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Antifungal Potential of Ethanol Extract of Ketapang (*Terminalia catappa* L) Leaves in Inhibiting the Growth of *Candida albicans* ATCC 10231 and Active Compound Profile

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ABSTRACT. *Candida albicans is a pathogenic fungus that causes candidiasis and has developed resistance to several antifungal drugs. Ketapang (Terminalia catappa L.) leaves contain active compounds with antimicrobial properties which can developed as an alternative antifungal agents. This study aimed to analyze the antifungal activity and minimum inhibitory concentration (MIC) of ethanol extract of ketapang leaves against C. albicans ATCC 10231 and identify its active compounds. Antifungal assays and MIC tests were conducted using the well diffusion method. Extract partitioning, column chromatography, and Liquid Chromatography–Mass Spectrometry (LC-MS) were used to identify active compounds. Terminalia catappa leaves extract showed the highest inhibition at 20% concentration with a 20.32 ± 0.39 mm inhibition zone and an MIC of 0.1% with a 9.19 ± 0.04 mm zone. The active compounds were found to be polar. Column chromatography yielded seven fractions, none of which exhibited antifungal activity individually. However, when all fractions were combined, a 14.60 ± 0.35 mm inhibition zone was observed. LC-MS analysis identified nine bioactive compounds, five of which, methoxybenzoic acid, cyanidin, pelargonidin 3,5-di-O-glucoside, pelargonidin 3-(6-p-coumaroyl)glucoside, and adamantane-1-carboxylate, are known for antifungal properties. These findings suggest that ketapang leaf extract holds potential as an antifungal agent against C. albicans ATCC 10231, with its activity relying on the combined effect of its active compounds.*

Keywords: *MIC, pathogen, resistance, synergistic*

INTRODUCTION

Fungal infections are among the most easily spread diseases in tropical regions such as Indonesia. The humid environmental conditions present in such climates create a highly favorable environment for the growth of these microorganisms. Public health factors such as age, physiological changes, pregnancy, immune disorders, personal hygiene, obesity, and frequent contact with individuals infected by fungi also contribute to the increasing incidence of fungal infections. A 2023 study conducted at Dr. Hasan Sadikin Hospital reported 40 cases (0.04%) of fungal infections out of 100,000 patients, with most cases found in individuals with a history of HIV/AIDS [1]. This indicates that fungal infections are more likely to occur in individuals with compromised health conditions.

One of the most common fungal pathogens that causes infections in humans is *Candida albicans*. This fungus is frequently cited as the leading cause of fungal infections and is recognized as the primary agent responsible for candidiasis [2]. *C. albicans* is a part of the normal microbiota found on the skin, respiratory tract, and digestive tract of healthy individuals but often causes candidiasis in those with weakened immune systems [3]. A study conducted between 2013 and 2016 at Dr. Soetomo Hospital in Surabaya reported 298 candidiasis cases, predominantly affecting female patients and individuals with a history of diabetes mellitus [4].

Treatment of infections caused by *C. albicans* remains challenging despite the availability of several classes of antifungal drugs. This is mainly due to the growing resistance of *C. albicans* to many commonly used antifungal agents. A 2022 study revealed that *albicans* has developed high resistance to miconazole and econazole and lower levels of resistance to amphotericin B, nystatin, flucytosine, and caspofungin [5]. Antifungal resistance in *C. albicans* is often associated with the improper and unsupervised use of antifungal

medications, including incorrect dosage and treatment duration. Additionally, it is influenced by the virulent characteristics of *C. albicans*, which enable it to survive in extreme environmental conditions [6].

One strategy to address antifungal resistance in *C. albicans* is the development of alternative antifungal treatments. Alternative antifungal compounds can be sourced from natural ingredients, one of which is the leaf of the ketapang tree (*Terminalia catappa* L.). Ketapang leaves are known to contain secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids, which have the ability to inhibit the growth of various microorganisms, including both bacteria and fungi [7].

The antimicrobial efficacy of ketapang (*Terminalia catappa*) leaf extract is supported by existing literature. For instance, recent studies from 2024 have demonstrated the inhibitory activity of its ethanol extract against fungi such as *Aspergillus terreus* and *Trichoderma mantas* [8], while its methanol and aqueous extracts have shown effectiveness against *C. albicans* ATCC 10231 [9]. Beyond its antifungal capacity, the extract has also been confirmed to inhibit bacterial pathogens like *Staphylococcus aureus* [7]. Building upon these findings, the present study was designed to specifically investigate the antifungal potential of the ethanolic extract of ketapang leaves against *C. albicans*. The primary objectives were to determine its Minimum Inhibitory Concentration (MIC) and to perform a phytochemical characterization of its active compounds via Liquid Chromatography–Mass Spectrometry (LC-MS).

RESEARCH METHODS

Extraction of Ketapang Leaves

Samples of mature ketapang leaves (*Terminalia catappa*, folia (7–12) were procured from the campus vicinity of the Chemistry Study Program, Udayana University, Bukit Jimbaran, Bali. Samples were identified in the Plant Taxonomy Laboratory of Biology Study Program, Udayana University. The sample preparation procedure commenced with washing the leaves under running water to eliminate surface contaminants, followed by draining. Subsequently, the leaves were sliced to increase the surface area and dried under ambient conditions for three days. The desiccated material was then mechanically pulverized into a fine powder [10]. The extraction of bioactive compounds was performed via static maceration using 96% ethanol as the menstruum. The powdered simplicia was immersed in the solvent at a 1:10 solid-to-solvent ratio (w/v) within a glass container for a period of 3 × 24 hours [11]. The resulting suspension was filtered through Whatman No. 1 filter paper to separate the filtrate from the solid residue (marc). The final stage involved concentrating the filtrate via rotary evaporation under reduced pressure (Buchi R-300) at a controlled temperature of 40°C to yield a viscous crude extract [12].

Preparation of *C. albicans* Suspension

Colonies of *Candida albicans* ATCC 10231 that had been subcultured on SDA medium were taken using 1–2 inoculating loops and dissolved in 5 mL of 0.9% NaCl physiological solution, then homogenized. The suspension was adjusted to match the turbidity of the 0.5 McFarland standard [13].

Antifungal Activity Test and Minimum Inhibitory Concentration (MIC) of Ketapang Leaf Extract Against *C. albicans* ATCC 10231

The antifungal activity of ethanol extract of ketapang leaves against *Candida albicans* ATCC 10231 was tested using the well diffusion method. A total of 15 mL of *Sabouraud Dextrose Agar* (SDA) medium was poured into sterile Petri dishes (15 mm × 90 mm) and allowed to solidify. The prepared *C. albicans* suspension was inoculated onto the SDA medium using a sterile *cotton swab* and left to dry [14]. Several wells with a 5 mm diameter were created in the agar using a sterile *cork borer*. Then, 20 µL of extract at concentrations of 20%, 15%, 10%, and 5% (w/v), 1% (v/v) Nystatin as positive control, and 96% ethanol as negative control were added into each well using a micro-pipette and allowed to absorb. The Petri dishes were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured using a caliper [15]. The presence of a clear zone indicates the extract's ability to inhibit the growth of the tested pathogenic fungi. The inhibition zone diameter (h) can be used to determine the strength of the antifungal activity, with the following classifications: h < 10 mm (weak), 10–15 mm (moderate), 16–20 mm (strong), and > 20 mm (very strong) [16]. MIC testing was performed by reducing the concentration of the extract that was still capable of inhibiting *C. albicans*, using the same method as the antifungal activity test.

Partition and Determination of the Active Phase of Ketapang Leaf Extract

Partitioning was conducted to separate the polar and non-polar compounds using two solvents of different polarities. A total of 8 grams of crude ketapang leaf extract was dissolved in 400 mL of

n-hexane and 400 mL of methanol and mixed in a separating funnel. The solution was left undisturbed in the separating funnel for 24 hours until two layers were formed. Each layer was collected separately and evaporated using a vacuum rotary evaporator (Buchi R-300) at 40°C to obtain thick extracts of the n-hexane fraction (non-polar) and methanol fraction (polar) [17]. Both fractions were tested for antifungal activity using the well diffusion method, similar to the crude extract test, to determine the active phase. This analysis was important to identify which fraction (polar or non-polar) contained the active compounds associated with antifungal activity.

Fractionation and Determination of Active Fraction of Ketapang Leaf Extract

Fractionation was carried out using column chromatography with a column measuring 30 cm in length and 1.5 cm in diameter. The column was prepared using the wet packing method with 20 g of silica gel 60 mesh, mixed with n-hexane to form a slurry and left to stand for 24 hours [18]. One gram of the active thick extract from the partitioning step was loaded into the prepared column, followed by elution using the step-gradient polarity method. Solvents of increasing polarity: n-hexane, chloroform, ethyl acetate, and methanol was used for elution. The eluted fractions were collected in 30 mL glass containers and labeled. All fractions were analyzed using Thin Layer Chromatography (TLC) to determine which fractions shared the same retention factor (Rf) value. Fractions with the same TLC profile were considered to contain similar compounds and were combined into grouped fractions [19]. These combined fractions were then used for their inhibitory activity test using the well diffusion method to determine the most active fraction. The most active fraction was analyzed using *Liquid Chromatography – Mass Spectrometry* (LC-MS) to identified their active compound.

Analysis of Active Compound in Ketapang leaf Extract Using *Liquid Cromathography – Mass Spectrometry* (LC-MS)

Phytochemical profiling was conducted on a Waters UPLC-MS system, comprising an ACQUITY UPLC® H-Class and a Xevo G2-S QTof mass spectrometer. Chromatographic separation was achieved on an ACQUITY UPLC® HSS C18 column (1.8 µm, 2.1 × 100 mm) maintained at 50°C. The mobile phase, delivered at 0.2 mL/min, consisted of 5 mM ammonium formate in water (A) and 0.05% formic acid in acetonitrile (B), applied in a 23-minute step-gradient. A 5 µL aliquot of the sample, pre-filtered with a 0.2 µm syringe filter, was injected for analysis. The mass spectrometer was operated in positive electrospray ionization (ESI+) mode, scanning a mass-to-charge ratio (m/z) range of 50–1200. Key operational parameters included source and desolvation temperatures of 100°C and 350°C, respectively, and desolvation gas flow of 793 L/h (cone gas was off). Collision energy was ramped from 4 to 60 eV. The MassLynx v4.1 software suite was used for instrument control and data analysis [20].

Data Analysis

Quantitative data from antifungal activity tests, *minimum inhibitory concentration* (MIC) testing, active phase testing, and fractionation testing (in the form of inhibition zone diameters in millimeters) were analyzed using *Analysis of Variance* (ANOVA) at a significance level of 5% ($P \geq 0.05$). If the results showed significant differences ($P \geq 0.05$), the analysis was continued with *Duncan's Multiple Range Test* (DMRT) at a 95% confidence level ($P \leq 0.05$). Statistical analysis was conducted using IBM *SPSS for Windows* Version 30.0.

Qualitative data obtained from the LC-MS analysis were further analyzed using MassLynx 4.1 software to obtain compound mass *spectra*, chromatograms with compound *peaks*, and molecular formulas. The resulting molecular formulas were then identified using the websites (https://www.chemspider.com) and (https://pubchem.ncbi.nlm.nih.gov) to determine the names of the active compounds.

RESULT AND DISCUSSION

Antifungal Activity and Minimum Inhibitory Concentration (MIC) of Ketapang Leaf Extract in Inhibiting *C.albicans* ATCC 10231

The ethanolic extract of ketapang leaves demonstrated significant antifungal activity against *C. albicans* ATCC 10231, evidenced by the formation of clear inhibition zones across a concentration range of 5% to 20% (Figure 1). The inhibitory effect was dose-dependent, with the highest concentration (20%) yielding the largest zone of inhibition at 20.32 ± 0.39 mm. Notably, the extract retained its inhibitory capacity even at the lowest initial concentration of 5%, which produced an inhibition zone of 12.40 ± 0.42 mm. This prompted further serial dilutions to ascertain the Minimum Inhibitory Concentration (MIC). The MIC value was subsequently established to be 0.1%, a concentration that corresponded to an inhibition zone of 9.19 ± 0.04 mm. A comprehensive summary of these antifungal

activity and MIC results is provided in Tables 1 and 2.

Table 1. Antifungal Activity of Ketapang Leaf Extract Againts *C.albicans* ATCC 10231

Extract Concentration	Inhibition Zone Diameter (mm)**	Inhibitory Category
Positive Control	25.62 ± 0.22[a]	Very strong
20%	20.32 ± 0.39[b]	Very strong
15%	16.50 ± 0.33[c]	Strong
10%	14.90 ± 0.18[d]	Moderate
5%	12.40 ± 0.42[e]	Moderate
Negative Control	0.00 ± 0.00[f]	None

**Values in Table 1 are means ± standard deviations from four replicates. Values followed by the same letter in the same column indicate no significant difference ($p > 0.05$) based on Duncan's Multiple Range Test following Anova.

Table 2. Minimum Inhibitory Concentration (MIC) of Ketapang Leaf Extract in Inhibiting the Growth of *C.albicans* ATCC 10231

Extract Concentration	Inhibition Zone Diameter (mm)**	Inhibitory Category
Positive Control	25.58 ± 0.76[a]	Very strong
4%	12.20 ± 0.04[b]	Moderate
3%	12.12 ± 0.02[c]	Moderate
2%	11.93 ± 0.02[d]	Moderate
1%	11.82 ± 0.02[e]	Moderate
0,9%	11.67 ± 0.02[f]	Moderate
0,8%	11.27 ± 0.02[g]	Moderate
0,7%	11.08 ± 0.03[h]	Moderate
0,6%	10.80 ± 0.02[i]	Moderate
0,5%	10.55 ± 0.02[j]	Moderate
0,4%	10.25 ± 0.02[k]	Moderate
0,3%	9.53 ± 0.02[l]	Weak
0,2%	9.33 ± 0.07[m]	Weak
0,1%	9.19 ± 0.04[n]	Weak
Negative Control	0.00 ± 0.00[o]	None

** Values in Table 2 are means ± standard deviations from three replicates. Values followed by the same letter in the same column indicate no significant difference ($p > 0.05$) based on Duncan's Multiple Range Test following Anova.

The results obtained in this study are consistent with several previous studies which reported that ketapang leaf extract has the ability to inhibit the growth of several dermatophyte fungi *(Trichophyton mentagrophytes, Aspergillus fumigatus*, and *Malassezia pachydermatis)* that cause skin infections [21]. Another study also reported that red and yellow methanol extracts of ketapang leaves exhibit antimicrobial activity and are able to inhibit the growth of pathogenic fungi *C. albicans* and *Aspergillus niger*, as well as several pathogenic bacteria [22].

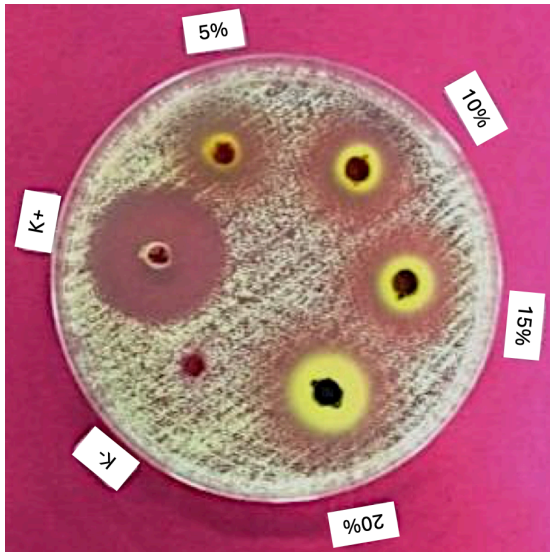


Figure 1. Antifungal Activity of Ketapang Leaf Extract Againts *C.albicans* ATCC 10231.

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Based on the data in Table 1, the strongest antifungal activity of the ethanol extract of ketapang leaves against *C. albicans* ATCC 10231 was observed at a concentration of 20%. However, its inhibitory capacity did not surpass that of the positive control, Nystatin. The inhibition ability of the extract also decreased in line with the reduction in the tested concentrations. This decline in antifungal activity at lower concentrations is closely related to the concentration of active compounds present in the tested extract. The lower the extract concentration, the fewer active compounds are present, thus weakening its inhibitory ability [23].

The MIC test results showed that the ketapang leaf extract was effective at concentration 0.1% as

their minimum concentration, with an inhibition zone diameter of 9.19 ± 0.04 mm. A study conducted in Nigeria in 2024 showed methanolic extract of ketapang leaves was able to inhibit the growth of *A. terreus* with an MIC value of 250 mg/mL and T. mantas on MIC value of 125 mg/mL [8]. Another study from 2015 in Cameroon reported that ketapang leaf extract showed different MIC values against various *Candida* species when extracted with different solvents [24]. The differences in MIC results between this study and previous ones suggest that the MIC values of ketapang leaf extract against fungi may be influenced by several factors, including differences in the fungal species and their pathogenicity, the solvent used for extraction, and the geographic origin of the sampled leaves used for extract preparation.

The results of the MIC test of the ketapang leaf extract against *C. albicans* ATCC 10231 in this study showed better results compared to the n-Hexane extract of the kembang bulan (*Tithonia diversifolia* (Hemsl.) Gray) herb with an MIC value of 400 mg/mL [25]. The MIC value of ketapang leaf extract in this study was also obtained at a lower concentration compared to aloe vera extract. The MIC value of aloe vera extract in inhibiting *C. albicans* ATCC 10231 was obtained at a concentration of 20% [26]. This shows that ketapang leaf extract has better potential than the kembang bulan herb extract and aloe vera extract.

Determination of the Active Phase from the Partitioned Ketapang Leaf Extract

Based on the separation of ketapang leaf extract using methanol and n-hexane solvents, two phases were obtained: a polar phase (methanol fraction) and a non-polar phase (n-hexane fraction), which were then used for antifungal activity test. The results of the antifungal activity test of the partitioned ketapang leaf extract are show in Table 3 and Figure 2.

The antifungal activity results in Table 3 show that antifungal activity was observed in the polar phase of the methanol extract, with an inhibition zone diameter of 20.18 ± 0.11 mm. However, its inhibitory capacity remained lower than that of the positive control, Nystatin. These results indicate that the antifungal active compounds in ketapang leaf extract tend to be polar in nature. This is likely due to the fact that the active compounds in the extract share a relative polarity with the methanol solvent, and are thus more effectively extracted by the polar methanol compared to the non-polar n-hexane, based on the principle of *"like dissolves like"* [27].

Table 3. Antifungal Activity of Partitioned Ketapang Leaf Extract

Treatment	Inhibition Zone Diameter (mm)**
Positive Control	25.79 ± 0.10^{a}
Polar Phase (Methanol)	20.18 ± 0.1^{b}
Non-Polar Phase (N-Hexan)	0.00 ± 0.00^{c}
Methanol Negative Control	0.00 ± 0.00^{c}
N-hexane Negative Control	0.00 ± 0.00^{c}

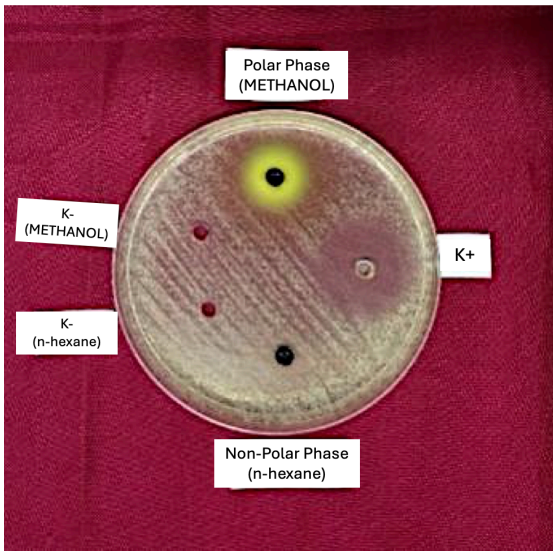


Figure 2. Antifungal Activity of Partitioned Ketapang Leaf Extract

Determination of the Active Fraction from Ketapang Leaf Extract Fractionation Using Column Chromatography

Based on the separation of active compounds in the methanol phase using gradient column chromatography, a total of 43 fractions were obtained. These fractions were then analyzed by thin-layer chromatography (TLC) using chloroform:ethyl acetate:methanol (3:6:1) as mobile phase and Silica Gel GF254 as the stationary phase. The resulting fractions were grouped based on similarities in profiles (spots, color, and Rf values), as shown in Table 4. Visualization of the TLC results is shown in Figure 3.

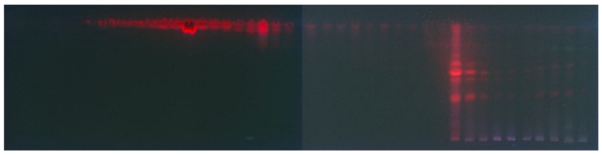


Figure 3. Visualization of TLC Results of Column Chromatography at UV Light 366 nm Wavelength.

Table 4. Thin-Layer Chromatography Results from Column Chromatography

Fractions No.	Retention factor (Rf)	Spot Color Under UV Light (λ=366 nm)	Combined Fractions
1-6	-	Non-fluorescent	FI
2-20	-	Red	FII
21-22	0.8	Purple	FIII
23-33	-	Red	FIV
34	0.32 0.49 0.53 0.60 0.66 0.75	Red Red Red Red Red Red	FV
35-40	0.38 0.50 0.55	Red Red Red	FVI
41-43	0.39 0.55 0.60 0.75	Red Red Red Green	FVII

Based on the TLC profiles of the fractions, the isolated components were grouped into 7 combined fractions. These combined fractions were subsequently tested for their antifungal activity against *C. albicans* ATCC 10231 to identify active antifungal components. The results of antifungal activity testing of ketapang leaf extract fractions are presented in Table 5.

Table 5. Antifungal Activity of Ketapang Leaf Extract Fractions Againts *C. albicans* ATCC 10231.

Fraction	Inhibition Zone Diameter (mm)	Inhibitory Category
FI	0.00 ± 0.00	None
FII	0.00 ± 0.00	None
FIII	0.00 ± 0.00	None
FIV	0.00 ± 0.00	None
FV	0.00 ± 0.00	None
FVI	0.00 ± 0.00	None
FVII	0.00 ± 0.00	None

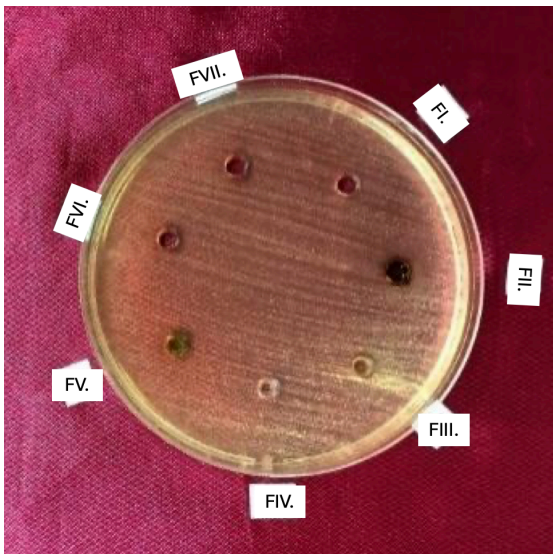


Figure 4. Antifungal Activity of Ketapang Leaf Extract Fractions Againts *C. albicans* ATCC 10231

The results above indicate that ketapang leaf extract did not exhibit antifungal activity after undergoing fractionation, as evidenced by the absence of inhibition zones around the wells in the antifungal testing of all separated fractions (Figure 4.). Consequently, further testing was carried out by recombining all fractions (FI–FVII), followed by antifungal testing using the well diffusion method. The results of the antifungal activity test of the combined fractions (FI–FVII) are shown in Table 6 and Figure 5.

Table 6. Antifungal Activity of Combined Fractions Againts *C. albicans* ATCC 10231

Fractions	Inhibition Zone Diameter (mm)	Inhibitory Category
FI-FVII	14.60 ± 0.35	Moderate

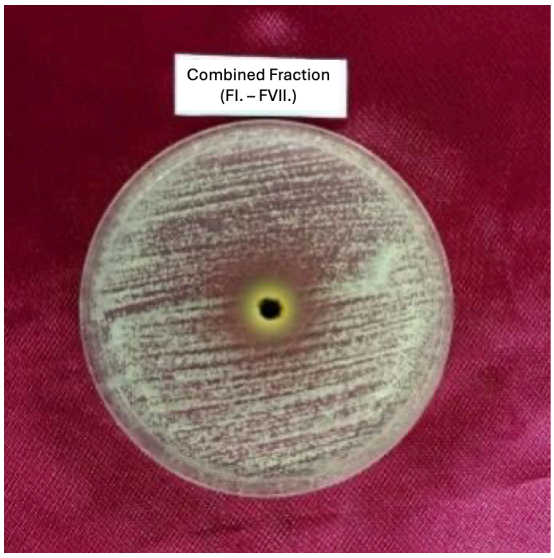


Figure 5. Antifungal Activity of Combined Fractions (FI-FVII) Against *C. albicans* ATCC 10231.

The antifungal activity test of the combined fractions (FI–FVII) against *C. albicans* ATCC 10231 showed inhibitory activity with an inhibition zone diameter of 14.60 ± 0.35 mm (Table 6). Although an inhibitory effect was observed in the combined fractions, the antifungal activity was lower than that of the unfractionated extract. This was indicated by the smaller inhibition zone compared to the methanol extract used in the fractionation process (20.19 ± 0.11 mm) or the ethanol extract at 20% concentration (20.32 ± 0.39 mm). These results suggest that the antifungal potential of ketapang leaf extract is stronger in its crude (unfractionated) form rather than after further purification through fractionation.

These findings align with previous research on the effect of fractions and sub-fraction combinations of *Tamarindus indica* extract on its antimicrobial activity against various Gram-negative and Gram-positive bacterial groups. That study reported that separated fractions and sub-fractions exhibited varying inhibitory abilities against different tested isolates. Several tests demonstrated reduced or lost antibacterial activity following further separation. However, some sub-fractions that lost activity regained antibacterial properties when combined, although still not as effective as before separation. Interestingly, a few fractions exhibited enhanced antibacterial activity after further separation [28]. This phenomenon is closely related to the synergistic interactions among active compounds in plant extracts. Mixtures of plant bio-active compounds can result in synergistic, antagonistic, or neutral effects [29].

The loss of antifungal activity of ketapang leaf extract against *C. albicans* ATCC 10231 after further separation using column chromatography suggests that the active compounds in the extract act synergistically. Fractionation can alter the composition of active compounds within each resulting fraction [30]. potentially separating synergistically acting components into different fractions, thus reducing the overall antifungal activity. The mechanism of synergy of extract compounds can be further explained based on the active compounds identified by LC-MS

Active Compound Content of Ketapang Leaf Extract Based on *Liquid Cromathography – Mass Spectrometry* (LC-MS)

Based on the results of the antifungal activity test of the fractionation products, antifungal activity was observed in the combined fractions. This result was further analyzed using LC-MS analysis to determine the active compound profile contained within. The results of the active compound content analysis of the combined fractions (FI–FVII), which showed antifungal activity, are presented in Table 7. The chromatogram from the LC-MS analysis is shown in Figure 6.

Based on the analysis of the active compounds in the combined fractions of Ketapang leaf extract on Table 7, nine (9) bio-active compounds were identified. Five of these compounds are known from previous studies to possess antifungal properties. These compounds are methoxy benzoic acid, cyanidin, pelargonidin 3,5-di-O-glucoside,

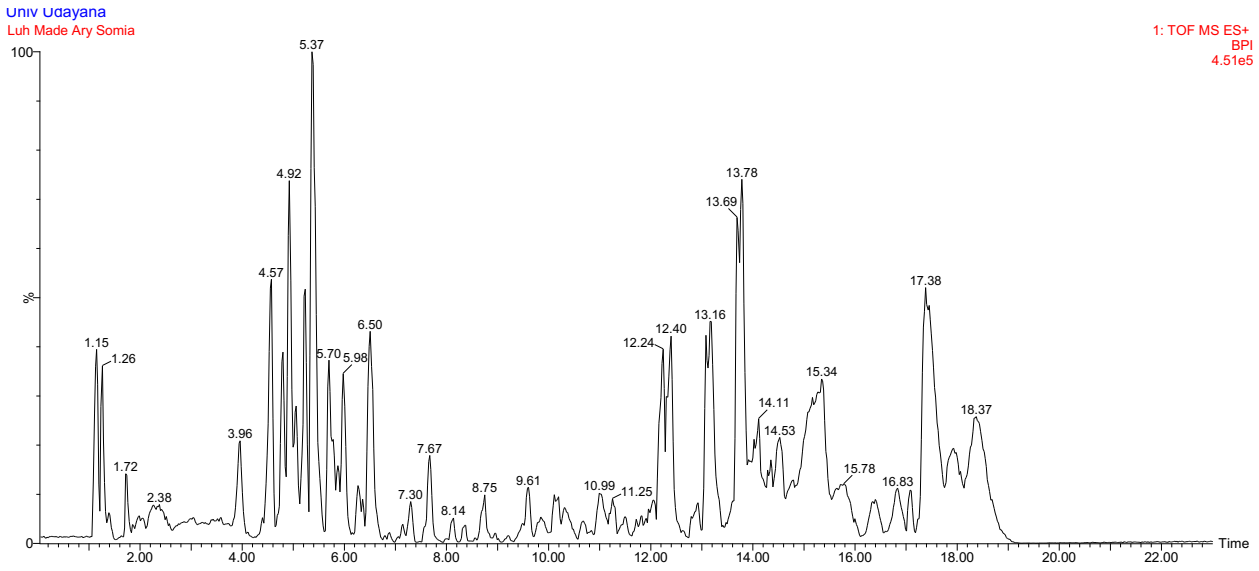


Figure 6. Base Peak Intensity (BPI) Chromatogram of LC-MS Analysis of Combined Ketapang Leaf Extract Fractions

Table 7. Active Compound Content of Combined Ketapang Leaf Extract Fractions Based on LC-MS Results

Retention Time	Ion Mass	Molecular Formula	Compound Name	Biological Role
1.148	115.0370	$C_8H_7O_3$	Methoxy benzoic acid **(PubChem CID 3783514)**	Antifungal [31]
1.261	203.0548	$C_8H_{12}O_6$	Gadusol **(PubChem CID 195955)**	Antioxidan [32]
5.366	287.0567	$C_{15}H_{11}O_6$	Cyanidin **(PubChem CID 128861) (ChemSpider ID 114193)**	Antioxidan and anticancer [33]; antifungal [34]
5.697	595.1658	$C_{27}H_{31}O_{15}$	Pelargonidin 3,5-di-O-glucoside **(PubChem CID 4999763)**	Neuroprotective, antioxidant, and antinflammatory [35]; Antifungal [34]
6.504	179.1082	$C_{11}H_{15}O_2$	Adamantane-1-carboxylate **(PubChem CID 3637755)**	Antimicrobial [36]
7.320	579.1546	$C_{30}H_{27}O_{12}$	Pelargonidin 3-(6-pcoumaroyl)glucoside **(PubChem CID 23724733)**	Antioxidant, antiinflammatory [37]; Antifungal [34]
7.672	207.1403	$C_{13}H_{19}O_2$	Hydron;(2S)-2[4-(2-methylpropyhl) phenyl] propanoic acid **(PubChem CID 67241072)**	Analgesic [38]
9.605	181.1247	$C_{11}H_{18}O_2$	MethylGeranate **(PubChem CID 5365910)**	Aromatic agen [39]
12.242	277.2174	$C_{18}H_{29}O_2$	Pinolenate (**PubChem CID 35027726)**	Antiinflammatory and antiaterogenic [40]

pelargonidin 3-(6-p-coumaroyl)glucoside, and adamantane-1-carboxylate. The presence of these compounds is associated with the formation of inhibition zones in the antifungal activity test of the combined fractions against *C. albicans* ATCC 10231 in this study.

Methoxy benzoic acid is a derivative of benzoic acid known to have antifungal properties. It has been reported to inhibit the growth of *Aspergillus flavus, A. fumigatus*, and *A. terreus* [31]. The antifungal mechanism of methoxy benzoic acid involves undissociated lipophilic acid penetrating the plasma membrane, increasing intracellular acid levels, leading to oxidative stress, lipid peroxidation, and disruption of membrane transport [41].

Cyanidin, pelargonidin 3,5-di-O-glucoside, and pelargonidin 3-(6-p-coumaroyl)glucoside are naturally occurring anthocyanins known for their antioxidant and anti-inflammatory activities [42][43]. In addition to their antioxidant effects, cyanidin and pelargonidin derivatives also exhibit antifungal activity against *Botrytis cinerea*. These compounds inhibit *germ tube* elongation and mycelial diameter. Their antifungal effect increases with higher tested concentrations [33]. Certain small phenolic compounds like cyanidin and pelargonidin can disrupt fungal cell membrane function and bind or polymerize within the hyphal cell wall, thereby inhibiting fungal growth [44].

Adamantane-1-carboxylate is a derivative of adamantane, a compound known to have antimicrobial properties. A 2014 study reported that 14 adamantane derivatives inhibited the growth of several Gram-positive and Gram-negative bacteria, as well as *Candida* spp. The strongest inhibition was observed against *S. epidermidis* ATCC 12228 among bacteria and *C. Albicans* ATCC 10231 among fungi [33]. Adamantane compounds work by increasing membrane permeability. Excessive increase in membrane permeability can cause microbial cell leakage, eventually leading to cell death [45]. This supports the results obtained in the present study, where adamantane-1-carboxylate was found in the combined fractions (FI–FVII), which exhibited inhibitory activity against *C. albicans* ATCC 10231.

The presence of active compounds Methoxy benzoic acid, cyanidin, pelargonidin 3,5-di-O-glucoside, pelargonidin 3-(6-pcoumaroyl) glucoside and adamantane-1-carboxylate with

different working mechanisms shows the synergy of compounds in the extract working with a multi-target mechanism [46]. The mechanism includes disruption of cell membrane permeability by adamantane compounds, thus facilitating other active compounds to enter more easily and disrupt cell function [45]. The next mechanism is followed by an increase in intracellular acid levels which causes oxidative stress and disruption of ion transport in the cell membrane by methoxy benzoic acid compounds, resulting in metabolic disruption in fungal cells [41]. Inhibition of fungal growth is also caused by the presence of cyanidin and pelargonidin compounds that polymerize in the fungal cell wall, thus disrupting the growth of fungal hyphae [45]. The mechanism of disruption of the cell membrane, cell wall, and ion transport system that occurs simultaneously by several compounds in the extract causes more efficient cell death compared to a single compound [46].

CONCLUSION

Ketapang leaf extract has antifungal potential against *C. albicans* ATCC 10231, with an inhibition zone of 20.32 ± 0.39 mm (very strong inhibition) and a minimum inhibitory concentration (MIC) of 0.1% (9.19 ± 0.04 mm). Nine active compounds were identified in the combined fractions of the extract. Among them, methoxy benzoic acid, cyanidin, pelargonidin 3,5-di-O-glucoside, pelargonidin 3-(6-p-coumaroyl) glucoside, and adamantane-1-carboxylate exhibit antifungal properties.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest realetd to this research.

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