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Inhibition Zone Test of Ethyl Acetate Extract of White Frangipani (*Plumeria acuminata*) Against *Candida albicans*

Sila Paramitha Dayang Ganjar*, Novitarini, I Nyoman Bagus Aji Kresnapati
*E-mail of Corresponding Author: siladayang@gmail.com
Program Studi S1 Farmasi, Fakultas Imu Kesehatan, Universitas Bumigora

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ABSTRACT

Candida albicans infection is a common health problem in Indonesia, particularly due to the tropical climate that supports fungal growth. The prevalence of candidiasis in Indonesia is approximately 20–25%. The use of synthetic antifungals such as ketoconazole is becoming increasingly limited because of the emergence of resistance and adverse side effects, creating the need for safer natural alternatives. One potential solution is the utilization of white frangipani (Plumeria acuminata), which is known to contain antifungal compounds such as flavonoids, alkaloids, and tannins. This study aimed to evaluate the effectiveness of ethyl acetate extract from white frangipani flowers against the growth of Candida albicans, as measured by the inhibition zones formed. The method used was a laboratory experimental design with well diffusion assays, applying various extract concentrations (25%, 50%, 75%, and 100%) against Candida albicans, followed by measuring the inhibition zone diameters. The results showed that increasing extract concentrations correlated with larger inhibition zone diameters, ranging from 16 mm at 25% concentration, 20.1 mm at 50%, 23.8 mm at 75%, and 27.5 mm at 100%. Based on these findings, it can be concluded that white frangipani flowers have potential as a natural antifungal alternative at all tested concentrations, with particularly strong effectiveness at 50%, 75%, and 100%. Therefore, white frangipani shows promise for further development as a herbal medicine.

Keywords: Antifungal; White frangipani, *Candida albicans,* Fungal infection, Inhibition zone

I. INTRODUCTION

Indonesia is an archipelagic country located along the equatorial line. Its geographical position results in a tropical climate characterized by relatively high temperatures and humidity, conditions that favor the growth and proliferation of infectious diseases caused by both non-pathogenic and pathogenic fungi (Herkamela, 2022). Candidiasis is an opportunistic pathogenic fungal infection, one of which is caused by the species *Candida albicans*, and can be acute, subacute, or chronic. Sepsis caused by *Candida albicans* has a mortality rate of around 40%, which is higher than sepsis caused by bacteria (Macias-Paz *et al.*, 2023). The prevalence of candidiasis in Indonesia ranges from 20–25%. If fungal infections are not treated promptly, they may

develop into chronic infections (Sophia & Suriani, 2024).

Candida albicans is the fungal species most frequently responsible for human infections, primarily affecting mucocutaneous surfaces such as the vagina, oral cavity, esophagus, and, less commonly, the nails. Mucosal candidiasis, particularly vulvovaginal candidiasis, may occur in individuals with intact immune function; however, immunocompromised individuals face a higher risk of increased infection frequency, severity, and recurrence. Cutaneous candidiasis is relatively rare and is typically associated with specific congenital immune disorders. Under certain conditions, *Candida albicans* can cause systemic candidiasis involving normally sterile sites, including the bloodstream, central nervous system, liver, spleen, heart, kidneys, and intra-abdominal compartments. Moreover, excessive colonization of *Candida albicans* in the gastrointestinal tract, often following antibiotic use, may facilitate translocation across the intestinal mucosa into the systemic circulation (Lopes & Lionakis, 2022).

Candida albicans is a yeast-like fungus commonly found in the environment and as part of the normal microbiota of human skin and mucosal surfaces. Under normal physiological conditions, it exists as a commensal organism; however, it can become an opportunistic pathogen in individuals with compromised immune systems. Transmission of *Candida albicans* occurs through direct contact with infected individuals or contaminated surfaces, including person-to-person contact, sexual contact, and exposure to contaminated objects or materials (Giraffa & Oliveira, 2024).

Treatment of candidiasis generally involves antifungal therapy from the azole class. Azole antifungals are synthetic compounds with broad-spectrum activity, classified based on their nitrogen atom content (Minarni *et al.,* 2020). However, inappropriate use of antifungal drugs can lead to resistance and cause side effects such as gastrointestinal disorders and abnormalities in liver enzymes (Marbun, 2021). These treatment challenges have encouraged the development of research on natural antifungal agents derived from plants available in Indonesia (Izzati *et al.,* 2022).

One plant proven to have antifungal activity is the white frangipani (*Plumeria acuminata*). This plant contains tannins, flavonoids, saponins, and alkaloids, which support its potential as a natural antibiotic (Nurhapit *et al.,* 2023). Previous studies have shown that white frangipani flower extract exhibits a very strong inhibitory effect against the growth of *Candida albicans*, as indicated by the formation of a clear zone measuring 25.8 mm (Permatasari & Sari, 2019). Therefore, this study will vary the concentration of the extract and use ethyl acetate as the solvent to determine the inhibition zone against *Candida albicans*.

In this study, ethyl acetate was employed as the extraction solvent based on findings reported by Monica et al. (2019), who demonstrated that ethyl acetate produced superior inhibitory

effects against *Candida albicans* compared to ethanol or n-hexane. This effectiveness is attributed to the semi-polar nature of ethyl acetate, which enables it to dissolve a wide range of secondary metabolites, including both polar and non-polar compounds. Furthermore, Wisnianti et al. (2024) reported that ethyl acetate exhibited greater antifungal inhibitory activity against *Candida albicans* than n-hexane. These differences are associated with the types of bioactive compounds selectively extracted by each solvent.

II. METHOD

The method used in this study was True Experimental (post-test only control group design). The research stages included: extraction of white frangipani (*Plumeria acuminata*) simplicia using the maceration method, phytochemical screening of the extract, and antifungal activity testing of the ethyl acetate extract of white frangipani flowers against *Candida albicans*.

The equipment used in this study included: laminar air flow (LAF), autoclave, incubator, rotary evaporator, spirit burner, oven, volumetric pipette, filter paper, stirring rod, petri dishes, inoculating loop, thermometer, hot plate, analytical balance, and other glassware.

The materials used in this study included: white frangipani flower extract, *Potato Dextrose Agar* (PDA) medium, ethyl acetate, pure culture of *Candida albicans*, ketoconazole, aluminum foil, gloves, label paper, sterile distilled water, $FeCl_3$ solution, 2N *hydrochloric acid* (HCl), concentrated HCl, acetic acid (CH_3COOH), concentrated sulfuric acid (H_2SO_4), Mayer's reagent, and Dragendorff's reagent.

Fresh white frangipani flowers weighing 2.343 kg were separated and washed thoroughly with running water. They were then dried by air-drying without direct sunlight. Once dried, the flowers were ground using a blender. The extraction process began with 200 grams of simplicia powder placed in a glass jar, then soaked in 1000 ml of ethyl acetate (ratio 1:5) with occasional stirring. Maceration was carried out for 24 hours over 3 consecutive days. The entire filtrate from the maceration process was combined in a maceration vessel and concentrated using a rotary evaporator.

For phytochemical screening, 0.5 grams of thick extract was dissolved in 50 ml of ethyl acetate. This solution was then subjected to phytochemical screening tests for flavonoids, alkaloids, tannins, and saponins. Flavonoid test: The test solution was placed in a test tube and heated for about 5 minutes. After heating, a small amount of magnesium ribbon and several drops of concentrated HCl were added. A positive result for flavonoids was indicated by a color change from orange to red. Tannin test: The test solution was reacted with $FeCl_3$ solution. A positive result for tannins was indicated by the appearance of a dark blue color. Alkaloid test: 1 ml of the test solution was evaporated to obtain a residue. The residue was dissolved in 3 ml of 2N HCl, placed in a test tube, and 4 drops of Dragendorff's reagent were added. A positive result for alkaloids was indicated by the formation of a red precipitate.

The antifungal test used the well diffusion method with PDA medium. A fungal inoculum of *Candida albicans* equivalent to 0.5 McFarland standard was spread evenly across the surface of the PDA medium using sterile cotton swabs. The inoculated medium was then perforated using a No. 4 cork borer to make 6 wells. Each well was filled with 50 µL of the prepared test samples, which included ethyl acetate extract of white frangipani flowers at concentrations of 25%, 50%, 75%, and 100%, ketoconazole 1%, and ethyl acetate. The inoculated media containing the test solutions were incubated for 24 hours at 37°C in an incubator. The clear zones formed were then observed and measured using a caliper.

III. RESULT AND DISCUSIONS

This study obtained the following results: 1) The extraction process using the maceration method from 200 grams of white frangipani (*Plumeria acuminata*) simplicia yielded 22 grams of thick extract, with an extract yield percentage of 11% and 2) The results of the phytochemical screening of the ethyl acetate extract of white frangipani flowers are presented in Table 1.

Table 1. Phytochemical Screening Result

Secondary Metabolit	Testing Methode	Standard Color	Result	Image
Flavonoids	Mg Powder + concentrated HCl	Redish Orange	Orange, red, and foamy (+)	
Alkaloids	2% HCl + 5 drops of Mayer reagent	White precipitate	No color change (-)	
Tanins	FeCl3	Dark bluish-black	Dark bluish-black coloration (+)	
Alkaloids	2% HCl + 3 drops of Dragon dorff reagent	Red	Red coloration (+)	
Saponins	10 mL distilled water	Foam	No foam formation (-)	

The inhibition zone results of the white frangipani flower extract against *Candida albicans* are presented in Table 2.

Table 2. Inhibition Zone Diameter of the Ethyl Acetate Extract of White Frangipani Flowers

Candida albicans						
Test Sample	**Concentration**	**Inhibition Zone Diameter(mm)**			**Mean Inhibition Zone Diameter**	**Inhibition Zone Criterion**
		I	**II**	**III**		
Ethyl Acetat extract of white frangipani flower (*Plumeria acuminata*	25%	17	15	15,5	16	Strong
	50%	20	22	8,5	20,1	Very strong
	75%	24,25	21,5	25,75	23,8	Very strong
	100%	27	24	31,75	27,5	Very strong
K(+) Ketoconazol		31	32	33,75	32,5	Very strong
K(-) Ethyl acetat		0	0	0	0,0	No Inhibition zone

The extraction process using the maceration method for 200 grams of white frangipani (*Plumeria acuminata*) simplicia yielded 22 grams of thick extract, with an extract yield percentage of 11%. The yield of white frangipani flower extract is considered good because it exceeds 10%. The higher the extract yield percentage obtained, the greater the amount of extract

produced. The yield of an extract can be influenced by several factors, one of which is the extraction method used (Eka Kusuma & Aprileili, 2022).

The positive control (+) employed in this study was ketoconazole. Ketoconazole was selected due to its higher sensitivity compared to other azole compounds, including miconazole, in exerting effective antifungal activity against dermatophytes and yeasts such as *Trichophyton, Epidermophyton, Microsporum, Candida albicans*, and *Malassezia furfur* (Tilu *et al.,* 2023). In addition, ketoconazole demonstrates broad-spectrum antifungal efficacy against a wide range of fungal species, encompassing both superficial and systemic fungal infections (Pharmacia *et al.,* 2025).

Phytochemical screening results of the ethyl acetate extract of white frangipani flowers tested positive for flavonoids, tannins, and alkaloids. The principle of the flavonoid test is the addition of magnesium powder and concentrated HCl to the sample to reduce the benzopyrone core, form a flavilium salt, and release H_2 gas, indicated by a color change to orange or red as an indication of the presence of flavonoids (Kumala *et al.,* 2019). The principle of the tannin test is the addition of $FeCl_3$ to the sample, which reacts with Fe^{3+} ions to form a dark blue complex as an indicator of the presence of tannins (Nasrul & Chatri, 2024). The principle of the alkaloid test involves adding concentrated HCl to dissolve alkaloids into soluble salts, then testing with Mayer's reagent (positive result indicated by a white precipitate) and Dragendorff's reagent (positive result indicated by an orange or brick-red precipitate due to the formation of potassium tetraiodobismuthate complexes) (Maisarah *et al.,* 2023).

The tests showed that the ethyl acetate extract of white frangipani flowers (*Plumeria acuminata*) exhibited inhibitory activity against *Candida albicans*, which increased with higher extract concentrations. The average inhibition zone diameters from three replications at concentrations of 25%, 50%, 75%, and 100% were 16 mm, 20.1 mm, 23.8 mm, and 27.5 mm, respectively. All concentrations were classified as strong to very strong, with 100% being the most effective concentration because it approached the inhibitory power of ketoconazole (32.5 mm), although its effectiveness was still lower than that of the drug. The negative control (ethyl acetate) showed no inhibition zone (0.00 mm) (Maisarah *et al.,* 2023).

Statistical analysis using the Shapiro–Wilk and Levene tests showed that the data were normally distributed and homogeneous (P-value > 0.05). The One-Way ANOVA test produced a P-value of 0.001 (< 0.05), indicating a significant difference between concentrations. These findings demonstrate a positive correlation between extract concentration and antifungal activity, as well as the potential of the extract as a natural antifungal alternative.

IV. CONCLUSION

The ethyl acetate extract of white frangipani flowers (*Plumeria acuminata*)

demonstrates significant antifungal activity against *Candida albicans*. The inhibitory effect increases in line with higher extract concentrations, indicating a concentration-dependent antifungal response. Notably, even at moderate concentrations, the extract exhibits a strong inhibitory capacity comparable to that of the standard antifungal agent, suggesting its promising potential as an alternative antifungal source. The observed antifungal activity is closely associated with the presence of secondary metabolites, particularly flavonoids, alkaloids, and tannins, which are known to contribute to the inhibition of fungal growth through various biochemical mechanisms.

REFRENCES

Eka Kusuma, A., & Aprileili, D. A. (2022). Pengaruh Jumlah Pelarut Terhadap Rendemen Ekstrak Daun Katuk (Sauropus androgynus L. Merr). *SITAWA: Jurnal Farmasi Sains Dan Obat Tradisional*, *1*(2), 125–135. https://doi.org/10.62018/sitawa.v1i2.22

Giraffa, G., & Oliveira, M. (2024). Editorial: enterococcus spp. transmission, pathogenesis, host pathogen interaction, prevention and treatment. Frontiers in microbiology, 15. https://doi.org/10.3389/fmicb.2024.1411790

Herkamela, S. W. Y. (2022). Jurnal kedokteran syiah kuala xx (x): 1xx-1xx ,Aprxx 20xx. Jurnal Kedokteran Syiah Kuala, 20(10), 121–127.

Izzati, B. D. W., Nurbaety, B., Leny Nopitasari, B., & Suprianto, A. (2022). Tingkat Pengetahuan, Sikap, dan Tindakan Dalam Pemanfaatan Tanaman Sebagai Obat Tradisional Oleh Masyarakat Di Desa Pringgabaya Kabupaten Lombok Timur. *Lumbung Farmasi: Jurnal Ilmu Kefarmasian*, *3*(1), 29–33. https://doi.org/10.31764/lf.v3i1.6357

Kumala, O., Yulianita, & Siwi, F. R. (2019). Activity of Antifungal Ethanol Extract 50% and Ethanol 96% Leaf henna Nail Lawsonia inermis L. Against Trichophyton mentagrophytes. *Ekologia*, *19*(1), 12–19. https://journal.unpak.ac.id/index.php/ekologia/article/view/1657

Lopes, J. P., & Lionakis, M. S. (2022). Pathogenesis and virulence of candida albicans. Virulence, 13(1),89–121. https://doi.org/10.1080/2150594.2021.2019950

Macias-Paz, I. U., Pérez-Hernández, S., Tavera-Tapia, A., Pedro Luna-Arias, J., Eugenio Guerra-Cárdenas, J., & Reyna-Beltrán, E. (2023). *Candida albicans* the main opportunistic pathogenic fungus in humans. *Revista Argentina de Microbiologia*, *55*(2), 189–198. https://doi.org/10.1016/j.ram.2022.08.003

Maisarah, M., Chatri, M., Advinda, L., & Violita. (2023). Characteristics and Functions of Alkaloid Compounds as Antifungals in Plants. *Serambi Biologi*, *8*(2), 231–236.

Marbun, R. A. T. (2021). Uji Aktivitas Ekstrak Daun Pirdot (Sauraia vulcani Korth.) Terhadap Pertumbuhan *Candida albicans* Secara In Vitro. *Jurnal Bios Logos*, *11*(1), 1–6. https://doi.org/10.35799/jbl.11.1.2021.30564

Minarni, A., Widarti, & Rahman. (2020).

Uji Daya Hambat Beberapa Jenis Obat Antijamur Pada Jamur Yang Di Isolasi Dari Kuku Kaki. *Jurnal Media Analis Kesehatan*, *11*(2), 119–126. https://doi.org/10.32382/mak.v11i2.1784 126

Nasrul, P. I., & Chatri, M. (2024). Peranan Metabolit Sekunder sebagai Antifungi. *Jurnal Pendidikan Tambusai* , *8*(1), 15832–15844.

Nurhapit, E. R., Nurfadhilla, F., Pratama, I. B., Jured, M. M., Harahap, S. R. R., & Fariza, W. (2023). Phytochemical Analysis and *Potential* Pharmacological Activity of Frangipani Flower Simplicia (Plumeria spp.) as a Source of Medicinal Ingredients Evi. *Eureka Herba Indonesia*, *5*(1), 388–393. https://doi.org/10.37275/ehi.v5i1.104

Permatasari, A. A. A. P., & Sari, N. K. Y. (2019). Efektivitas Antijamur Ekstrak Etanol Bunga Kamboja Putih (Plumeria acuminata) Terhadap Pertumbuhan *Candida albicans*. *Seminar Ilmiah Nasional Teknologi, Sains, Dan Sosial Humaniora (SINTESA)*, *2*, 375–378. https://doi.org/10.36002/snts.v0i0.859

Pharmacia, J., Waluya, M., Pharmacia, J., Waluya, M., No, V., Ratte, R. A., Nasir, N. H., Hasanuddin, S., Kunci, K., & Ratte, R. A. (2025). Uji aktivitas antijamur ekstrak etanol bunga kembang sepatu (hibiscus rosa-sinensis l.) Terhadap candida albicans dan malassezia furfur antifungus activity test of hibiscus flower (hibiscus rosa-sinensis l.) Extract on Candida albicans and Malassezia. 4(3).

Sophia, A., & Suriani. (2024). Efektivitas Perasan Daun Meniran Phyllanthus niruri. L Sebagai Antifungi Terhadap Pertumbuhan Jamur *Candida albicans*. *Bioma: Jurnal Biologi Makasar*, *9*(1), 133–161. https://doi.org/10.1163/9789004612204_010

Tilu, M. A., Pusmarani, J., & Juliansyah, R. (2023). Uji aktivitas antijamur ekstrak etanol biji keben (barringtonia asiatica l.) Terhadap jamur malassezia furfur. Jurnal Pharmacia Mandala Waluya, 2(4), 199-210. https://doi.org/10.54883/jpw.v2i4.23

Wisnianti, D., Nasir, N., & Fitriah, W. O. I. (2024). Uji aktivitas antijamur fraksi n- heksan, etil asetat dan air daun rambutan aceh (nephelium lappaceum l.) Terhadap pertumbuhan candida albicans. Jurnal Pharmacia Mandala Waluya, 3(6), 352–363. https://doi.org/10.54883/jpmw.v3i6.158