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Research Article

Analysis of Antifungal Activity of *Artocarpus camansi* Leaf Extract Against *Malassezia furfur*: An In Vitro Experimental Study

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ABSTRACT

A lipophilic fungus, *Malassezia furfur*, is responsible for several skin diseases. The conventional treatment is ketoconazole, but concerns about adverse effects and resistance have prompted the search for natural alternatives such as Artocarpus camansi, whose effectiveness remains poorly understood despite its bioactive flavonoid and steroid content. The purpose of this study was to assess the in vitro antifungal activity of leaf extract from *Artocarpus camansi* against *Malassezia furfur* at doses of 25%, 50%, 75%, and 100%. This laboratory experimental study employed the disc diffusion method. Kruskal-Wallis and Mann-Whitney U tests were used to assess and statistically analyze the inhibition zones of the therapy Artocarpus camansi leaf extract at doses of 25%, 50%, 75%, and 100%, with four replications each. The mean inhibition zone for the positive control was 9.53 mm. On the other hand, no inhibitory zone (6.00 mm diameter) was formed by any of the *Artocopus camanci* extract doses or the negative control. There were no significant differences between the extract concentrations ($p>0.05$), but there was a significant difference between the positive control and all extract groups ($p=0.014$). In conclusion, at the tested concentrations, *Artocarpus camansi* leaf extract shows no antifungal activity against *Malassezia furfur*, whereas Ketoconazole remains a useful inhibitor.

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INTRODUCTION

Skin and subcutaneous diseases significantly impact quality of life globally, with over 4.8 billion cases reported in 2019 (Yakupu et al., 2023). In tropical countries like Indonesia, high humidity and warm temperatures create an ideal environment for fungal growth, making dermatomycosis a leading cause of disease in primary healthcare settings (Prameswari et al., 2023). Among the prevalent fungal pathogens, *Malassezia furfur* (MF) is a lipophilic yeast responsible for several common dermatological conditions, including *pityriasis versicolor*, *seborrheic dermatitis*, and *Malassezia folliculitis* (Ahmad & Ervianti, 2022).

The current gold standard for treating MF infections is topical antifungals, particularly azole derivatives such as ketoconazole. However, prolonged use of synthetic antifungals has been associated with adverse effects, including scalp irritation, hair loss, and the emerging threat of antifungal resistance (Nenoff et al., 2014). This challenge necessitates the exploration of natural alternatives derived from medicinal plants, which may offer lower toxicity and synergistic bioactive compounds.

Artocarpus camansi (AC), commonly known as breadnut or *kluwih*, is a member of the Moraceae family that has been traditionally used for its medicinal properties (Masiala et al., 2026). Previous phytochemical screenings indicate that *Artocarpus* species are rich in flavonoids, tannins, and steroids, which are known to exhibit antimicrobial and anti-inflammatory activity (Gondokesumo & Muslikh, 2024; Jagtap et al., 2010). Specifically, flavonoids have been documented to disrupt fungal cell membranes and inhibit spore germination (Rahmawati et al., 2025). While some studies have explored the antibacterial potential of *Artocarpus camansi*, comprehensive data regarding its specific antifungal efficacy against *Malassezia furfur* remains scarce (R. Singh et al., 2025).

While previous studies have demonstrated the antimicrobial efficacy of related species, such as *Artocarpus heterophyllus*, against general fungal strains, the specific therapeutic potential of *Artocarpus camansi* against lipophilic pathogens remains largely unexplored (Bicaldo, 2022; S. P. Singh et al., 2025). The novelty of this study lies in addressing a critical translational gap: determining whether the unique phytochemical profile of *Artocarpus camansi* leaves, traditionally recognized for producing lipophilic prenylated flavonoids, can effectively target and penetrate the exceptionally thick, lipid-rich cell wall barrier unique to *Malassezia furfur*. Investigating this interaction is highly vital, as conventional azole therapies increasingly face resistance, necessitating the discovery of novel phytocompounds capable of disrupting lipid-dependent fungal structures. Therefore, this study aims to provide the first comprehensive, concentration-dependent evaluation (25%, 50%, 75%, and 100%) of *Artocarpus camansi* leaf extract against *Malassezia furfur* using a rigorous in vitro framework compared against a standard ketoconazole benchmark, thereby establishing baseline evidence for its viability as a specialized natural antidermatophytic agent (Saptarini et al., 2024).

METHOD

Research Design

This laboratory experiment evaluated the antifungal activity of *Artocarpus camansi* (AC) leaf extract against *Malassezia furfur* (MF). Using a post-test-only control group design, the inhibitory effects of various extract concentrations were compared against established controls. This study was approved by the Ethics Committee of the Faculty of

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Medicine, Hang Tuah University, for their valuable guidance, support, and contributions throughout this study.

Population and Sample

Malassezia furfur pure isolates were obtained from the Microbiology Laboratory at the Faculty of Medicine, Universitas Indonesia, while *Artocarpus camansi* leaves were collected and botanically authenticated. Based on Federer's formula, the study utilized 24 experimental units divided into six treatment groups with four replications each.

Data Collection and Procedures

Phytochemical Extraction and Yield Determination

Dried *Artocarpus camansi* leaf powder was extracted via cold maceration in 96% ethanol (1:10 w/v) for 72 hours at room temperature with periodic agitation. The mixture was filtered (Whatman No. 1), concentrated using a rotary evaporator at 40°C, and vacuum-desiccated to a constant weight to ensure complete solvent removal. The extraction yield was calculated relative to the starting dry weight. This crude extract (100% stock) was then diluted to prepare experimental concentrations of 25%, 50%, and 75% (Iacovozzi et al., 2026).

Phytochemical Screening and Quantitative Methods

Qualitative screening for flavonoids, tannins, and steroids was performed using standard colorimetric assays (Wilstätter/cyanidin and Liebermann-Burchard tests). Spectrophotometric quantification determined total phenolic content (TPC; Folin-Ciocalteu method at 765 nm), total flavonoid content (TFC; $AlCl_3$ assay at 415 nm, yielding 24.66 mg/mL), and total steroids (at 520 nm, yielding 45.66 mg/mL). Primary antifungal screening used the Kirby-Bauer disc diffusion method, widely adapted for crude plant extracts against lipophilic yeasts. Preliminary assays yielded a uniform 6.00 mm baseline across all extract concentrations (no clearing zones around the 100% discs), confirming complete ethanol evaporation and rendering an isolated solvent control unnecessary (Liu et al., 2022).

Media Preparation and Environmental Parameters

The in vitro screening used agar supplemented with 1.0% (v/v) sterile commercial olive oil to support the lipophilic growth of Malassezia, which was poured into 90 mm petri dishes. Commercial ketoconazole discs (15 µg), used as the positive control, were stored under vacuum with desiccants at -20°C in absolute darkness and equilibrated to room temperature immediately prior to use (Cassola et al., 2024).

Inoculum Preparation and Disk Diffusion Assay

Actively growing, 72-hour-old *Malassezia furfur* ATCC 14521 colonies were suspended in sterile 0.9% NaCl and adjusted spectrophotometrically to a 0.5 McFarland standard. Following a modified CLSI M44 protocol for lipophilic species, the suspension was swabbed in three directions onto oil-supplemented PDA plates. Within 15 minutes, treatment discs were applied: AC extracts (25%, 50%, 75%, and 100%), a positive control (15 µg ketoconazole), and a negative control (sterile distilled water). After aerobic incubation at 37°C for 48–72 hours, inhibition zone diameters were measured macroscopically using a calibrated digital caliper (mm) (Li et al., 2024).

Data Analysis

SPSS was used for statistical analysis. The Kruskal–Wallis test was used to assess differences between groups due to the non-parametric distribution of the data (caused

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by zero-inhibition values), and the Mann–Whitney U test was used for post-hoc comparisons ($p < 0.05$).

RESULT

Antifungal Inhibition Zone Results

Data collection and laboratory procedures were conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Hang Tuah, Surabaya, in September 2025. This study evaluated the inhibitory potential of *Artocarpus camansi* (AC) extract against *Malassezia furfur* (MF) ATCC 14521 using the disc diffusion method. The experimental units were divided into six distinct treatment groups: a positive control (ketoconazole 15 µg), four concentration-based groups of AC leaf extract (K1: 25%, K2: 50%, K3: 75%, and K4: 100%), and a negative control (sterile distilled water).

Table 1. Results of the Growth Inhibition Zone of *M.furfur* Fungus.

Sample \ Replication	Inhibition Zone Diameter (mm)				Mean
	I	II	III	IV	
Positive control (+)	9.61	9.56	9.50	9.46	9.5325
K1	6.00	6.00	6.00	6.00	6.0000
K2	6.00	6.00	6.00	6.00	6.0000
K3	6.00	6.00	6.00	6.00	6.0000
K4	6.00	6.00	6.00	6.00	6.0000
Negative control (-)	6.00	6.00	6.00	6.00	6.0000

Table 2. Descriptive data of minimum, maximum, average, and standard deviation of inhibition zones for each group

Group	N	Inhibition Zone Diameter (mm)			
		Minimum	Maximum	Mean	Std. Dev
Positive Control	4	9.46	9.61	9.5325	0.6602
K1	4	6.00	6.00	6.0000	0.0000
K2	4	6.00	6.00	6.0000	0.0000
K3	4	6.00	6.00	6.0000	0.0000
K4	4	6.00	6.00	6.0000	0.0000
Negative Control	4	6.00	6.00	6.0000	0.0000.

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Table 1 presents the measured inhibition zones for MF across four replications for each treatment group. In the positive control group, all replications consistently demonstrated inhibition zones exceeding 9 mm (ranging from 9.46 to 9.61 mm), with a mean diameter of 9.5325 mm. Conversely, all experimental groups (K1–K4) and the negative control yielded a measurement of 6.00 mm, indicating an absence of an inhibition zone beyond the disc diameter.

Descriptive statistics, including the minimum, maximum, mean, and standard deviation (SD) of the inhibition zone diameters, are summarized in Table 2. For the positive control group, the minimum and maximum values were 9.46 mm and 9.61 mm, respectively, with a mean of 9.5325 mm (SD = 0.6602). In contrast, groups K1 (25%), K2 (50%), K3 (75%), K4 (100%), and the negative control each exhibited a minimum, maximum, and mean value of 6.00 mm, with a standard deviation of 0.0000. Comprehensive measurement data for all groups are detailed in the tables below.

Statistical Analysis

Due to the absolute homogeneity and lack of variance observed across all experimental extract groups (25%, 50%, 75%, and 100%) and the negative control, in which every replication uniformly yielded a baseline measurement of 6.00 mm (indicating zero inhibition), inferential statistical analyses were substantially simplified. Standard normality and homogeneity metrics were not computable for these groups due to the complete absence of standard deviation. Consequently, comparative hypothesis testing was restricted to evaluating the stark dichotomy between the active positive control group and the inactive treatment/ negative control cluster. While a formal non-parametric Kruskal-Wallis framework technically confirms a mathematical difference across the dataset, the deterministic nature of these uniform bioassay results renders extensive post-hoc *p-value* matrices redundant. The statistical significance is completely driven by the absolute efficacy of ketoconazole (9.53 mm), contrasting against the total physiological inactivity of the extract matrix (6.00 mm).

Table 3. Mann-Whitney U post-hoc results

	Negative Control	Concentration 25%	Concentration 50%	Concentration 75%	Concentration 100%
Negative Control	-	1.000	1.000	1.000	1.000
Concentration 25%		-	1.000	1.000	1.000
Concentration 50%			-	1.000	1.000
Concentration 25%				-	1.000
Concentration 25%					-
Positive Control	0.014	0.014	0.014	0.014	0.014

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DISCUSSION

This study aimed to evaluate the in vitro antifungal efficacy of *Artocarpus camansi* (AC) leaf extract against *Malassezia furfur* (MF) using the disc diffusion method. MF was selected as the target pathogen due to its role as the primary causative agent of pityriasis versicolor. *A. camansi* was investigated based on its traditional use in Indonesia and its taxonomic relationship with *Artocarpus champeden*, which contains prenylated flavonoids with documented antifungal properties (Hu et al., 2023; Leong et al., 2021).

From a biological standpoint, the uniform lack of growth inhibition across all Artocarpus camansi extract concentrations indicates that the plant's secondary metabolites failed to exert a disruptive effect on the viability of *Malassezia furfur* under these experimental parameters (Goh et al., 2022). Rather than focusing heavily on statistical p-values, the interpretation must emphasize the pathogen's evolutionary structural defenses. *Malassezia furfur* possesses a highly unique, rigid outer cell wall dominated by an intricate invaginated lipid layer. This thick, hydrophobic shield regulates structural influx and efflux very strictly, making it structurally distinct from other common pathogenic yeasts (Laokor & Juntachai, 2021).

The phytochemical screening profile indicates that, while the extract contains abundant flavonoids, these compounds are predominantly polar in this crude ethanolic form. Biologically, polar molecules face immense thermodynamic resistance when attempting to partition into or cross the intensely lipophilic and waxy outer envelope of *Malassezia furfur* (Yanthi et al., 2022). Furthermore, the high concentration of steroids detected (45.66 mg/mL) exerts no direct disruption on the fungal ergosterol synthesis pathway, unlike the positive control ketoconazole, which chemically halts cell membrane formation by targeting the lanosterol 14-α-demethylase enzyme (Sogandi & Amelia, 2020). This structural mismatch explains why increasing the extract concentration from 25% to 100% resulted in a flat biological response curve. The fungal cells remain fully structurally viable, indicating that metabolic inhibition in this species requires highly specific, nonpolar, lipophilic vehicles or targeted enzymatic disruptors rather than generic, high-dose crude flavonoid exposure (Wakano et al., 2022).

The lack of antifungal activity observed in this study may be attributed to the complex structural characteristics of the MF cell wall. *Malassezia* species possess a highly hydrophobic cell surface and the ability to form thick biofilms that act as physical barriers against antimicrobial agents (Angiolella et al., 2018). The cell wall of MF is exceptionally lipid-rich and significantly thicker than that of other fungi (Ashbee & Evans, 2002; Celis Ramírez et al., 2020).

Its lipidomic profile, which comprises triglycerides, sterols, fatty acids, and phospholipids, can regulate membrane permeability and influence susceptibility to antifungal compounds (Celis Ramírez et al., 2020; Li et al., 2025).

Phytochemical analysis in this study revealed a flavonoid content of 24.66 mg/mL. Evidence suggests that non-polar compounds penetrate the lipid-dense cell wall of MF more effectively than polar compounds (Saptarini et al., 2024). Since flavonoids are predominantly polar, their capacity to traverse the hydrophobic and lipid-rich barrier of MF is likely limited, preventing the active constituents from disrupting fungal growth. Furthermore, the extract contained a higher concentration of steroids (45.66 mg/mL). Interestingly, the positive control (ketoconazole) produced a relatively small inhibition zone (9.53 mm) compared to standard

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reports, which often cite zones ≥ 28–33 mm for yeasts. This discrepancy underscores that even potent synthetic antifungals face challenges when tested against the robust cell wall of MF (Sanchez-Rodriguez et al., 2023).

The relatively small zone of inhibition observed in the positive control (ketoconazole 15 µg, mean diameter of 9.53 mm) in this study can be explained by several compounding methodological and physicochemical factors. Primarily, ketoconazole is a high-molecular-weight, highly lipophilic imidazole compound (Park et al., 2024). When evaluated using agar-based methods, its hydrophobic nature considerably reduces its lateral diffusion coefficient in the aqueous agar gel matrix. This diffusion restriction is further pronounced by the mandatory inclusion of olive oil in the culture media; the lipid droplets increase medium viscosity and establish a physical partitioning effect that entraps the non-polar ketoconazole molecules, preventing their rapid radial migration from the paper disc (Juntachai et al., 2026).

The biological growth kinetics of Malassezia furfur play a critical role: as a fastidious, slow-growing yeast that typically requires prolonged incubation (48 to 72 hours), the rate of initial cell duplication outpaces the slow passive diffusion of the antifungal agent. Consequently, a stable cell lawn forms before a critical inhibitory concentration gradient can materialize across the agar interface (Juntachai et al., 2026). These combined dynamics justify why agar-based diffusion assays frequently yield tightly constrained inhibition zones for standard azole controls when tested against lipid-dependent fungi, reinforcing that a modest zone diameter does not correlate linearly with reduced intrinsic potency (Jiang et al., 2017).

Interpretation of the results obtained from the disc diffusion method requires careful consideration regarding the technical and biological limitations inherent to testing *Malassezia species*. The absolute absence of a visible inhibition zone around all *Artocarpus camansi* extract discs (yielding a persistent 6.00 mm measurement) should not be definitively interpreted as a complete lack of intrinsic antifungal activity. As outlined in the technical limitations of CLSI M44 and EUCAST agar diffusion protocols, the assay relies heavily on unimpeded passive diffusion of bioactive compounds through the agar matrix. Because *Malassezia furfur* necessitates the mandatory addition of lipid supplements, such as olive oil, into the growth medium to satisfy its lipophilic requirements, the increased viscosity and altered density of the lipid-supplemented agar can significantly impede the lateral diffusion of high-molecular-weight or highly hydrophobic phytochemical constituents (Querequincia, 2025).

This limitation is strongly supported by Rojas et al. (2016), who demonstrated that variables in agar diffusion cannot be modeled by a linear correlation model for Malassezia, meaning that inhibition zone values frequently fail to accurately predict true susceptibility or to reflect exact minimum inhibitory concentrations (MIC). Bioactive components in the crude *Artocarpus camansi* extract, despite their potential fungistatic activity, likely failed to diffuse at a rate sufficient to outpace the rapid initial growth phase of the yeast cell lawn. Consequently, relying solely on standardized agar diffusion protocols may underrepresent the genuine therapeutic efficacy of the extract's polar flavonoids and steroids, cementing the need for specialized broth-based microdilution methods in future updates (Rojas et al., 2017).

Several limitations of this study should be addressed to chart clear directions for future research. First, while phenotypic characterization was used, definitive

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verification of the fungal strain via molecular sequencing (such as ITS rDNA profiling) is recommended to ensure absolute isolate authenticity. Strategically, because the crude ethanolic extract showed no growth-inhibitory zones due to diffusion barriers and complex chemical interference, subsequent studies should focus on bioassay-guided fractionation. Partitioning the crude matrix into distinct non-polar and polar fractions (e.g., using n-hexane, ethyl acetate, and water) could successfully isolate and concentrate the specific lipophilic prenylated flavonoids required to traverse the hydrophobic cell wall of *Malassezia furfur*.

Furthermore, investigating the synergistic dynamics between *Artocarpus camansi* fractions and sub-inhibitory concentrations of synthetic azoles like ketoconazole presents a promising therapeutic avenue; such combinations may disrupt the cell wall matrix or reverse localized tolerance mechanisms, even if the plant metabolites lack robust standalone potency. Lastly, because agar diffusion assays are structurally limited and cannot evaluate specialized fungal lifestyles, future work must transition to broth microdilution assays to establish precise Minimum Inhibitory Concentration (MIC) values, coupled with dedicated biofilm inhibition and eradication assays to determine whether the phytocompounds can effectively impair the protective extracellular polymeric substance (EPS) matrix characteristic of chronic Malassezia skin infections.

CONCLUSION

In conclusion, *Artocarpus camansi* crude ethanolic leaf extract (25%–100%) shows no in vitro antifungal activity against *Malassezia furfur*, likely because its polar metabolites fail to penetrate the fungus's lipid-rich cell wall. Conversely, ketoconazole (15 µg) maintains robust inhibition. To address these limitations, future studies should transition to bioassay-guided fractionation using n-hexane, ethyl acetate, or chloroform to isolate lipophilic compounds. Additionally, researchers should implement liquid broth microdilution assays to determine precise MIC/MFC values, investigate synergistic interactions with ketoconazole using FICI indexing, and evaluate the extract's efficacy against resilient *M. furfur* biofilms.

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