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Antifungal Activity of Butterfly Pea Flower (*Clitoria Ternatea L.*) against Candida Albicans Causes of Dandruff

Rizki Nisfi Ramdhini[1], Dwi Aula Ramdini[2], Dinda Shava Kamila[1]

[1]Diploma of Pharmacy (D3), Cendikia Farma Husada Pharmacy Academy, Lampung, Indonesia
[2]Pharmacy, Faculty of Medicine, University of Lampung, Indonesia

Corresponding Author: Rizki Nisfi Ramdhini
Email: rizkinisfi2020@gmail.com

Abstract. *Dandruff is a common scalp problem characterized by flaking, itching, and redness. One of the causes of dandruff is the fungus Candida albicans. Under certain conditions, such as excessive oil production, this fungus can grow and become pathogenic. Butterfly pea flower is a plant known to contain antifungal compounds, including tannins, flavonoids, saponins, and alkaloids. This study aims to determine the antifungal activity of ethanol extract from butterfly pea flower against Candida albicans using SDA (Sabouraud Dextrose Agar) medium with the disk diffusion method. The concentrations of butterfly pea extract used were 5%, 15%, 30%, 60%, and 90%. The positive control used was ketoconazole, while the negative control was 1% CMC-Na. The results of the study indicate that the butterfly pea extract exhibits antifungal activity, as evidenced by the formation of an inhibition zone at a concentration of 90%, with an average inhibition zone of 3.7 mm. The positive control showed an inhibition zone of 14.38 mm, categorized as strong. In contrast, the negative control did not show any inhibition zone due to the lack of antifungal activity. Based on these results, it can be concluded that butterfly pea (Clitoria ternatea L.) has the potential for weak antifungal activity against Candida albicans.*

Keywords: *Candida albicans, Telang flower (Clitoria ternatea L.), Antifungal*

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INTRODUCTION

Dandruff is a common problem on the scalp characterized by white, flaky skin and the appearance of itching. This condition can pose a serious issue if the amount of dandruff on the scalp becomes excessive. This can occur due to the continued growth of the fungus Candida albicans resulting from excessive oil secretion. Dandruff is a type of seborrheic dermatitis commonly experienced by people living in tropical countries like Indonesia. The tropical climate can make the scalp more prone to moisture, which can increase oil secretion on the skin, thereby elevating the risk of dandruff, which ultimately can lead to hair damage, hair loss, and discomfort on the scalp (Istiqomah et al., 2016; Laelasari & Musfiroh, 2022).

So far, the prevention of excessive dandruff on the scalp can be done through hair care using anti-dandruff shampoo. However, frequent use of anti-dandruff shampoo, especially in the long term, carries the risk of damaging the hair, such as hair loss, breakage, and changes in hair color, or even more serious effects. This is due to the presence of chemical compounds often found in anti-dandruff shampoos, including sulfur, salicylic acid, selenium sulfide, zinc pyrithione, and piroctone olamine (Punyani et al., 2021). To avoid these risks, people are increasingly inclined to

adopt a back to nature lifestyle, using traditional remedies as an alternative. Traditional medicines derived from nature are considered to have a lower risk of side effects and are safer compared to chemical-based products. Therefore, dandruff prevention can be done not only with chemical-based products but also with traditional remedies (Sahraie-Rad et al., 2015). One natural ingredient that has the potential to be used as a raw material for traditional anti-dandruff medicine is the butterfly pea flower (Clitoria ternatea L.). The butterfly pea flower is known to contain secondary metabolites with antifungal properties against the growth of Candida albicans, a cause of dandruff (Rezaldi et al., 2022).

The butterfly pea flower (Clitoria ternatea) is a type of endemic plant originating from Southeast Asia. It belongs to the Fabaceae family and has various health benefits due to its content of compounds such as tannins, phlobatannins, saponins, triterpenoids, flavanol glycosides, alkaloids, anthraquinones, anthocyanins, essential oils, and steroids. These compounds are known for their antibacterial and antifungal properties (Budiasih, 2017; Ramdhini & Dewi, 2024; Sesilia et al., 2024). Previous research has reported that a 70% ethanol extract from butterfly pea flowers exhibits antibacterial activity against Pseudomonas aeruginosa at a concentration of 10% and Bacillus cereus at a concentration of 30%. This antibacterial activity is indicated by the formation of clear zones, demonstrating the ability of the butterfly pea extract to inhibit bacterial growth (Riyanto & Suhartati, 2019). Additionally, the extract also shows antifungal activity against the growth of Alternaria solani at a 15% concentration (Suganda et al., 2020). Given the potential of butterfly pea flower as a natural substance capable of inhibiting microbial growth, further antifungal activity testing is necessary, particularly against other fungi such as Candida albicans, which causes dandruff. It is hoped that the butterfly pea flower's antimicrobial properties can exhibit equal or even greater effectiveness. The results of this research are expected to contribute to the development of anti-dandruff shampoo products derived from natural ingredients like the butterfly pea flower.

METHODS

The type of research conducted is laboratory experimental research. This study involves five stages of work. The first stage is preparation, which includes the sterilization of equipment and media to be used. The second stage is the extraction of ethanol from butterfly pea flower using the maceration method. The third stage involves preparing the media for fungal growth. The fourth stage is the implementation of testing the activity of butterfly pea flower extract against *Candida albicans.* The fifth stage is observing the results of the antifungal test of the ethanol extract of butterfly pea flower against *Candida albicans*, the cause of dandruff. The replication calculation in this study uses the Federer formula. The data obtained from the antifungal activity test is the size of the inhibition zone formed on the agar medium. The data is analyzed descriptively and quantitatively, where the data is presented in the form of tables and figures. Then, a Shapiro-Wilk test is conducted to determine whether the research data is normally distributed or not. If the data is normal, it is followed by a homogeneity of variance test to determine whether the data is homogeneous or not. If homogeneous, a one-way ANOVA test can be performed to determine whether there is any difference among all test solution groups

RESULT AND DISCUSSION

In this study, several tests were conducted, including organoleptic characterization of the simplicia and extract of the butterfly pea flower, phytochemical screening to determine the groups of secondary metabolites contained in the butterfly pea extract, and antifungal testing using the disk diffusion method to assess the ability of the butterfly pea extract to inhibit the growth of *Candida albicans*, indicated by the diameter of the inhibition zone formed.

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Table 1. Phytochemical Screening of Butterfly Pea

Phytochemical Test	Reagent/Treatment	Result
Alkaloids	Mayer's reagent	+
Terpenoids	Vanillin-sulfuric acid	+
Flavonoids	HCl + Magnesium powder	+
Saponins	HCl 2N	+
Tannins	$FeCl_3$ + 1% gelatin	+

Table 1 shows the results of the phytochemical screening of butterfly pea. The phytochemical screening was conducted with the aim of qualitatively detecting secondary metabolites in the material (Wulan Kusumo et al., 2022). This provides an overview of the active compounds present in butterfly pea. Based on the screening results, it was found that butterfly pea contains alkaloids, terpenoids, flavonoids, saponin and tannins.

Table 2. The Organoleptic Properties of Butterfly Pea Simplicia and Extract.

Sample	Results
Simplicia	Form: Dry Taste: Sweet Color: Blue Aroma: Characteristic of flowers
Extract	Form: Thick Taste: Tasteless Color: Dark brown Aroma: Characteristic of flowers

Table 2 shows the results of the organoleptic test of butterfly pea simplicia and extract. The organoleptic test was conducted as a physical evaluation using the senses to describe the shape, smell, color, and taste (Depkes RI, 2000). Based on the organoleptic test results, the simplicia had a dry form, sweet taste, blue color, and characteristic flower aroma. The extract had a thick consistency, tasteless, dark brown color, and characteristic flower aroma. These results indicate that both the simplicia and extract are in good condition with no quality degradation.

Tabel 3. Inhibitory Diameter of Butterfly Pea Extract Against *Candida albicans*

Treatment	Inhibition Diameter (mm) Replication					Mean ± Standard Deviation (%)	P-Value
	1	2	3	4	5		
5%	0	0	0	0	0	0	<0,001
15%	0	0	0	0	0	0	
30%	0	0	0	0	0	0	
60%	0	0	0	0	0	0	
90%	3,9	3,5	3,7	3,6	3,8	3,7±0,15	
Positive Control	14,2	14,4	14,5	14,7	14,1	14,3±0,23	
Negative Control	0	0	0	0	0	0	

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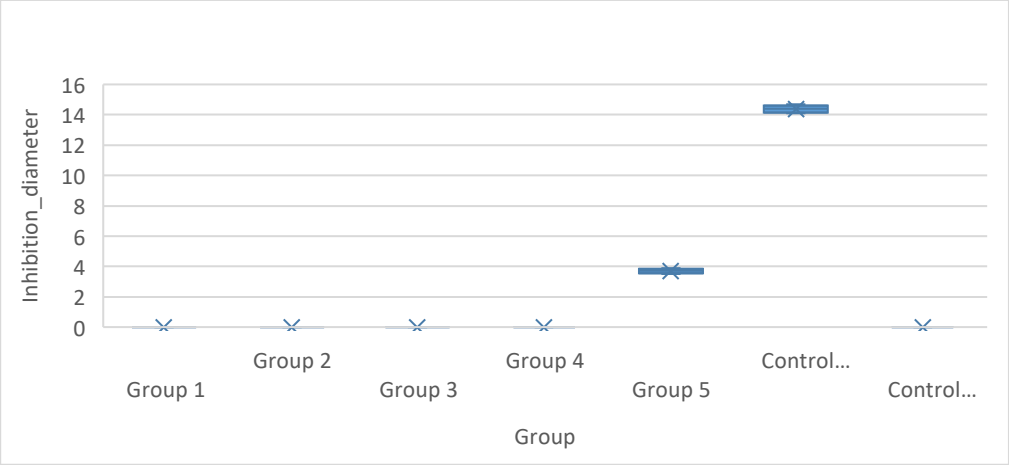

Figure 1. Graph of Antifungal Activity Based on Concentration of Butterfly Pea Extract

Table 3 shows the results of the antifungal activity test of ethanol extract of butterfly pea against Candida albicans. At a concentration of 90%, there is antifungal activity with an average inhibition zone of 3.7 mm formed, while at concentrations of 5%, 15%, 30%, and 60%, no inhibition zones were formed. In this study, the results of the normality test indicated a p-value < 0.05, which means the data did not meet the requirements for the One-Way ANOVA test. After conducting a non-parametric alternative test, the Kruskal-Wallis test yielded a p-value < 0.001, indicating a significant difference. This shows that a significant difference was found in Group 5 with the treatment of 90% extract concentration compared to the positive control, as indicated in Figure 1. These results indicate that there is no significant difference among the other groups because no inhibition zones were formed.

The antifungal activity test against *Candida albicans* was conducted using the disk diffusion method. The disk diffusion method involves using paper disks with a diameter of 6 mm. The paper disks serve as a medium to absorb the antifungal sample. In this method, the area of the clear zone formed around the paper disk is measured to determine antifungal activity. The clear zone indicates the microbial sensitivity to the antimicrobial sample used as the test material, represented by the width of the inhibition zone diameter (Gutiérrez et al., 2009). A test sample can be concluded to have antibacterial activity if it can inhibit the growth of the test bacteria based on the inhibition zone formed around the test sample. The ability of the test sample to inhibit bacterial growth can be categorized based on the diameter of the inhibition zone formed, including very strong level if the inhibition zone is ≥20 mm, strong level of 10-20 mm, moderate level of 5-10 mm, and weak level of ≤5 mm (Davis & Stout, 1971).

The positive control used was 0,1% ketoconazole tablets. The positive control yielded an average inhibition zone of 14.38 mm, categorized as strong. Ketoconazole is the most effective azole group in inhibiting the growth of *Candida albicans* because it is a broad-spectrum antifungal that inhibits the synthesis of fungal cell membranes, leading to increased permeability of the cell wall, making it susceptible to osmotic pressure (Kalsum & Ayu, 2019). The negative control used was a 1% CMC-Na (sodium carboxymethyl cellulose) solution. This negative control was employed to determine whether the solvent used could inhibit fungi. If the results obtained do not inhibit fungal growth, it indicates that the extract of butterfly pea effectively inhibits the growth of *Candida albicans* (Kusumawati et al., 2020). CMC-Na was chosen because it does not have antifungal activity. Additionally, CMC-Na was used to dissolve the test sample due to its characteristic of easily dissolving the extract compared to aquadest. In the negative control, no inhibition zone was formed because it does not possess antifungal activity.

The ability of butterfly pea extract to inhibit *Candida albicans* is attributed to its content of secondary metabolite compounds, including alkaloids, terpenoids, flavonoids, saponins and tannins. Alkaloid compounds inhibit microbes by disrupting the components that make up

peptidoglycan in fungal cells, resulting in an incomplete cell wall formation and ultimately causing cell death (Pertiwi et al., 2022; Robinson, 1995). Terpenoid compounds exert their antimicrobial effects by inhibiting fungal growth, either through the cytoplasmic membrane or by interfering with the development and growth of fungal spores (Ismaini, 2011). Flavonoids have three mechanisms for providing antimicrobial effects, including inhibiting fatty acid synthesis, disrupting cytoplasmic membrane function, and inhibiting metabolism (Nugrahini et al., 2019). Saponins inhibit microbes by having monosaccharide groups and their derivatives, which can act as detergents with structures that can interact with both hydrophilic and lipophilic molecules, thereby damaging the cytoplasmic membrane and killing fungi. Tannins inhibit microbes by precipitating proteins and damaging fungal cell membranes, which disrupts fungal growth (Budiasih, 2017).

In this study, the antifungal activity of the ethanol extract of butterfly pea (Clitoria ternatea) is categorized as weak according to Davis and Stout (1971). This is in contrast to the results of tests on bacteria, which indicated that the butterfly pea extract has antibacterial activity against *Staphylococcus aureus*, characterized by the formation of a strong inhibition zone. The difference is due to the more complex cell structure of fungi compared to bacteria (Widhowati et al., 2022). The defenses of *Candida* can be seen in the structure of its cell wall, which consists of five layers. This structure includes an outer plasma membrane composed of lipids, as well as an ergosterol membrane, which is a double phospholipid membrane that can resist lysis due to osmotic pressure. At 37°C, *Candida albicans* can form chlamydospores, which have very thick and strong spore walls, making them difficult for secondary metabolites to penetrate. Unlike antibacterial agents, bacteria belong to the prokaryote group, so the target cells of antibacterial agents are not found in mammalian cells. This has led to more advanced development of antibacterial drugs compared to antifungal drugs (Widhiasih *et al.*, 2017).

The mechanism of inhibiting the growth of *Candida albicans* involves disrupting or damaging the cell membrane, inhibiting the biosynthesis of ergosterol in fungal cells, and inhibiting fungal mitosis. actors that may contribute to the lack of inhibition at concentrations of 5%, 15%, 30%, and 60% of butterfly pea extract include the insufficient quantity of secondary metabolites in the extract to exert antifungal activity against *Candida albicans*. Additionally, the sensitivity of the organism, culture medium, incubation conditions, and agar diffusion rate can also affect antifungal efficacy. The virulence factors of *Candida albicans* can also influence the antifungal activity of the ethanol extract of butterfly pea. These virulence factors play a crucial role in the pathogenesis of *Candida albicans*. Some of these factors include morphological changes, tissue adhesion capability, Secreted Aspartyl Proteases (SAP), phospholipase secretion, phenotypic changes, and biofilm formation (Tyasrini et al., 2006).

CONCLUSION

The butterfly pea extract is capable of inhibiting the growth of Candida albicans with a weak inhibition zone. The most effective concentration of the ethanol extract of butterfly pea as an antifungal against Candida albicans was found to be at a concentration of 90%, with an average inhibition zone of 3.7 mm, while other concentrations did not show any inhibition zone against Candida albicans. These results indicate that butterfly pea can be used as a raw material for traditional medicine to prevent dandruff caused by Candida fungi, even at high concentrations. Therefore, further research is needed to conduct combinations to achieve a synergistic effect, as well as to perform safety and side effect tests.

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