

Antifungal Test of Telang Flower Ethanol Extract (*Clitoria Ternateal*) as a Mouthwash Against *Candida*

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ABSTRACT

Candidiasis is a disease of the oral cavity caused by fungal changes *Candida albicans* from saprophytes to pathogens. *Cl love albicans* live in the human body as saprophytes and can turn into pathogens if there are risk factors such as poor oral hygiene, low immunity, and other factors. So that preventive action can be done by using mouthwash that can inhibit the growth of bacteria or fungi, one of which is the use of butterfly pea flower extract. Objective: To determine the effectiveness of ethanol flower extract mouthwash in inhibiting growth *Candida albicans*. Research methods: This research is a true quantitative experimental laboratory test only control group design with a total of 36 samples consisting of positive control, negative control, and basic mouthwash added with 20%, 30%, 40%, and 50% ethanol extract. Research result: There were significant differences between the butterfly pea flower ethanol groups at concentrations of 20%, 30%, 40%, and 50% to the negative control group. So that the ethanol extract of butterfly pea flowers has inhibitory or antifungal power on growth *Candida albicans*. Conclusion: Mouthwash ethanol extract of butterfly pea flower concentration of 50% is the best concentration in inhibiting growth *Candida albicans* compared to other groups.

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1. INTRODUCTION

Candidiasis is a disease of the oral cavity caused by fungal changes *Candida albicans* from saprophytes to pathogens [1]. *Candida albicans* live in the human body as saprophytes and can turn into pathogens if there are risk factors such as low body immunity, presence of endocrine disorders, long-term use of endocrine therapy, smoking, chemotherapy, and bad oral hygiene [2]. Diseases caused by *Candida albicans* one of which is associated with poor oral hygiene [3].

Indonesia is one of the countries with a tropical climate which has a high humidity level, lead *Candida albicans* easier to grow and develop [2]. Data from the Directorate General of Disease Control and Environmental Health (P2PL) of the Ministry of Health of the Republic of Indonesia in 2018, reported that candidiasis ranks second highest of the 10 diseases accompanying people with HIV/AIDS (PLWHA), totaling 266 cases [4].

Chronic candidiasis will develop into a systemic infection through the lymph flow which can attack vital organs such as the kidneys, lungs, brain, blood vessel walls. And can develop towards malignancy such as leukoplakia which causes squamous cell carcinoma. So that patients need preventive measures to reduce the prevalence, especially in conditions of oral candidiasis [5]. Prevention efforts that can be done is to use mouthwash that can inhibit the growth of bacteria or fungi [2]. The active ingredients contained in mouthwash consist of: *tween 80* (5%), *glycerin* (2,5%), *coat* (0,1%), *Of Sakari* (0,2%), *Na Benzoat* (0.4%), and *aquades* (100 ml) [6]. Mouthwashes on the market today contain more than one active ingredient to support oral hygiene, one of which is alcohol with a concentration of 70% which acts as a solvent and preservative and functions as an antiseptic. However, long-term use causes dry mouth, reduces saliva production, and damages the tissues in the oral cavity [5]. Therefore, this research is an experimental study of mouthwash without alcohol content, without side effects, and can be used for a long time.

[7] The treatment of oral candidiasis usually uses the synthesis of first-line antifungal drugs namely *Nistatin*, *Amphotericin B*, and *Clotrimazole* and second-line treatment such as *Ketokonazol*, *Flukonazol*, and *Itraconazole*. However, using this drugs causes several side effects such as nausea, vomiting, diarrhea, and headaches. The use of traditional medicine is generally considered safer than modern medicine, one of the herbs used as an alternative antifungal is butterfly pea flower.

Butterfly pea flower (*Clitoria ternatea L*) grows and spreads in areas with subtropical and tropical climates, one of which is Indonesia. Butterfly pea flower has many benefits with its composition, namely acid *phenolicas* antibacterial, antiviral, anticarcinogenic, and anti-inflammatory, *flavonols* as antioxidants, antibacterial, antiviral, anti-inflammatory, and hypoallergenic, as well as compound *squercetin* and *malvidin* as a *antihistamines*, anti-inflammatory, anti-diabetic, anti-inflammatory, and anticancer [8].

[9] Previous research showed that the ethanol extract of butterfly pea flowers with a concentration of 70% from the roots, leaves, stems and seeds of butterfly pea flowers has the ability as an antifungal. Other studies also prove that telang flower kombucha (*Clitoria ternatea L*) has a secondary metabolic content that is *flavonoid*, *alkaloid* and *saponin*, where these compounds have cellular mechanisms that can inhibit microbial growth. *Alkaloid* works by leaking the cell membrane, *flavonoid* works by destroying cell membranes, as well *aponin* works by inhibiting cell wall synthesis which causes lysis of fungal cells and inhibits the growth of pathogenic fungi [10]. Therefore, butterfly pea flower is an alternative that can be used as a mouthwash to inhibit growth *Candida albicans*. The purpose of this study was to determine the effectiveness of ethanol flower extract mouthwash in inhibiting growth of *Candida albicans*.

2. METHOD

This research is a true quantitative experimental laboratory post test only control group design. This study consisted of the independent variable, namely the ethanol extract of butterfly pea mouthwash and the dependent variable, namely the zone of inhibition of fungal growth of *Candida albicans*.

The sample calculation technique uses the Feeder formula with the results of 36 samples. The research sample was divided into six groups, namely two control groups and four intervention groups. The control group consisted of a positive control group, namely 0.2% chlorhexidine solution and a negative control group, namely a mouthwash solution without butterfly pea flower extract. While the intervention group is the mouthwash basic formula and additional ethanol extract of butterfly pea flower with concentrations of 20%, 30%, 40% and 50%. The research was conducted in the laboratory of Setia Budi University, Surakarta City in May-June 2023.

Research tools in this study using test tube, measuring cup, tween, erlenmeyer, micropipette, glass funnel, bunsen burner, marker, handsoon, mask, calipers, 6 mm cork borer, yellow tip, wire loop, spectrophotometer, incubator, autoclave, analytical balance, stove, and pan stainless. And the material consists of two ingredients, namely the main ingredient ethanol extract of butterfly pea flowers and media Sabouraud Dextrose Agar (SDA) *candida albicans*. Supporting materials are Aquades, chlorhexidine antifungal was used as a positive control, 0.5 standard solution *Mc. Farland*, and NaCl 0,9%.

For data analysis, this study used a nonparametric test *Kruskal Wallis* and advanced test using *Post Hoc Dumn Test*, because based on the previous test it showed that the data were not normally distributed with a p value <0.05 and were not homogeneous.

3. RESULTS AND DISCUSSION

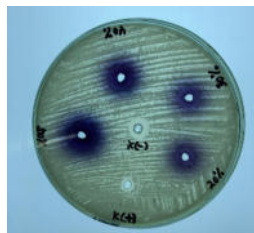


Figure 1. Antifungal activity of ethanol extract of butterfly pea flowers against *Candida albicans*

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Information:

20% : Ethanol extract of butterfly pea flower 20% concentration

30% : Ethanol extract of butterfly pea flower 30% concentration

40% : Ethanol extract of butterfly pea flower 40% concentration

50% : Ethanol extract of butterfly pea flower 50% concentration

Positive control : Chlorhexidine solution 0.2%

Control negative: Mouthwash solution without butterfly pea flower extract

Table 1. Average and Standard Deviation of Resistance Measurement Results

Sample Group	N	Mean	Std. Deviation
Concentration 20%	6	14.600	.6293
Concentration 30%	6	16.017	.0408
Concentration 40%	6	17.350	.1975
Concentration 50%	6	20.017	.0408
Positive Control	6	14.017	.0408
Negative Control	6	17.017	.0408

Table 2 shows that the 50% concentration group has the highest mean value of inhibition, which is 20,017 mm with a standard deviation of 0,0408.

 Table 2. Nonparametric Test Results *Kruskal-Wallis*

	Diameter of Antifungal Power
Kruskall Wallis H	33.664
df	5
Asymp. Sig.	0.000

The table above shows the Asymp values. Sig is 0.000 ($p < 0.05$) which means that H_0 is rejected and H_a is accepted. There is a significant difference between the six sample groups in the inhibition of growth of *Candida albicans*.

 Table 3. Test Results *Post Hoc Dunn*

Sample Group	20%	30%	40%	50%	Positive Control	Negative Control
20%	-	.562	.229	.022	.002*	.000*
30%	.075	-	.229	.275	.058	.003*
40%	.000*	.002*	-	.058	.423	.275
50%	.000*	.000*	.003*	-	.058	.275
Positive Control	.562	.075	.064	.000*	-	.000*
Negative Control	.064	.022	.275	.423	.058	-

The table 3 shows that there is a significant difference in antifungal power between the concentration groups of 20%, 30%, 40%, and 50% of the ethanol extract of butterfly pea flowers to the control group with p value $< 0,05$. And there was a significant difference between the 50% concentration of butterfly pea flower ethanol and the positive control group. Based on the results of the analysis above, the ethanol extract of butterfly pea flowers has inhibitory or antifungal power on growth of *Candida albicans*.

Discussion

The results showed that there were differences between the six sample groups in their potency as an antifungal, and significantly the butterfly pea flower had an effect on inhibiting growth of *Candida albicans*. In line with previous research that the butterfly pea flower (*Clitoria Ternatea L*) functionally

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has benefits for the human body and is able to inhibit microba growth [11]. This is because the butterfly pea flower has the ability to act as a *antioxidant*, *antidiabetic*, *antiobesity*, *anticancer*, *anti-inflammatory*, *antibiotic* and protects liver tissue.

Butterfly pea flowers contain bioactive components that are lipophilic and hydrophilic, and contain bioactive components such as *flavonol glycosides*, *anthocyanins*, *flavones*, *flavonols*, *phenolic acids*, *terpenoids*, *alkaloid*, as well as compounds of *cyclic peptide or cyclotide*. Butterfly pea flower has advantages and benefits which can be used as a potential ingredient for functional food, nutraceuticals and medicine (Abdullah 2020).

Previous studies have also shown the use of butterfly pea flower extract (*Clitoria ternatea*) and butterfly pea flower kombucha fermented solution is effective in inhibiting the growth of bacteria and *Candida albicans*, where the researchers also used butterfly pea flower extract with a concentration of 50% [12]. The higher the concentration of the mouthwash preparation, the more its ability to inhibit growth increases *Candida albicans*. This is also influenced by the content *flavonoid* which work cellularly to prevent the growth of fungi by means of protein denaturation and coagulation of proteins found in fungal cells [11].

The content of secondary metabolites such as alkaloids and saponins also found in the butterfly pea flower. These compounds have cellular mechanisms in which each compound has the ability to inhibit microbial growth [10]. The content of alkaloids carry out their duties by leaking the cell membrane, then compounds *flavonoid* works by destroying the cell membrane, and *saponin* works by inhibiting cell wall synthesis, resulting in lysis of fungal cells which has an effect on inhibiting the growth and development of pathogenic fungi [13].

Stability test of butterfly pea flower ethanol extract mouthwash has also been evaluated to see the quality of the extract in previous studies. The results showed that the yield value was 52.05% [6]. The examinations carried out were organoleptic examination of PH and Viscosity. Butterfly pea flower ethanol extract mouthwash with a concentration of 50% has the highest inhibition of growth *Candida albicans*. And organoleptic tests were also carried out including examination of shape (in the form of a watery liquid), examination of smell (smells of mint), examination of color (dark blue in color) and examination of taste (tastes coarse).

The pH of the ethanol extract mouthwash of the butterfly pea flower was examined at a concentration of 50% where a pH value of 5.99 was obtained which indicated that the pH value was acidic. The pH value of the resulting mouthwash must be in the pH range of the oral cavity which ranges from 5.5 to 7.9 so that when the preparation is consumed it does not cause irritation to the oral mucosa [14]. Examination of the viscosity of the ethanol extract mouthwash of butterfly pea flowers was carried out using a VT-04 viscometer (*high viscosity*). The results of the viscosity analysis showed that the viscosity value of the ethanol extract mouthwash formula of butterfly pea flower with a concentration of 50% had a range of 1.550 cP – 1.552 cP. Where this range is close to the viscosity of pure water (1002 μ Pa.s or around \pm 1 cps) [15].

The viscosity of a formula greatly affects the level of viscosity of the product when it is used to gargle in the mouth. The closer the viscosity level of a formula product to the viscosity level of water, the easier and more comfortable the product is to use for gargling [16].

4. CONCLUSION

Concentration of 50% in mouthwash preparations of ethanol extract of butterfly pea (*Clitoria ternatea L*) is the best concentration in inhibiting growth *Candida albicans*, compared to negative control, positive control, and concentrations of 20%, 30% and 40%.

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