PHARMACON
Jurnal Farmasi Indonesia
Pharmaceutical Journal of Indonesia

Pharmacon: Jurnal Farmasi Indonesia
Vol. 22 No. 2, 2025, pp. 151–159
DOI:10.23917/pharmacon.v22i2.10551
p-ISSN: **1411-4283**, e-ISSN: **2685-5062**

Antifungal Potential of *Melastoma malabathricum* L. Leaf Extract against *Candida albicans*: A Phytochemical Approach

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ARTICLE HISTORY:
Submitted : 2025-05-21
Accepted : 2025-12-30
Published : 2025-12-31

KEYWORDS:
Antifungal activity; *Candida albicans;* *Melastoma malabathricum*; Infectious diseases; Phytochemical screening

Citation:
Mayasari, D., Meiliani, S. (2025). Antifungal Potential of Melastoma malabathricum L. Leaf Extract against Candida albicans: A Phytochemical Approach. Pharmacon: Jurnal Farmasi Indonesia, 22(2), 151-159. https://doi.org/10.23917/pharmacon.v22i2.10551

ABSTRACT

Candida species are opportunistic fungi, capable of causing acute and chronic infections in the gastrointestinal tract, vagina, and oral mucosa among which *Candida albicans* is the most important species. *Melastoma malabathricum* L. is one of the most important traditional medicinal plants in Sumatera Island, Indonesia used to treat infectious diseases. The main of this work was to evaluate phytochemical screening of ethanol extract of *M. malabathricum* leaf, qualitative analysis of mixture compounds by TLC analysis and investigation antifungal activity against *C. albicans.* The antifungal activity of plant extract at concentrations of 20%, 40%, 60% and 80% were assessed using agar-well diffusion method. Flavonoids, saponins and tannins were secondary metabolites found in ethanol extract. The extract of *M. malabathricum* leaf formed a zone of inhibition of 6.45 ± 0.002, 6.35 ± 0.023, 6.30 ± 0.050 at concentrations of 20%, 40%, 60%, respectively. In conclusion, *M. malabathricum* leaf had weak antifungal activity. In addition, ethanolic extract had a variety of secondary metabolites that possibly have antifungal activities. Thus, the present findings support the folklore use of *M. malabathricum* for the treatment of different fungal infections.

INTRODUCTION

Candida albicans is one of approximately 200 species in the genus *Candida* that can cause opportunistic infection in the body. Mostly 50% infection of *C. albicans* caused candidiasis, a secondary infection in immunocompromised individuals, and followed by common inhabitant in the oral cavity, bronchial secretions, skin folds, feces, urines and vagina (Talapko et al., 2021). They can cause infectious only when favorable conditions, such as warmer and more humid places for oral and the vaginal cavity (Bonifácio et al., 2019). For hence, vulvovaginal candidiasis (VVC), considered the most common infection affecting 75% of women at least once in their life time, which could also present frequent episodes of recurrence (Ramos et al., 2015). Another example, infection caused by candidiasis affecting mouth called thrush, it has symptom including soreness and difficulty in swallowing (Vanani et al., 2019).

Herbal medicines are still commonly used by about eighty percent of the global population for primary healthcare, particularly in developing countries (Mayasari et al., 2022a). As a tropical country with rich in biodiversity, Indonesia possesses a wide variety of medicinal plants for threating *C. albicans* infections. Several species from Zingiberaceae family including *Alpinia galanga*, *Curcuma longa, Curcuma aeruginosa*, *Zingiber officinale* var. *rubrum* and *Curcuma xanthorrhiza* have demonstrated antifungal potential against *C. albicans* (Prastiyanto et al., 2021). Another example is Cymbopogon citratus from Poaceae family exhibited strong antifungal activity with the lowest minimum inhibitory concentration (MIC) and minimum fungicidal

concentration (MFC) values of 3.91 mg/mL (Geraldi et al., 2022).

The Melastomataceae also includes several medicinal plant species, one of which is *Melastoma malabathricum* L., commonly known as "Senduduk" in Sumatera Island, Indonesia. This species is widely distributed throughout South East Asia, India, Malaysia, Australia, Indonesia and other tropical countries (Mayasari et al., 2021). Traditionally, the leaves of *M. malabathricum* have been used to treat various ailments such as dysentery, diarrhea, hemorrhoids, cuts and wounds, infection during confinement, stomachache, sore legs and thrush (Mayasari et al., 2021a). The plant is often applied topically to stop bleeding or consumed as an herbal remedy for gastrointestinal disorders.

Previous studies reported the chemical components of *M. malabathricum* extracts, including flavonoids (quercetin, kaempferol), flavonoid glycosides (rutin, quercitrin, naringin), tannins (malabathrin A, B, C, D, nobotanins B, D, G, H, casuarictin, strictinin, pterocarinin, pedunculagin and patriscabatrin) (Yoshida et al., 1992). Several studies have been reported regarding biological activity of *M. malabathricum* extracts including antioxidant, antibacterial, cytotoxic, hepatoprotective, antiinflammation, antidiabetic, antihyperlipedemic (Balamurugan et al., 2014; Kamisan et al., 2013; Mayasari et al., 2021b; Mayasari et al., 2022b; Roslen et al., 2014).

Previous investigations on the antifungal potential of *M. malabathricum* have produced varying results. Zhafira et al., 2022 reported that ethanolic leaf extract of *M. malabathricum* tested on concentration 1% to 50% exhibited no inhibitory effect against *C. albicans*. In contrast, Hasan et al. 2019 isolated several endophytic fungal strains from the leaves, roots, and stems of *M. malabathricum*, all of which demonstrated antifungal activity against *Colletotrichum gloeosporioides* with the highest inhibition zones recorded at 57.89% and 52.63% (Hasan & Sudin, 2019). Moreover, previous study by Maji et al. 2010 reported that various solvent extracts of *M. malabathricum* leaves: acetone, aqueous and benzene extracts, exhibited antifungal activity with inhibition zone diameters of 8, 4, 7 mm, respectively. Furthermore, Wiart et al. 2004 observed antifungal activity of the leaf extract against several microorganisms including *Bacillus cereus*, *Bacillus subtilis*, *C. albicans*, *Escherichia coli*, *Pseudomonas aerugiunosa*, and only inhibition for *Bacillus subtilis* with diameter inhibitor of 7 mm (Maji et al., 2010).

Although there are several studies focusing on the biological activity of *M. malabathricum*, there are varying outcomes concerning bioactive substances. This is regarding where the plants grow (habitat) which influenced medicinal plants and phytochemistry. Therefore, here we have further evaluated the antifungal activity of *M. malabathricum* extract against strains of *C. albicans*, the most common etiological agent of candidiasis.

METHODS

Materials

All solvents: ethanol, n-butanol, acetic acid, chloroform, benzene, and methanol were of analytical grade supplied from Merck (Merck, Darmstadt, Germany). Ferric (III) chloride, sulfuric acid, Meyer, Dragendroff were used for the phytochemical screening assay. Extracts were monitored by TLC silica gel 60 F 254 nm plates (Merck). Ketoconazole was used as a positive control, and aquadest was used for negative control for antifungal test. All chemical solvents used were analytical grade. The microbial media Sabouraud dextrose agar (SDA) was procured from HiMedia Laboratories, India (Mumbai, India), whereas nutrient agar (NA) was obtained from Thermo Fisher Scientific (Basingstoke, England).

Collection and identification of plant material

Figure 1. *Melastoma malabathricum* leaves (Author's documentation, 2022)

Fresh leaves of *Melastoma malabathricum* L. (**Figure 1**) were collected from Sukaramai,

Tapung Hulu district, Kampar Regency, Riau Province, Indonesia in December 2022. The plant specimens were identified and authenticated at the Department of Biology, Faculty of Math and Science, Universitas Riau. The samples were dried in a hot air oven at 50°C, homogenized to a fine powder, and stored for further analysis.

Preparation extracts of *M. malabathricum* leaf

Five hundred grams (500 g) of *M. malabathricum* leaf powder were macerated with 1,8 liters ethanol 96% for three days. The extract was filtered, and the residue was re-macerated for an additional two days with ethanol. The filtrates were combined and evaporated using a rotary vacuum evaporator to obtain a viscous semi-solid mass. The yield of the extract was calculated and recorded. The crude ethanol extract was subjected to thin-layer-chromatography (TLC) analysis, phytochemical screening and antifungal assay.

Thin layer chromatography

TLC analysis was performed on silica gel 60 F254 plates (10x5 cm) with several mobile phases containing: n-butanol: acetic acid: water (4: 1: 5) (1), chloroform: methanol: water (13: 7: 2) (2), chloroform: benzene: ethanol (45: 45: 10) (3). The plates were placed in a saturated chamber for 20 minutes prior to development. After eluation, the TLC plates were dried under a warm air and visualized at wavelengths of 254 nm and 365 nm.

Phytochemical screening

Qualitative phytochemical screening for the plant extract was performed to determine the present of saponin, alkaloid, phenolic, tannin, steroid, terpenoid and flavonoids, according to the standard qualitative analysis of phytogenic compounds described by Harborne et. al. (Harborne, 1998).

Preparation of extract concentrations for antifungal test

The ethanol extract was dissolved in dimethyl sulfoxide (DMSO) to prepare concentrations of 20%, 40%, 60% and 80% (w/v).

Media preparation and inoculum standardization

The fungal medium was prepared following the manufactures' directions and specifications. *Candida albicans* (ATCC 10231) was used in this study. The fungal turbidity of each species was prepared and standardized by the Clinical and Laboratory Standard Institute (CLSI) guidelines. Candida species were sub-cultured in Sabouraud Dextrose Agar (SDA). 0.5 McFarland standard was employed to balance the turbidity of the Candida inoculum.

Antifungal assay

The antifungal activity of the *M. malabathricum* ethanol extract was evaluated through agar well diffusion method, with modifications based on Asmerom et al., 2020. In short, the actively growing *C. albicans* broth cultures was standardized to a density of 0,5 McFarland standard. The media for the Candida species were MHA supplemented with 2% glucose and 0.5 ⌈g/mL methylene blue. Wells of 6 mm diameter were bored aseptically into the agar using a sterilized cork borer and labeled according to the test concentrations. Each well was filled with 20%, 40%, 60% and 80% of the extract solution. Ketoconazole (dissolved in DMSO) served as the positive control. Plates were allowed to stand for 2 hours at room temperature for diffusion, then incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters (mm) using digital caliper. The experiment was performed in triplicate, and the mean were recorded. The average zone of inhibition was calculated for extract and the standard antibiotics.

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA; licensed), followed by post-hoc tests where applicable. Results were considered statistically significant at $p < 0.05$.

RESULT AND DISCUSSION

Preparation extract of *M. malabathricum* leaves

The leaf parts of the plant were shade dried and made into coarse powder. Extraction process was done by maceration method using ethanol as solvent. Maceration extraction method is extraction technique in which coarsely powdered drug material, either leaves, stem bark or root bark, is placed inside a container, the menstruum is poured on top until completely covered the drug material (Abubakar & Haque, 2020). Extracts were concentrated by removal of solvent under reduced pressure and finally residue was dried in vacuum. The yield of extraction process resulted 16.876 g extract with percentage of rendemen 3.375%w/w as shown in **Table 1**.

Table 1. Preparation of ethanol extract of *M. malabathricum* leaf

Powder leaf (g)	Ethanol extract (g)	Rendemen (%)
500	16.876	3.375

Phytochemical analysis

Extract was subjected to preliminary phytochemical screening to identify phytoconstituents through qualitative chemical reagents. The relationship between biological activity and secondary metabolites was investigated in the phytochemical screening test. The phytochemical tests revealed the presence of flavonoid, tannin, and saponin. In contrast, there is no presence of alkaloids and triterpenoids in ethanol extract of *M. malabathricum*. The results of preliminary of phytochemical test were given in **Table 2**.

Table 2. Qualitative phytochemical screening of extract od *M. malabathricum* leaf

Phytochemical test	Reagent	Result	Explanation
Alkaloids	Dragendorf	-	No alkaloids
Flavonoids	Mg + HCl	+++	Presence of flavonoids
Triterpenoids	Vanillin $H2SO_4$	-	No triterpenoids
Tannins	$FeCl_3$ 10%	+++	Presence of tannins
Saponin	Hot water + 2N HCl	+++	Presence of saponin

Phytochemical screening is an initial step to determine the secondary metabolite compounds contained in the plants. The phytochemical screening test was carried out using a color reagent. The ethanol extract of *M. malabathricum* was tested for phytochemical screening which included tests for alkaloids, flavonoids, triterpenoids/steroids, tannins and saponins. Based on the phytochemical screening test, *M. malabathricum* extract is positive containing flavonoids, tannins and saponins. This finding is related to inhibitory effect of *M. malabathricum* extract on the growth of microorganisms especially antifungal activities, due to the presence of secondary metabolite. Previous studies showed that antifungal properties were reported due to the presence of saponins and flavonoids (Tan et al., 2022).

Flavonoids are secondary metabolites that is identifies as broad classes of polyphenol found plenty in plants. Flavonoids have been found to be effective antifungal agents against a wide range of pathogenic organisms. Several flavonoid compounds showed antifungal activities against *C. albicans* including isoflavane; glabridin (MIC 6.3-12.5 µg/mL), flavononones; hesperetin (MIC 7.8-500 µg/mL), flavonol; vincetoxicoside B (MIC 64 µg/mL), rutin (MIC 256 µg/mL), quercitrin (MIC 7.8-256 µg/mL), quercetin (MIC 31.2-125 µg/mL), myricitrin (MIC 3,9-83 µg/mL), isoquercitrin (MIC 2.5-5.0 µg/mL), galangin (MIC 15,6-1000 µg/mL), fisetin (MIC 8-128 µg/mL), avicularin (MIC 2-32 µg/mL) (Aboody & Mickymaray, 2020).

Flavonoids inhibit fungal growth with various underlying mechanisms, including plasma membrane disruption, the introduction of mitochondrial dysfunction, and inhibiting the following: cell wall formation, cell division, RNA and protein synthesis, and the efflux mediated pumping system (Al Aboody & Mickymaray, 2020). Tannin is one of the active compounds of secondary metabolites which has antifungal activity with binding to the fungal membrane sterol. Two mechanisms of antifungal action involving ergosterol, first, bind to membrane ergosterol forming pores in the structure, second, inhibit enzymes in the synthesis ergosterol (Carvalho et al., 2018).

Thin layer chromatography

Thin layer chromatography (TLC) of the ethanol extrcat of *Melastoma malabathricum* was performed using different solvent systems.

The chromatogram revealed distinct spots with varying intensities under UV light at 254 nm and 365 nm, indicating the presence of multiple secondary metabolites (**Figure 2**). The Rf values of the detected spots are summarized in **Table 3**.

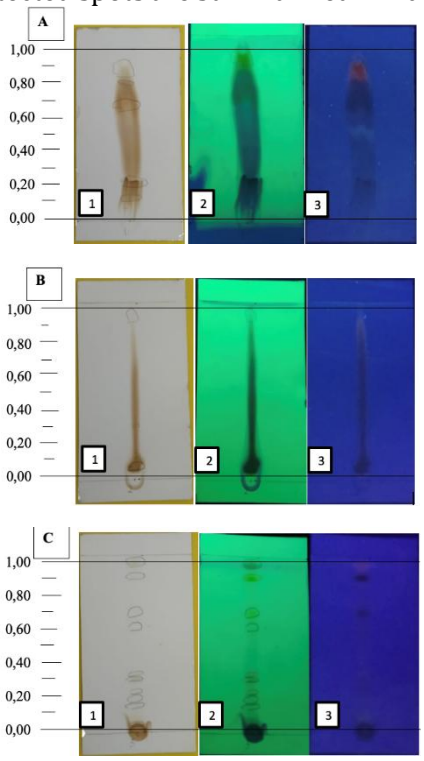

Figure 2. TLC profiles of *M. malabathricum* leaf extract in three solvent systems: A) n-butanol: acetic acid: aquadest (4: 1: 5 v/v/v); B) chloroform: methanol: aquadest (13: 7: 2 v/v/v); C) chloroform: benzene: ethanol (45: 45: 10 v/v/v). The baseline, solvent front, and sample lanes are indicated on each plate. Major separated bands are marked and visualized under visible light (1), UV 254 nm (2), and UV 365 nm (3). In the third mobile phase (C), the compounds appear to be better separated, showing clearer and more distinct to other solvent systems.

Among the solvent systems tested, chloroform: benzene: ethanol (45: 45: 10, v/v/v) provided the best separation pattern, producing several well-defined spots. The variations in Rf values reflect the differences in compound polarity, which is typical of plant extracts rich ini flavonoids and tannins. These findings are consistent with the results of the phytovhemical screening, confirming that M. malabathricum contains a complex mixture of polyphenolic compounds. Generally, compounds with higher polarity tend to migrate more slowly due to stronger interactions with the polar stationary phase, while less polar compounds move faster with the solvent front (Astefanei et al., 2017).

Table 3. TLC analysis of ethanol extract of *M. malabathricum* leaf

Mobile phase	Number of spots	Rf value	Intensity
n-butanol: acetic acid: aquadest (4: 1: 5 v/v/v)	5	0.13; 0.25; 0.7; 0.81; 0.91	strong
chloroform: methanol: aquadest (13: 7: 2 v/v/v)	4	0.08; 0.13; 0.31; 0.93	strong
chloroform: benzene: ethanol (45: 45: 10 v/v/v)	11	0.06; 0.15; 0.2; 0.23; 0.3; 0.33; 0.61; 0.68; 0.85; 0.88; 0.97	moderate

The presence of multiple spots in the TLC profile suggest that *M. malabathricum* leaves contain diverse bioactive constituents that may act synergically to produce antifungal effects. Previous studies reported that flavonoids and tannins identified in this extract,can disrupt fungal cell membranes and inhibit ergosterol synthesis leading to growth suppression of *C. albicans* (Aboody & Mickymaray, 2020). Therefore, the separation profile obtained from TLC supports the observed antifungal acitovoty and indicates that the active compounds responsible for inhibition zones. Further chromatographic purification and compound isolation are required to confirm the spesific constituents contributing to the antifungal effects.

Antifungal activity

Antifungal activity was assesssed using the agar-well diffusion method against *C. albicans* at extract concentrations of 20%, 40%, 60% and 80%, presented in **Table 4**. The inhibition zone values are also illustrated in **Figure 3**, which clearly shows the decreasing trend of antifungal activity with increasing extract concentration. In this finding, the extract exhibited weak antifungal activity, with inhibition zone diameters of 6.45 mm, 6.35 mm, and 6. 30 mm for concentration s of 20%, 40%, and 60%, respetively. No inhibition zone was observed at 80% concentration. Based on classification by Ouchari et al. 2018, inhibition zone< 10 mm indicate weak antifungal activity. Responses to

inhibition of fungal growth were classified based on the diameter of the clear zone, namely <10 mm (weak); 10-15 mm (medium); 16-20 mm (strong) and >20 mm (very strong). (Ouchari et al., 2018).

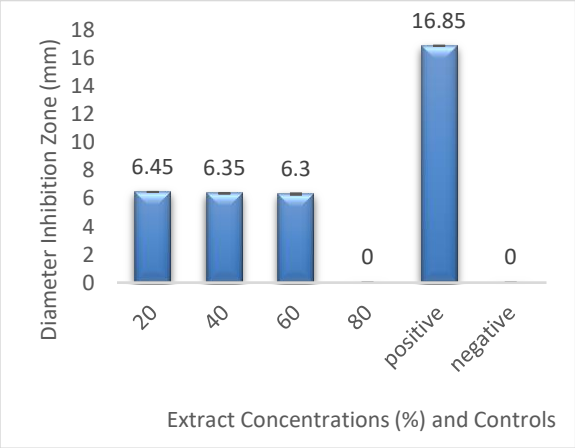

Figure 3. Inhibition zone diameters (mm) produced by the extract at various concentrations (%) against *C. Ibicans*, compared to positive and negative controls. Data are presented as mean values from triplicate assays.

Table 4. Diameter inhibition zone of ethanol extract of *M. malabatahricum* leaf against *C. albicans*

No	Sample	Diameter inhibition zone (mm)
1.	20%	6.45 ± 0.002
2.	40%	6.35 ± 0.023
3.	60%	6.30 ± 0.050
4.	80%	-
5.	Positive control	16.85 ± 0.002
6.	Negative control	-

Values are expressed as mean ± SEM (n=3), and the statistical analysis was performed using one-way ANOVA ($p<0.05$).

Interestingly, the lower extract concentration (20%) produces slightly larger inhibition zone compared to the higher concentrations. This unexpected pattern may be attributed to the limited diffusion of the viscous extract at higher concentrations, which can restrict the movement of active compounds through the agar medium (Nostro, 2020). Highly concentrated extracts tend to be more saturated and less soluble, reducing the effective diffusion rate and availability of antifungal constituents on the agar surface.

These findings are consistent with the previous describing in the inherent limitations of diffusion-based assay. The discrepancies highlight that steric hindrance within the solid agar structure can impede the diffusion of test substances, leading to an apparent reduction in activity even when the compound possesses bioactive potential (Fernandes et al., 2022). Similar limitations have been documented when diffusion assays are applied to poorly soluble molecules (Balouiri et al., 2016). This phenomenon does not necessarily indicate a lack of antifungal efficacy but rather reflects restricted leaching or poor diffusion through agar medium. Physicochemical factors such as molecular weight, solubility and diffusion rate strongly influence the ability of test substances to migrate and exert their antifungal action (Puxeddu et al., 2025).

Although the ethanol extract of *M. malabathricum* showed weak inhibition against *C. albicans*, the results support its potential antifungal properties and traditional use. Future studies involving purified fractions or alternative assay may provide a more accurate assessment of antifungal activity and overcome the limitations of diffusion-based methods.

CONCLUSIONS

The qualitative and quantitative analysis provide essential information about the plant. The findings confirm the presence of several key bioactive compounds and indicate that the leaves also possess medicinal value. As a species with notable morphological variability, these data are useful for distinguish *M. malabathricum* from closely related taxa for quality control purposes. These results support efforts in standardization, identification and further research on lef-based *M. malabathricum*, used in both traditional and modern therapy. As this study serves as a preliminary investigation, additional research is needed to isolate and characterize the bioactive components in *M. malabathricum* leaves, with the goal of identifying lead compounds that could advance future efforts in plant-based antifungal discovery and development.

ACKNOWLEDGMENT

The authors graciously acknowledge the support given by Department of Pharmacy, Natural Product laboratory and Microbiology laboratory Universitas Abdurrab for institutional support and assistance.

AUTHORS' CONTRIBUTIONS

Dian Mayasari: designed the experimental, analyzed the data and wrote the original manuscript; Siska Meiliani: conducted the research.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

ETHICAL CONSIDERATION

Plagiarism

No portion of this work has been reproduced without proper acknowledgement of its original source. All citations and references have been provided in full compliance with academic standards.

Data Fabrication

All data presented in this study was obtained directly from the research conducted and have not been altered, fabricated, or manipulated in any form.

Multiple publications

This research represents original work that has not published previously and is not under consideration for publication in any other journals simultaneously.

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