCl solution (Sidoretno & Gustari, 2021).

Matoa Leaf Extract Inhibitory Test

The effect of the ethyl acetate fraction of *matoa* leaves on the growth of *Trichophyton rubrum* was tested using the well diffusion method. The fungal suspension was applied with cotton wool and spread evenly on SDA media. A 6 mm diameter hole was made in each plate to create a well, and the ethyl acetate fraction was added at concentrations of 10% and 20%. 2% ketoconazole cream served as a positive control, while DMSO served as a negative control. This test was performed in duplicate. The media were then incubated at 37°C for 24 to 48 hours. The size of the inhibition zone was measured using a ruler (Afifah & Nurwaini, 2018).

Data analysis

The inhibition zone diameter measurement data were statistically analyzed using SPSS version 25 software. The initial stage of the analysis was performed using the Shapiro-Wilk normality test to determine data distribution. Based on the results of the normality test, if the data were normally distributed ($p>0.05$), a parametric

One-Way ANOVA was performed. However, if the data were not normally distributed or the homogeneity assumption was not met, the analysis continued using the non-parametric Kruskal-Wallis test to determine significant differences between all treatment groups (10%, 20% concentration, Positive Control, and Negative Control).

If the Kruskal-Wallis test showed a significance value of $p<0.05$, the analysis continued with the Mann-Whitney test as a post-hoc test to determine specific differences between pairs of treatment groups. All hypothesis decisions were based on a 95% confidence level ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Sample Extraction

Using ethanol as a solvent to extract *matoa* leaves (Pometia pinnata J.R. Forst & G. Forst) yielded 26.27 grams of extract. The amount of matoa leaf extract produced is shown in Table 1.

Table 1. Extract Yield

Powder weight (Kg)	Condensed extract weight	Yield
200 gram	26,27 gram	13,13%

The yield of matoa leaf extract, at 12–20%, met the criteria shown in the table.

Phytochemical Screening Test

The results of the phytochemical analysis of the matoa leaf fractions are shown in Table 2.

Table 2. Phytochemical Screening Test

Test	Ethanol-Water Fraction	Ethyl Acetate Fraction	N-Hexane Fraction
Flavonoids	+	+	+
Terpenoids	+	+	-
Phenolic	+	+	+
Saponins	+	-	+
Alkaloids	+	+	-
Tannins	+	+	+

The table above shows that the ethanol fraction has a higher concentration of secondary metabolites compared to the ethyl acetate and n-hexane fractions.

Antifungal Activity Analysis

The antifungal activity of the ethyl acetate fraction of *Pometia pinnata* leaves against *Trichophyton rubrum* was evaluated using the disc diffusion method, with results presented in Table 3. The fraction demonstrated a strong inhibitory effect on the fungus, with the diameter of the zone of inhibition increasing commensurately with the concentration. Specifically, the fraction yielded a zone of inhibition of 18.25 mm at 10% and 19.75 mm at 20%. These diameters classify the fraction as having a strong category of antifungal activity (Sungkar et al., 2024).

Table 3. Inhibition Zone Diameter of Matoa Leaf Ethyl Acetate Fraction.

Treatment	Replication (mm)		Mean (mm)	Category
	I	II		
10% concentration	17	19,5	18,25	Strong
20% concentration	19	20,5	19,75	Strong
Positive control	63	67	65	Very Strong
Negative control	0	0	0	Weak

The table shows the diameter of the inhibition zone of the ethyl acetate fraction of matoa leaves on Trichophyton rubrum, showing the highest diameter at 19.75 mm at a concentration of 20%, categorized as strong.

The analysis of the data utilized a non-parametric approach. The Kruskal-Wallis test was employed to determine overall differences among the multiple treatment groups (10%, 20%, Positive Control, and Negative Control). The test indicated a statistically significant difference in activity among the groups ($p < 0.05$). Subsequent Mann-Whitney U tests were performed as post-hoc analysis. The results confirmed that, while descriptively the 20% concentration showed a larger zone, the difference between the 10% and 20% concentrations was not statistically significant ($p > 0.05$). This finding suggests that even at the lower concentration (10%), the active compounds are present in sufficient quantity to exert a powerful inhibitory action, and further increases in concentration lead only to marginal, non-significant gains in zone diameter.

Interestingly, post-hoc analysis revealed no statistically significant difference between the 10% and 20% concentrations ($p > 0.05$), despite a descriptive increase in the inhibition zone. This finding is clinically significant as it indicates that the ethyl acetate fraction possesses high potency. The active metabolites, particularly flavonoids, appear to reach an effective therapeutic threshold even at a concentration of 10%. Consequently, this suggests that the fraction is efficient,

offering strong fungal inhibition without the necessity of using higher concentrations. This efficiency is advantageous for future pharmaceutical formulation, as it minimizes the required dosage while maintaining maximum efficacy.

The control groups performed as expected: the negative control (solvent) showed no zone of inhibition, while the positive control, 2% Ketoconazole cream, produced an exceptionally large inhibitory zone with a mean diameter of 65 mm. This value, while extreme, is crucial as it validates the susceptibility of the *T. rubrum* strain used and confirms the efficacy of the experimental conditions. The substantial difference between the synthetic drug (Ketoconazole) and the natural fraction is anticipated, given that Ketoconazole is a pure, single-target compound with high bioavailability, while the ethyl acetate fraction is a natural isolate. Nevertheless, the fact that the *P. pinnata* fraction achieved a "strong" category of inhibition demonstrates its significant potential as a crude natural source.

Correlation between Phytochemistry and Bioactivity

The strong antifungal activity observed is directly correlated with the phytochemical profile of the ethyl acetate fraction. Table 4 shows that this fraction contains the highest concentration of total flavonoids, measured at 198.679±1.02 mg QE/g extract.

Table 4. Total Flavonoid Content of Matoa Leaf Fractions.

	Measurement	KTF	Mean	SD	KTF ± SD
Ethanol	0.311	104.039	104.954	1.57	104.954 ± 1.57
	0.319	107.176			
	0.310	103.647			
	Measurement	**KTF**	**Mean**	**SD**	**KTF ± SD**
Ethyl Acetate	0.551	198.156	198.679	1.02	198.679 ± 1.02
	0.550	197.764			
	0.556	200.117			
	Measurement	**KTF**	**Mean**	**SD**	**KTF ± SD**
N-Hexane	0.146	39.333	40.379	0.73	40.379 ± 0.73
	0.150	40.901			
	0.150	40.901			

The highest yield was obtained from the ethyl acetate fraction, with a KTF value of 198.679 ± 1.02 mg QE/g extract.

The robust antifungal activity observed is directly attributed to the high concentration of secondary metabolites partitioned into the semi-polar solvent. Quantitative analysis confirmed that the ethyl acetate fraction contained the highest total flavonoid content (198.679 ± 1.02 mg QE/g) compared to other fractions. Flavonoids exert their fungicidal effect through complex mechanisms. They are known to disrupt cell membrane integrity, leading to increased membrane permeability and the leakage of essential intracellular constituents and eventual cell lysis (Cushnie & Lamb, 2005). Furthermore, the presence of tannins and alkaloids likely provides a synergistic effect by inhibiting fungal enzymatic activity and interfering with protein synthesis, which prevents the mycelial growth of *T. Rubrum* (Sari et al., 2020; Rochaeni et al., 2021).

Furthermore, the presence of alkaloids, as confirmed by initial phytochemical screening, also contributes to the observed antifungal effect. Alkaloids are basic chemicals containing one or more nitrogen atoms, often arranged in a ring. These substances act as antifungals by inhibiting the respiration process in fungal cells (Putri et al., 2022). Alkaloids exert their antimicrobial action by preventing cell wall formation, altering membrane function, and inhibiting protein synthesis (Razoki, 2023). Thus, the potent activity of the ethyl acetate fraction is likely due to the synergistic action of its major components—flavonoids, saponins, and alkaloids—attacking the fungal cell structure through multiple pathways.

This result compares favorably with previous research. Sidoretno and Gustari (2021) showed that the crude ethanol extract of Matoa leaves inhibited *T. mentagrophytes* with zones ranging from 13.0 mm to 14.7 mm. The significantly larger inhibition zones achieved in the current study with the ethyl acetate fraction (up to 19.75 mm) against the clinically more relevant *T. rubrum* suggest that the fractional separation successfully isolated and concentrated the most active anti-dermatophyte components, making the ethyl acetate fraction a superior material for future development.

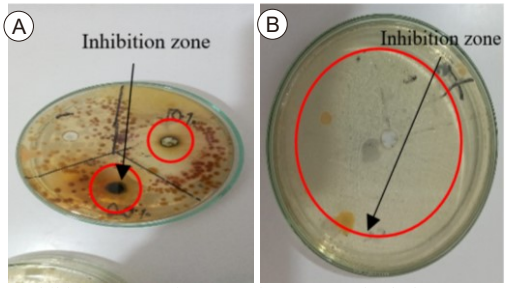

Figure 1. Inhibition Zone Diameter of Matoa Leaf Ethyl Acetate Fraction Description: (a): Inhibition zone at concentrations of 10%, 20%, and the negative control. (b): Inhibition zone in the positive control.

CONCLUSIONS

The ethyl acetate fraction of *Pometia pinnata* leaves possesses strong antifungal activity against *Trichophyton rubrum*. The highest inhibitory activity was achieved at a 20% concentration with a mean inhibition zone diameter of 19.75 mm, followed closely by the 10% concentration with 18.25 mm. This potent activity is strongly correlated with the high concentration of total flavonoids (198.679 ± 1.02 mg QE/g extract) partitioned within the semi-polar ethyl acetate fraction.

Based on this compelling *in vitro* efficacy, the ethyl acetate fraction of *P. pinnata* leaves demonstrates significant potential as a source of alternative natural antifungal agents for dermatophytosis. However, further research, including determination of the Minimum Inhibitory Concentration (MIC) and necessary toxicological assessments, is recommended before proceeding to topical formulation development.

Competing Interests: The authors declare that there are no competing interests.

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