narra j

Short Communication

Antifungal activity of *Durio zibethinus* Murray peel extract against *Candida albicans*: A preliminary study

Syifa S. Siregar[1,2], Cut A. Adella[1,2*] and Seyi S. Enitan[3]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [2]Department of Obstetrics and Gynecology, H. Adam Malik General Hospital, Medan, Indonesia; [3]Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Nigeria

*Corresponding author: cutadeya@usu.ac.id

Abstract

The incidence of antifungal resistance to *Candida albicans* infections has been growing over the past years; therefore, innovations are required to develop medicinal plants with antifungal properties such as durian fruit peels (*Durio zibethinus* Murray) that contain significant of bioactive compounds with antifungal properties. The aim of this study was to determine the antifungal activity of *D. zibethinus* fruit peel extract against *C. albicans* by analyzing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A post-test only control group experiment was conducted from July to October 2020. *D. zibethinus* peel was collected from Simalungun Regency, Medan, Indonesia, and extracted by maceration technique using 70% ethanol to obtain *D. zibethinus* peel ethanol extract (DPEE). Samples of *C. albicans* were obtained from the Laboratory of Microbiology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. The diffusion method was used to determine the antifungal activity. Six groups with different concentrations of DPEE (6.25%, 12.5%, 25%, and 50%), ketoconazole (positive control) and dimethyl sulfoxide (negative control) were exposed to *C. albicans* in six replicates. Six lower concentrations (12.5%, 6.25%, 3.12%, 3%, 1.56%, and 0.78%) were divided to perform the liquid dilution method to obtain the MIC and affirmation test for MBC. The diameter of the inhibition zone was analyzed using one-way ANOVA and the Tukey post-hoc test for differences between concentrations. Our data indicated that the DPEE 6.25% had the largest inhibition zone (17.26±5.64 mm) and the inhibition zones were significant different among concentrations of DPEE ($p<0.05$). Furthermore, the DPEE had a MIC of 0.78% and MBC of 3.125% against *C. albicans*. This study highlights that the ethanol extract of *D. zibethinus* has potential antifungal activity against *C. albicans*. However, a further study is needed to determine its antifungal activities in more precise manner.

Keywords: Candidiasis, antifungal activity, durian peel extract, inhibitory, medicinal plant

https://narraj.org/

Introduction

The most common cause of fungal infection is candidiasis, caused by *Candida*, particularly *Candida albicans* (*C. albicans*). According to the Ministry of Health of Indonesia, the candidiasis prevalence rate in the country ranged from 25% to 50% in 2010 [1]. From 2011 to 2013, the prevalence of genital infections, specifically vulvovaginal candidiasis, had a significant rise,

Received: August 27, 2023 - Accepted: January 29, 2024 - Published online: March 2, 2024

reaching of 30%–35% [1]. The clinical manifestations of candidiasis vary widely, ranging from acute to subacute and chronic to episodic. These fungal infections can be localized in the gastrointestinal tract, respiratory tract, integumentary system and reproductive organs. The infection may progress to systemic levels resulting in septicemia, endocarditis, and meningitis [1,2].

Antifungal resistance poses a significant challenge due to antifungal development lags behind that of antibiotic developments [2]. An epidemiological study in 2022 showed increased cases of fungal infections due to antifungal resistance, and *Candida* species have been reported resistant to fluconazole [3]. Fluconazole resistance considerably increased over periods, from 31.8% in 2005–2008, to 37.7% in 2012–2015, and to 48.4% during COVID-19 period [3]. Another study revealed different levels of *C. albicans* resistance on several antifungals, such as fluconazole (34.1%), voriconazole (11%), ketoconazole (7.69%), itraconazole (6.59%), clotrimazole (2.19%), and amphotericin B (1.09%) [4].

Recent studies have focused on exploring the antifungal properties of medicinal plants. The peel of *Durio zibethinus* Murray (*D. zibethinus*), known as durian from Indonesia, contains phytochemicals with potential antifungal properties, such as alkaloids, flavonoids, saponins, quinones, tannins, and terpenoids [5,6]. The aim of the study was to determine the antifungal activity of *D. zibethinus* peel extract against *C. albicans* by analyzing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Method

Study design and setting

An experiment using post-test only control group study design was conducted at the Medicinal Plants Research and Development Laboratory of ASPETRI, Medan, Indonesia, by analyzing *D. zibethinus* antifungal properties against *C. albicans*. Antifungal activity analysis was performed at the Integrated Laboratory of Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. This study was conducted from July to October 2020.

D. zibethinus and *C. albicans* sources

Fresh peels of *D. zibethinus* were collected from a garden located in Sinda Raya, Pematang Siantar, Indonesia, in July 2020. The plant specimen was identified and authenticated at the Herbarium Medanese Laboratory, Universitas Sumatera Utara, with the registration number 5299/MEDA/2020. Samples of *C. albicans* were obtained from the Laboratory of Microbiology, Faculty of Medicine, Universitas Sumatera Utara.

Plant extract preparation

D. zibethinus peel extract was made by the maceration method using 70% ethanol. One kilogram of fresh durian peel was cleaned, washed, and dried under the sun for two weeks. Then, the peel was mashed into powder using a pestle, and mashed once more using a blender. The finely macerated peel was mixed with three liters of 70% ethanol and soaked for 24 hours. The extract solution was filtered to obtain the filtrate and pulp and soaked again using 70% ethanol for 24 hours. The filtrate was evaporated using an evaporator in a water bath to obtain a thick extract. The *D. zibethinus* peel ethanol extract (DPEE) formed with 100% concentration was then diluted using dimethyl sulfoxide (DMSO) to achieve different concentration levels of 6.25%, 12.5%, 25%, and 50%.

Fungal preparation and antifungal activity assessment

C. albicans culture was mixed with 0.9% NaCl and the McFarland standard of 0.5 of *C. albicans* was prepared. This study applied the diffusion method to test the antifungal activity of DPEE. Briefly, *C. albicans* was streaked on a Sabouraud dextrose agar (SDA) media and a whole was formed using a cork with a diameter of 7 mm [7]. Afterward, 0.2 ml of DPEE (50%, 25%, 12.5%, and 6.25% concentrations) was inserted into the hole and were incubated for 18–24 hours at 37°C. In this study, 2% ketoconazole was used as a positive control, and DMSO disc was used as negative control. Each sample was repeated six times, resulting in a total sample of 36 samples.

Minimum inhibitory concentration (MIC) and turbidity assessment

Results of the diffusion test were observed visually by measuring the diameter of the inhibition zone. The power of inhibition was classified into very strong (>20 mm), strong (16–20 mm), moderate (10–15 mm) and weak (<10 mm) [7]. The MIC was determined by using the liquid dilution method. After obtaining the inhibition zone, the range of inhibition zone concentrations was used to determine the MIC. The variation of the concentration was made based on the smallest concentration which provided the inhibition zone of the antifungal activity test. This test began by inserting 0.1 ml of *C. albicans* suspension and 0.9 ml of extract solution in various concentrations into a tube that contained 9 ml of Sabaroud Dextrose Broth (SDB) media. All of which was vortexed for homogeneity. Lastly, the solution was incubated at 37°C for 18–24 hours. The concentration series used were 12.5%; 6.25%; 3.12%; 3%; 1.56%; 0.78%; formalin as the positive control and DMSO as negative control. The concentration series was replicated three times using the same extract as the diffusion test. The determined MIC results were compared to observe the turbidity with formalin as the positive control and SDB as the negative control. Turbidity indicated the presence of fungal growth, while clear media indicated no fungal growth. Media that was very cloudy was given a notation (+++), cloudy (++), slightly cloudy (+) and media that was clear was given a notation (-) to facilitate the observation [7].

Minimum bactericidal concentration (MBC) and affirmation test

The clearest tube from the liquid dilution test (the two smallest concentrations selected) was subjected to an affirmation test. The affirmation test was carried out three times with six different concentrations: 0.78%, 1.56%, 3.00%, 3.12%, 6.25% and 12.50%. Formalin and DMSO were both used as the positive and negative controls, respectively. The affirmation test was conducted by streaking a loop on sterile SDA media, incubated for 24 hours and observed. Media that has no fungal growth was given the notation (-) and fungal growth presence was given the notation (+). MBC was achieved if there was no fungal growth around the streak plate scratches.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and continued with the Tukey post-hoc test. Normality test was conducted, where a $p>0.05$ was considered normally distribute data. Statistical significance was considered at a value of $p<0.05$. All statistical analyses were conducted using SPSS program version 25 (IBM, New York, USA).

Results

Inhibition zone of *D. zibethinus* peel ethanol extract (DPEE) against *C. albicans*

The lowest mean diameter of the inhibition zone was observed in the concentration of 50% (11.2±3.28 mm), with a weak inhibition power. Concentrations at 25%, 12.5% and 6.25% appeared to exhibit strong inhibition power with the mean diameter of inhibition of 15.9±2,47 mm, 16.78±2.80 and 17.26±5.64 mm, respectively. For comparison, ketoconazole revealed a very strong inhibition, as expected for positive control (**Table 1**). Our data indicated that the data were normally distributed.

Table 1. Inhibition zone diameter of DPEE against *C. albicans*

Group	Inhibition zone diameter (mm)						Mean±SD
	Repetition						
	1	2	3	4	5	6	
DPEE 50%	14.2	11.3	7.2	7.5	12.0	15.0	11.2±3.28
DPEE 25%	19.1	15.5	12.4	15.0	15.0	18.4	15.9±2.47
DPEE 12.5%	17.0	15.6	14.0	15.1	17.0	22.0	16.78±2.80
DPEE 6.25%	23.4	15.0	12.0	12.0	16.2	25.0	17.26±5.64
Ketoconazole	25.0	23.1	19.0	20.0	20.0	20.7	21.30±2.27
DMSO	0.0	0.0	0.0	0.0	0.0	0.0	0 ± 0

A significant difference was found in the concentration varieties between the groups, DPEE (6.25%, 12.5%, 25%, and 50%) and ketoconazole, in inhibiting the growth of *C. albicans* based on

the one-way ANOVA test, with $p<0.001$ (**Table 2**). Tukey post-hoc test results showed a significant mean difference between DMSO and other concentration extracts.

Table 2. Differences between variation of concentration

Inhibition zone diameter	Sum of squares	Mean square	*p*-value
Between DPEE groups and controls	1672.546	334.509	<0.001
Within groups of DPEE	308.722	10.291	<0.001

Minimum inhibitory concentration (MIC) of *D. zibethinus* peel ethanol extract (DPEE) against *C. albicans*

Clear results from the turbidity test were observed for all concentrations, except for 12.50%, across the three replications. The MIC was observed at the concentration of 0.78% as it represented the smallest concentration demonstrating clear turbidity (**Table 3**).

Table 3. Turbidity test of the dilution test for minimum inhibitory concentration (MIC)

Group	Turbidity		
	Replication		
	1	2	3
DPEE 0.78%	-	-	-
DPEE 1.56%	-	-	-
DPEE 3.00%	-	-	-
DPEE 3.12%	-	-	-
DPEE 6.25%	-	-	-
DPEE 12.50%	++	++	++
Formalin	-	-	-
DMSO	+++	+++	+++

Minimum bactericidal concentration (MBC) of *D. zibethinus* peel ethanol extract (DPEE) against *C. albicans*

The concentration of 3.12% was identified as the MBC since it demonstrated the absence of a growing fungal colony. Other concentrations of 0.78%, 1.56%, 3.00%, 6.25%, and 12.50% was observed with fungal growth, along with the negative control (**Table 4**).

Table 4. Growing colonies of the affirmation test for minimum bactericidal concentration (MBC)

Group	Growing colonies		
	Replication		
	1	2	3
DPEE 0.78%	+	+	+
DPEE 1.56%	+	+	+
DPEE 3.00%	+	+	+
DPEE 3.12%	-	-	-
DPEE 6.25%	+	+	+
DPEE 12.50%	+	+	+
Formalin	-	-	-
DMSO	+	+	+

Discussion

This study found concentrations at 25%, 12.5% and 6.25% of DPEE exhibited strong inhibition against *C. albicans*. A similar study that used a different solvent reported that at concentrations of 15%, 25%, 50%, 75%, and 50% had strong inhibition activities [8]. The results from a previous study found that the concentration of 25% durian peel extract had the most optimal inhibition activities against *C. albicans*, compared to other concentrations of 15% and 20% [6]. Lower concentrations contain minimal phytochemical compounds, potentially allowing the fungus to regrow [8]. Conversely, higher concentrations make it hard for phytochemical compounds to diffuse into the media [9]. The present study also demonstrated that the higher concentration of DPEE, the inhibition effects were diminished based on MIC and MBC tests.

The reported study employed a 96% ethanol solvent, which was less polar than 70% ethanol, the solvent used this present study. The phytochemical compounds found in *D. zibethinus* peel are polar and are inclined to dissolve more readily in 70% ethanol rather than 96% ethanol [10].

A combination of internal factors, such as genetic variations, and external factors, including light, temperature, humidity, pH, nutrient content in the soil, and altitude, may also contribute to the different contents of phytochemical compounds in the durian peel [11].

The inhibitory activity of DPEE against *C. albicans* is probably caused by the presence of active antifungal compounds contained in the durian skin, namely saponins, flavonoids, tannins, quinones, terpenoids, and alkaloids [12]. Flavonoids, saponins and quinones work in similar ways, as antimicrobial compounds, causes damage to the fungal cell membranes causing changes in the permeability and ultimately resulting in lysis of the fungal cell membranes [9,13,14]. Alkaloids inhibits the biosynthesis of fungal nucleic acids, which is responsible for cell development [15]. Terpenoids have hydrophobic or lipophilic properties that could cause membrane cytoplasmic damage, cell coagulation, and proton disruption in fungal cells [16]. Tannins prevents the synthesis of chitin, which is used for cell wall formation in fungi [17].

This study had some limitations. The phytochemical analysis was not conducted to detect phytochemical constituents contained in the durian peels, and a biochemical test was not carried out. It is recommended for future studies to employ gas chromatography-mass spectrometry (GC-MS) analysis to screen and identify the bioactive compounds. A comparison of solvents with a wide range of concentrations should also be carried out to determine which solvent show optimum advantage in exhibiting antifungal activities.

Conclusion

The ethanol extract of *D. zibethinus* demonstrated antifungal activity against *C. albicans*. The concentration of 6.25% was the most effective and there were significant differences between concentrations ($p<0.05$). Furthermore, the extract had a MIC of 0.78% and MBC of 3.125% against *C. albicans*.

Ethics approval

Ethical research has approved by Research Ethics Committee Universitas Sumatera Utara Medan, Sumatera Utara: 263/KEP/USU/2020.

Competing interests

The authors declare that there are no conflicts of interest.

Funding

This study received no external funding.

Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Acknowledgements

The authors would like to thank the support that given by all of the staff from Integrated Laboratory and Laboratory of Microbiology, Faculty of Medicine, Universitas Sumatra Utara and Medicinal Plants Research and Development Laboratory of ASPETRI.

How to cite

Siregar SS, Adella CA and Enitan SS. Antifungal activity of *Durio zibethinus* Murray peel extract against Candida albicans: A preliminary study. Narra J 2024; 4 (1): e429 - http://doi.org/10.52225/ narra.v4i1.429.

References

1. Suyoso S. Mucosal candidiasis. Airlangga University Publishing; 2013; 1(1):79-83
2. Canuto MM, Rodero FG. Antifungal drug resistance to azoles and polyenes. Lancet Infect Dis 2002;2(2):550-563.

3. Routsi C, Meletiadis J, Charitidou E, *et al.* Epidemiology of candidemia and fluconazole resistance in an ICU before and during the COVID-19 pandemic era. Antibiotics (Basel) 2022;11(6):771
4. Sharma PC, More SR, Raut SS, Rathod VS. In vitro antifungal susceptibility pattern of oropharyngeal and oesophageal Candida species in HIV infected patients. Int J Health Sci Res 2013;3(5):1-6.
5. Bahry B, Setiabudy R. Fungal medicine - Pharmacology and therapy fifth edition. FKUI Publishing. Jakarta 2011.
6. Setyowati H, Hanifah Z, Nugraheni RP, Setyani W. Durian (*Durio zibethinus* Murray L.) fruit peel cream as an herbal medicine for treatment of *Candida albicans* fungal infections. Farmasi Indonesia Media 2013;8(2):48-58.
7. Balouiri M, Moulay S, Saad K. Methods for in vitro evaluating antimicrobial activity: A review. J Pharma Analys 2016;6(2):71-79.
8. Syadiah L, Putri DR, Zahwa A, Trimulyono G. Effect of durian (*Durio zibethinus*) peel extract on the growth of pathogenic fungi on potato plants (*Phytophthora infestans*) in vitro. Biotropic 2019; 3(2):129-134.
9. Kawaroe M, Soedarma D, Effendi H, *et al.* Antibacterial activity of soft coral extract *Sarcophyton sp.* fragmented and unfragmented in the waters of Pramuka Island, Seribu Islands. Biota 2019;15(3):340-347.
10. Mubarak F, Sartini S, Purnawanti D. Effect of ethanol concentration on antibacterial activity of bligo fruit extract (*Benincasa hispida* Thunb) to *Salmonella typhi.* Indones J Pharma Sci Technol 2018:5(3):76-81.
11. Giupponi L, Leoni V, Pavlovic R, Giorgi A. Influence of altitude on phytochemical composition of hemp inflorescence: A metabolomic approach. Molecules 2020; 25(6):1381.
12. Anggraeni E V, Anam K. Identification of chemical ingredients and testing of peel antimicrobial potential. J Chem Sci Applications 2016;19(3):87-93.
13. Shamsudin NF, Ahmed QU, Mahmood S, *et al.* Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. Molecules 2022;27(4):1149
14. Tian, J., Huang, B., Luo, *et al.* The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand Mazz essential oil and its potential use as a food preservative. Food Chemistry 2012;130 :27-520.
15. Yan Y, Li X, Zhang C, *et al.* Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. Antibiotics (Basel) 2021;10(3):318
16. Natta L, Krittika O, Pantip P. Essential oil from Zingiberaceae for anti food borne bacteria. Int Food Res J 2008;15(3):337-346.
17. Wu XZ, Cheng AX, Sun LM, Lou HX. Effect of plagiochin E, an antifungal macrocyclic bis (bibenzyl), on cell wall chitin synthesis in *Candida albicans*. Acta Pharmacol Sin 2008;29:1478-1485.