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Research Article

Can *Moringa oleifera* Leaf Ethyl Acetate Extract Inhibit *Candida albicans* Planktonic Cell Growth and Biofilm Formation *In Vitro*?

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Abstract



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Introduction: *Candida albicans* (*C. albicans*) is the most common fungus found in the human oral cavity. This fungus has the ability to form a biofilm that causes infectious diseases in the oral cavity. Nowadays, the incidence of infectious diseases caused by *C. albicans* was increasing due to resistance to antifungal drugs. This study aimed to investigate the effect of *Moringa oleifera* ethyl acetate extract on the inhibition of *C. albicans* planktonic cell growth and biofilm formation in vitro. **Methods:** *C. albicans* (ATCC 10231) was the fungus used in this study. Determination of inhibition planktonic cell growth by microdilution method. The polystyrene microplate assay method was used to test the inhibition of *C. albicans* biofilm formation. The extract concentrations used in this study were 25%, 12.5%, 6.25%, 3.13%, and 1.57%, respectively. A crystal violet (CV) assay assessed the biofilm's inhibitory activity. **Results:** The minimal inhibitory concentration of *Moringa oleifera* leaf ethyl acetate extract against the planktonic form of *C. albicans* was found to be 1.57%. Starting at 6.25% concentration, *Moringa* leaf ethyl acetate extract inhibits the formation of *C. albicans* biofilm. **Conclusion:** Since *Moringa oleifera* leaf ethyl acetate extract inhibits *C. albicans* planktonic and biofilm formation, it has the potential to be developed as an alternative anti-fungal agent.

Keywords: *Moringa* leaf extract, planktonic cells, biofilm, *Candida albicans*

INTRODUCTION

The most common fungus in the human oral cavity is *C. albicans*.¹ This fungus is commensal in healthy people, but it can be a pathogen if the oral cavity biology was changed.² *C. albicans* is the most abundant species worldwide, accounting for 66% of all *Candida* species. The prevalence of *C. albicans* as the dominant species has increased from 37% in Latin America to 70% in Norway as a result of rising candidiasis incidence, increased populations of susceptible individuals, and treatment delays caused by antifungal drug resistance.³

In humans, *C. albicans* is the most virulent and pathogenic species. This species can produce biofilms that cause infectious diseases in the oral cavity.⁴ According to the research,⁵ *C. albicans* is one of the fungi that can cause disease in humans. The ability of this fungus to form biofilms is its most virulent aspect. Antifungal drugs are not effective against the biofilm that has formed. As a result, research is needed to develop alternative materials that can inhibit the formation of biofilms as a strategy for developing anti-fungal agents.

Moringa oleifera is a plant that originated in the southern Himalayan hills. *Moringa* is a long-lived plant that can reach a height of 7-12 meters. The leaves are long-stemmed compound leaves with alternating leaf arrangements. *Moringa* leaves are light green when young, but as they mature, the green color darkens.⁶ *Moringa* is a plant that has numerous

health benefits, including anti-cancer⁷, anti-inflammatory⁸, anti-oxidant⁹, anti-microbial¹⁰, and immune response¹¹. By denaturing proteins and causing cell membrane damage, phenols, particularly flavonoids, can inhibit fungi activity. This harm has the potential to kill fungal cells.¹²⁻¹⁴

In carrying out the extraction, the right solvent is needed to get the optimal extract. Factors that need to be considered in the selection of solvents include selectivity, toxicity, polarity, and ease of evaporation.¹⁵ Ethyl acetate is a solvent with low toxicity that is semi-polar, therefore it is expected to attract polar and non-polar compounds from *Moringa* leaves.

Although studies on the advantages of moringa leaves as herbal constituents have been conducted, there has not been much research on the advantages of moringa leaves as an antifungal, notably the inhibitory effect on the production of oral biofilms. The aim of this study was to examine the effects of ethyl acetate leaf extract from *Moringa oleifera* on *C. albicans*' planktonic cell growth and biofilm formation in vitro.

MATERIALS AND METHODS

Plant Collection and Extraction

The *Moringa* plant is harvested for its leaves in the Purwosari, Sinduadi, Mlati, Sleman regions, in Indonesia. The Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, conducted plant identification. The

extraction of the secondary metabolite from Moringa leaves was carried out according to ¹⁶ with slight modifications. After cleaning under running water, the moringa leaves dried in an oven for 48 h at 50°C. Then, turned into powder after drying. The powder was mixed with ethyl acetate in the ratio 1: 7 (w/v). for 24 h., after which the filtrate was filtered and concentrated at 60°C. The extracted substance weighed 20.1 gr. The basic extract solution was made by dissolving 5 grams of Moringa leaf extract in 5 ml of 2% DMSO. After measuring the extract's pH, the solution was filtered.

Determination of Inhibition Planktonic Cell Growth

To determine the concentration of moringa leaf extract that can inhibit planktonic cell growth using the Minimum Inhibitory Concentration (MIC) test. The test was performed at the Integrated Research Laboratory, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta. The initial inoculum of *C. albicans* (ATCC 10231) was inoculated into 5 ml of Sabouraud Dextrose Broth (SDB) medium, incubated for 24 h at 37°C, and adjusted to 0.5 Mc. Farland standard.

The MIC of moringa leaf extract was determined by the microdilution method. ¹⁷ The different concentrations of moringa leaf extract were plated on a 96-well flat-bottom microplate, including the positive control (Nystatin), and the negative control (Phosphate Buffer Saline = PBS). Furthermore, the fungal suspension was inoculated to the well that contains various concentrations of extract and control to obtain the final concentration of *C. albicans* 1 x 10⁶ CFU/mL. Then it was incubated for 24 h at 37°C in aerobic condition. MIC was indicated by the absence of turbidity after incubation and the absorbance was measured at 595 nm using a microplate reader. The experiment was performed in triplicate.

Determination of Inhibition Biofilm Formation

The inhibition of *C. albicans* biofilm formation was performed using a polystyrene microplate assay method according to ⁴ with modification. In the first, 100 µL/well standard *C. Albicans* suspension was inoculated in triplicate into wells of a sterile flat-bottomed microtiter plate and incubated for 90 minutes at 37 °C for initial adhesion. Following a 90-minute incubation period, the plate was carefully washed twice with 200 µL of sterile phosphate-buffered saline (PBS),

Furthermore, 50 µLSDB and 50 µL extracts of various concentrations of Moringa leaf extract or positive and negative control were added, then the plate was incubated for 24 hours at 37°C. The inhibitory activity of the biofilm formation was measured using a crystal violet (CV) assay.

For the CV assay, after incubating, the non-adherent cell was aspirated. A 100 µL of 1%, CV solution was added to each well and incubated for 20 minutes at 37 °C before the plate was carefully washed twice with sterile PBS. Finally, CV-stained cells were decolorized by adding 200 µL of 95% ethanol per well. The absorbance at 595 nm was measured using a microtiter plate reader after 100 µL of ethanol was transferred to a new microtiter plate.

Statistical analysis

A one-way analysis of variance was used to analyze the effect of various concentrations of Moringa leaf extract in inhibiting planktonic cells of *C. albicans* growth and biofilm formation, followed by the Post Hoc LSD test.

RESULTS

a. The result of plant identification:

Division: Tracheophyta

Sub Division: Spermatophyta

Class: Magnoliopsida

Super Order: Rosanae

Order: Brassicales

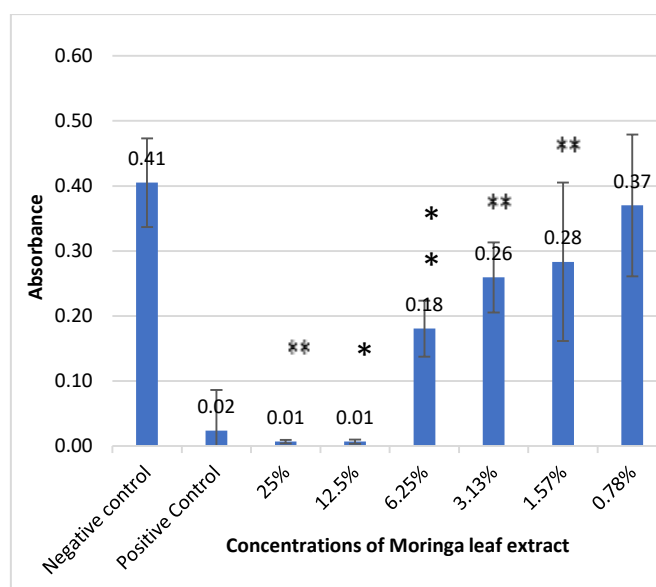
Family: Moringaceae

Genus: Moringa

Species: Moringa oleifera Lam.

b. Results of ethyl acetate extract of Moringa leaf against *C. albicans* in inhibiting planktonic cell growth.

The microdilution method was used to conduct the inhibiting planktonic cell growth using different concentrations of Moringa leaf ethyl acetate extract (25%, 12.5%, 6.25%, 3.13%, 1.57%, and 0.78%). Figure 1 displays the outcomes of the microplate reader-based absorbance measurement.



** sig. p < 0.01, Inhibit planktonic cell growth starting from 1.57% concentration,

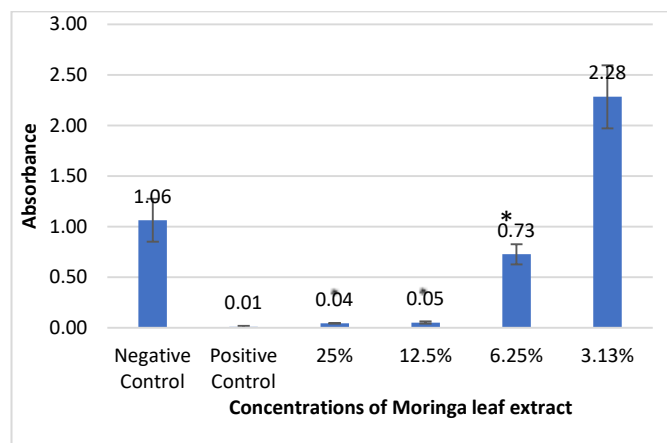
+ Control: Nystatin, - Control: PBS

Figure 1: Inhibition of Moringa leaf ethyl acetate extract against *C. albicans*

According to our findings that the growth of *C. albicans* could be greatly inhibited by an ethyl acetate extract of Moringa leaf at a concentration of 1.57% (MIC).

c. Results of ethyl acetate extract of Moringa leaf against *Candida albicans* in inhibiting biofilm formation.

The polystyrene microplate assay method was used to study the formation of *C. albicans* biofilms. This study used extract concentrations of 25%, 12.5%, 6.25%, and 3.13%. A crystal violet (CV) assay was assessed to determine the biofilm's inhibitory activity. Figure 2 shows the potential of Moringa leaf ethyl acetate extract in inhibiting a biofilm of *C. albicans*.



* Sig. $p < 0.05$, Inhibition of biofilm formation starting at 6.25% concentration,

Positive Control: Nystatin, Negative Control: PBS

Figure 2: The potential of Moringa leaf ethyl acetate extract in inhibiting *C. albicans* biofilm formation

Based on Figure 2. The absorbance of the ethyl acetate extract of Moringa leaf started at a concentration was 6.25% and upper, lower than the absorbance in the negative control group. This means that an ethyl acetate extract of Moringa leaf at a concentration of 6.25% can inhibit the formation of *C. albicans* biofilm.

DISCUSSION

Based on the results of this study (Figure 1), the lowest inhibitory concentration of the ethyl acetate extract of Moringa leaf was lower when compared to the ethanol extract of Moringa leaf in our previous study (MIC: 6.25%)¹⁸, which suggests that the ethyl acetate extract of Moringa leaf has greater potential as an antifungal *C. albicans*. This is probably due to the fact that ethyl acetate is a semi-polar solvent capable of attracting compounds with a wide polarity range from polar to non-polar.¹⁹ This ability causes the metabolites contained in the ethyl acetate extract of Moringa leaf to be attracted more than using ethanol as a solvent, such as alkaloids, tannins, saponins, and flavonoids. Flavonoids are the largest group of phenolic compounds, which have effective properties against bacteria, viruses, and fungi.¹²

This study supports work²⁰, which found that a moringa fruit extract can inhibit the growth of *C. albicans*. The research demonstrates that flavonoids are present in the fruit of the moringa plant. Because they cause protein denaturation and enhance the permeability of fungal cell membranes, flavonoids can limit the growth of fungi. This increase in membrane permeability causes damage to fungal cells which causes death in fungi. According to Dewi²¹, flavonoids have the potential to serve as antifungals because they include phenolic compounds that can combine with ergosterol to generate complex molecules in fungal cell membranes. This phenol and ergosterol compound make fungal cells' pores larger, which allows tiny molecules like nucleic acids and proteins to exit the cells and induce death. This study is also consistent with the work¹², which investigated the flavonoid baicalein's antifungal properties against various *Candida* species. The findings demonstrated that *C. albicans*, *C. tropicalis* and *C. parapsilosis* cell viability and proliferation were both suppressed by the flavonoid baicalein.

Alkaloids, like flavonoids, have antifungal properties. Alkaloids are semipolar metabolites.¹⁹ The mechanism of alkaloids as antifungals is to insert them between the cell wall and DNA, preventing fungal DNA replication and thus disrupting fungal

growth.²³ Moringa leaf ethyl acetate extract can attract tannin compounds. Tannins are included in the non-polar polyphenol group.¹⁹ The antifungal mechanism possessed by tannins is their ability to inhibit the synthesis of chitin which is used for the formation of cell walls in fungi and damage cell membranes, hence that fungal growth is inhibited. Tannins are lipophilic compounds that are easily attached to fungal cell walls.^{23,24} Saponins are also compounds that can be extracted with ethyl acetate as a solvent. Saponins have a glycosyl that functions as a polar group and a steroid group as a non-polar group.²⁵ The mechanism of saponins as antifungals is by disrupting the stability of cell membranes, resulting in the lysis of microbial cells.²³

Figure 2 showed that ethyl acetate extract of Moringa leaf at a concentration of 6.25% can inhibit the formation of *C. albicans* biofilm. According to²⁶, the formation of *C. albicans* biofilms is dependent on yeast cell attachment to a surface, followed by yeast cell attachment to each other. Hyphae formation is the main component of *C. albicans* biofilm formation. Secondary metabolites produced by ethyl acetate extract of Moringa leaf have the potential to reduce *C. albicans* to form hypha, preventing the formation of biofilm. This is consistent with the study of²⁷, which showed that flavonoids had a modest inhibitory effect on the growth of *C. albicans* hypha. Apigenin, apigenin, and isoquercitrin molecules demonstrated the highest capacity to suppress hypha development among the examined flavonoid groupings. Another theory is that yeast cells may be prevented from adhering to surfaces by the ethyl acetate extract of Moringa leaf, preventing biofilm formation. This is consistent with research done by²⁸, who examined the effectiveness of flavonoids derived from Moringa seed shells against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The results showed flavonoids can inhibit cell attachment and interfere with biofilm formation and biofilm metabolic activity.

Because the mechanism of inhibition of *C. albicans* biofilm formation by ethyl acetate extract of Moringa leaf is still unknown, more research is required. Furthermore, more research is needed to determine the compound of Moringa leaf ethyl acetate extract, which has the potential to inhibit the formation of *C. albicans* biofilm.

CONCLUSION

Based on this study, it can be concluded that since Moringa oleifera leaf ethyl acetate extract inhibits *C. Albicans* planktonic growth and biofilm formation, it has the potential to be developed as an alternative anti-fungal agent.

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CONFLICT OF INTEREST

The author declared that there is no conflict of interest

REFERENCES

- Lu SY. Oral Candidosis: Pathophysiology and Best Practice for Diagnosis, Classification, and Successful Management, Journal of Fungi, 2021; 7(555):1-26. <https://doi.org/10.3390/jof7070555>
- Gani BA, Alghassani AQ, Mubarak Z, Bachtar EW, Bachtar BM. Potensi Cigarette Smoke Condensate terhadap Peningkatan Pembentukan Biofilm Candida albicans Isolat ATCC 10261, J Syiah Kuala Dent Soc. 2017; 2(1):33-9.

3. Puspitasari A, Kawilarang AP, Ervianti E, Rohman A. Profil Pasien Baru Kandidiasis. Berkala Ilmu Kesehatan Kulit dan Kelamin. 2019; 31(1):24-34
4. Weerasekera MM, Wijesinghe GK, Jayarathna TA, Gunasekara CP, Fernando, Kottegoda N, et al. Culture media profoundly affect *Candida Albicans* and *Candida tropicalis* growth, adhesion and biofilm development. *Memorias do Instituto Oswaldo Cruz*. Fundacao Oswaldo Cruz, 2016; 111(1):697-702. <https://doi.org/10.1590/0074-02760160294>
5. Lohse, MB, Gulati, M, Arevalo, AV, Fishbum, A, Johnson AD, Nobile, CJ. Assessment and optimizations of *Candida albicans* in vitro biofilm assays. *Antimicrobial Agents, and Chemotherapy*. American Society for Microbiology. 2017; 61(5):02749-16. <https://doi.org/10.1128/AAC.02749-16>
6. Yang, S., Fu, Y., Wu, X., Zhou, Z., Xu, J., Zeng, X., Kuang, N., Zeng, Y. 2014. Baicalin prevents *Candida albicans* infection via increasing its apoptosis rate. *Biochemical and Biophysical Research Communications*, 451:36-41. <https://doi.org/10.1016/j.bbrc.2014.07.040>
7. Wiguna, I. Pasar dan Khasiat Kelor. Jakarta: Trubus Swadaya; 2018
8. Sreelatha P, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa Oleifera* leaf extract on human cancer cells. *Food and Chemical Toxicology*. 2011; 49(6): 1270-75 <https://doi.org/10.1016/j.fct.2011.03.006>
9. Cui C, Chen S, Wang X, Yuan G, Jiang F, Chen L, et al. Characterisation of *Moringa oleifera* Roots Polysaccharide MRP-1 with Anti-inflammatory Effect. *International Journal of Biological Macromolecules*. 2019; 132: 844-51 <https://doi.org/10.1016/j.ijbiomac.2019.03.210>
10. Charoensin S. Antioxidant and Anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plants Research*. 2014; 8(7):318-25 <https://doi.org/10.5897/JMPR2013.5353>
11. Ojiako EN. Phytochemical Analysis and Antimicrobial Screening Of *Moringa Oleifera* Leaves Extract. *The International Journal of Engineering and Science*. 2014; 3(3):32-35. Available at: www.theijes.com.
12. Minutolo A, Dotesta M, Roglia V, Cirilli M, Lacovelli F, Cerva C, et al. Plant MicroRNAs from *Moringa oleifera* Regulate Immune Response and HIV Infection. *Front Pharmacol*. 2021; 11:2253 <https://doi.org/10.3389/fphar.2020.620038>
13. Rahayu P. Konsentrasi Hambat Minimum Buah Belimbing Wuluh (*Averrhoa bilimbi*) terhadap Pertumbuhan Jamur *Candida albicans*. Skripsi.Universitas Hasanuddin, Makassar: 2013
14. Sulistiyani H, Fujita M, Miyakawa H, Nakazawa F. Effect of roselle calyx extract on in vitro viability and biofilm formation ability of oral pathogenic bacteria. *Asian Pacific Journal of Tropical Medicine*. 2016; 9(2):119-124 <https://doi.org/10.1016/j.apjtm.2016.01.020>
15. Savitri I, Suhendra L, Wartini NM. Pengaruh Jenis Pelarut pada Metode Maserasi terhadap Karakteristik Ekstrak *Sargassum polycystum*. *Jurnal Rekayasa dan Manajemen Agroindustri*. 2017; 5(3):93 - 101
16. Syahruramadhan M, Yanti NA, Darlian L. Aktivitas Antijamur Ekstrak Daun Kelor (*Moringa oleifera* Lamck.) dan Daun Kirinyuh (*Chromolaena odorata*) terhadap *Candida albicans* dan *Aspergillus flavus*. *JAMPIBI* 2016; 1(2):7-12
17. Borman AM, Muller J, Walsh-Quantick J, Szekeley A, Patterson Z, Palmer MD, et al. MIC Distributions for Amphotericin, B, Fluconazole, Itraconazole, Voriconazole, Flucytosine and Anidulafungin and 35 Uncommon Pathogenic Yeasts Species from The UK Determined using The CLSI Broth Microdilution method. *J. Antimicrob. Chemother*. 2020; 75(5):1194-1205. <https://doi.org/10.1093/jac/dkz568>
18. Nuryanti, S, Mustapa, K, Sudarmo, I.G., Uji Daya Hambat Ekstrak Buah Kelor (*Moringa oleifera* Lamk) terhadap Pertumbuhan jamur *Candida albicans*. *J.Akad.Kim* 2016; 5(4):178-184 <https://doi.org/10.22487/j24775185.2016.v5.i4.8067>
19. Sulistiyani H, Sulastri S, Agustina D, A'yun Q. Effect of *Moringa (Moringa oleifera)* Leaf Extract on in vitro Inhibition of *Candida albicans* Biofilm. *Dentino (in review)*
20. Putri WS, Warditiani NK, Larasanty LPF. Skrining Fitokimia Ekstrak Etil. Asetat Kulit Buah Manggis (*Garcinia mangostana* L.). *Jurnal Farmasi Udayana*. 2013; 2(4):56-60
21. Dewi RC. Uji Aktivitas Antijamur Ekstrak Buah Pare Belut (*Trichosanthes anguina*, L). Skripsi. Universitas Sebelas Maret, Surakarta: 2009
22. Serpa R, Franca EJG, Furlaneto-Maia L, Andrade CGTJ, Diniz A, Furlaneto MC. In vitro Antifungal Activity of The Flavonoid Baicalein Against *Candida* species. *Journal of Medical Microbiology*. 2012; 61(12):1704-1708. <https://doi.org/10.1099/jmm.0.047852-0>
23. Komala O, Yulianita, Siwi FR. Aktivitas Anti Jamur Ekstrak Etanol 50% dan Etanol 96% daun Pacar Kuku *Lawsonia inermis* L. Terhadap *Trichophyton mentagrophytes*. *Ekologia: Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*. 2019; 19(1):12 - 19 <https://doi.org/10.33751/ekol.v19i1.1657>
24. Santi M, Runtuwene MRJ, Simbala HEI, Makang VMA. Analisis Fitokimia Tumbuhan Obat di Kabupaten Minahasa Utara. *Chem. Prog*. 2008; 1(1):47-53.
25. Chong PP, Chin VK, Wong WF, Madhavan P, Yong VC, Looi CY. Transcriptomic and Genomic Approaches for Unravelling *Candida albicans* Biofilm Formation and Drug Resistance-An Update. *Genes*. 2018; 9(11):540 <https://doi.org/10.3390/genes9110540>
26. Ivanov, M., Kannan, A., Stojkovic, D.J., Glamoclija, J., Calhelha, R.C., Ferreira, I.C.F.R., Sanglard, D., Sokovic, M. Flavones, Flavonols, and Glycosylated Derivatives - Impact on *Candida albicans* Growth and Virulence, Expression of CDR I and ERG II, Cytotoxicity, Pharmaceuticals. 2021; 14(27):5-12. <https://doi.org/10.3390/ph14010027>
27. Onsare, J.G., Arora, D.S. Antibiofilm Potential of Flavonoids Extracted from *Moringa oleifera* seed coat against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, *Journal of Applied Microbiology*. 2014; 118(2):313-325. <https://doi.org/10.1111/jam.12701>
28. Marrufo, T., Encarna3ro, S., Silva, O.M.D., Duarte, A., Neto, F.F., Barbosa, F.M. Chemical Characterisation and Determination of Antioxidant and Antimicrobial Activities of The Leaves of *Moringa oleifera*. *International Network Environmental Management Conflicts*, 2013; 2(1):1-15