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Antimicrobial Test of Telang Flower Extract (*Clitoria ternatea L.)* against the Growth of *Candida albicans*

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Abstract

Candidiasis is an acute and subacute fungal infection caused by the Candida group, especially *Candida albicans*. Infectious diseases due to microbes can be treated using modern drugs, namely antifungals. In some cases, antimicrobials often develop resistance due to improper administration. The occurrence of such resistance encourages further research on new active ingredients that can be used as antimicrobial alternatives, especially from plant sources containing flavonoids. The purpose of this study is to determine the effect of antimicrobial content of ethanol extract 70% of Telang flowers on the growth of *C. albicans*. This research is a laboratory experimental research with post test control group design only method. Determination of antimicrobial activity is carried out by the pitting method. Telang flower extract that has been diluted with 10% DMSO, taken as much as 30µl then dripped into the well. Positive control using antimicrobial ketocenazole and negative control using DMSO 10% which was dripped on the well as much as 30µl. Based on the results of observations, the inhibition zone is only formed in positive control with an average of 15.5 $\pm$ 0.4 mm. The results of ethanol extract of 70% Telang flowers that have been conducted did not show antimicrobial activity against fungal growth *C. albicans*. The absence of this inhibitory zone is due to the unknown concentration of active compounds of Telang flowers that are responsible as antimicrobials, so it is necessary to conduct research on the search for concentrations of active compounds that have the ability as antimicrobials.

Keywords: Telang flower extract, candidiasis, Candida albicans

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INTRODUCTION

Indonesia is a warm and humid tropical country that allows for the growth of a wide variety of microbes that cause infection (Wahyuningsih et al., 2021). Infection is a disease that is still one of the major public health problems in developed and developing countries including in Indonesia (WHO, 2011). Infectious diseases due to microbes in Indonesia are mostly caused by fungal and bacterial infections. One of the microbes that cause infection is *Candida albicans* (Puspitasari et al., 2019).

C. albicans is an oblong fungus that reproduces by budding that produces pseudomycelium both in culture and in tissue and exudate (Tazkiatulmilla, 2020). Candida is a normal flora of the mucous membranes of the respiratory tract, gastrointestinal tract and female genitalia (Rumayar et al., 2020; Yulianingsih et al., 2022). In these places these fungi may become dominant and be associated with pathogenic States (Riedel et al., 2019).

C. albicans are the most common causative agents of mucosal infections and systemic infections, and are responsible for about 70% of fungal infections worldwide. *C. albicans* is the cause of candidiasis disease (Talapko et al., 2021). *C. albicans* causes canker sores,

lesions on the skin, vulvavaginistis, candida in the urine, gastrointestinal candidiasis that can cause gastric ulcer or can even be a complication of cancer (Riedel et al., 2019).

Death rate due to *C. albicans* infection account for nearly 40% (Nabilla Tahta Avwina Amir et al., 2021). Infectious diseases due to microbes can be treated using modern drugs, namely antifungals. In some cases, antimicrobials often cause resistance due to improper administration (Utami, 2012). The occurrence of such resistance, encourages further research on new active ingredients that can be used as antimicrobial alternatives, especially from plant sources containing flavonoids (Rahmawati, 2015; Zahro Iftikhonsa et al., 2021). Flavonoid Flavonoids are natural compounds known as antioxidants, antimicrobials and anti-inflammatories (Ismail & Wan Ahmad, 2019).

One plant that has a high flavonoid content is Telang flower. Telang flower is one of the plants in Indonesia that is often used as an ornamental and medicinal plant (Purba, 2020). According to (Styawan & Rohmanti, 2020), flavonoid levels in telang flower extract average 4.65%. Telang flowers also contain anthocyanins that play a role in the maintenance of eye tissue, antidiabetic, anti-inflammatory, maintain the immune system, and prevent platelet aggregation. Telang flower has considerable pharmacological potential, including as an antioxidant, antimicrobial, anti-inflammatory, analgesic, antiparasitic and antacid, antidiabetic, anticancer, antihistamine and immunomodulator (Ketut Ayu Martini et al., 2020).

Based on research conducted by (Rezaldi, Eman, et al., 2022)) on the potential of Telang flowers as antifungals with biotechnological methods of kombucha fermentation showed that telang flower kombucha at 40% sugar concentration has the potential to be the highest antifungals when compared with 20% sugar concentration and 30% sugar concentration. Where is the diameter of the inhibitory zone formed in fungus *C. albicans* by 21.24 mm. This is because the content of anthocyanin compounds in Telang flowers will be more stable if fermented by lactic acid bacteria (BAL), one of which is found in kombucha. Due to the fermentation process of kombucha Telang Flower takes quite a long time that is for $\pm$ 12 days, then the manufacture of ethanol extract telang flower. Where the manufacture of ethanol extract of telang flowers does not really require a long time.

Referring to the description, then conducted research on antimicrobial test of Telang flower extract (*Clitoria ternatea L*.) because these plants are considered to need in-depth testing related to the effectiveness of antimicrobial content of Telang flowers. The purpose of this study was to determine the effect of antimicrobial content of Telang flower extract on the growth of *C. albicans*.

METHOD

Research design :

This study has a laboratory experimental nature with the design used is post test control group design only. The method used to test the antimicrobial activity in this study using the pitting method. The research process was conducted in the Laboratory of Pharmaceutical Microbiology Falkultas Health Sciences University of dr. Soebandi.

Research tools and materials :

The tools used in this research are petri dish, incubator, test tube, micropipette, yellow tip, erlenmeyer, analytical balance, and bunsen. While the materials used in this study include telang flowers (*Clitoria ternatea L*.), Sabouraud Dextrose Agar (SDA), ketoconazole, DMSO 10%, *Candida albicans fungus* (ATCC 10231).

Population and sample :

The population of this study was *C. albicans*, while the sample in this study is a fungus *C. albicans* rejuvenated on Potato Dextrose Agar (PDA) media.

Repetition in this study is determined by using the Formula Federer :

$$(n-1)\ (t-1) \geq 15$$

n : number of replication
t : many treatments

Based on the results of the Federer formula, the minimum number of repetitions of each group is 5 times.

Manufacture of ethanol extract of telang flower :

Telang flower (*Clitoria ternatea L.*) is extracted using the maceration method. Maceration is a simple extraction process by using a solvent several times shaking or stirring at room temperature (Susanty & Bachmid, 2016).

Antimicobial activity test procedure :

Testing of antimicrobial activity in this study was divided into 5 groups, where each group was replicated 5 times. The division of the group is as follows :

1. Positive control group (+): testing of antimicrobial activity by administration of ketoconazole
2. Negative control group (-): antimicrobial activity testing with administration of DMSO 10%
3. Treatment group 1 (P1): antimicrobial activity testing with telang flower extract concentration 25%
4. Treatment group 2 (P2): antimicrobial activity testing with telang flower extract concentration 50%
5. Treatment group 3 (P3): antimicrobial activity testing with telang flower extract concentration 100%

Antimicrobial activity testing method in this study using the pitting method. The first step is to dilute the Telang flower extract using aquadest. The result of the dilution is then taken as much as 30µl which is then dripped into the well. The next process is bacterial density strandarization with McFarland 0.5. If the standardization process has been in accordance with the standard, followed by applying evenly smeared fungus *C. albicans* on Sabouraud Dextrose Agar (SDA) media. Then dripped Telang flower extract concentration according to the treatment group, DMSO 10% (Control-), and ketoconazole (Control+). The medium is incubated using an incubator at a temperature of 37^0C for 24 hours. The last step is to look at the resistance zone around the well and measured using a caliper by adding the diameter of the vertical and horizontal clear zones then divided by 2.

RESULTS AND DISCUSSION

Based on the antimicrobial activity test of Telang flower extract against fungus *C. albicans* (Table 1) shows if treatment 1, treatment 2, and treatment 3 do not form inhibitory zones. The inhibitory zone was formed in the positive control group (C+), that is, with the administration of ketoconazole. Administration of a dose of ketoconazole as much as 30 micrograms. The selection of ketoconazole as a positive control (C+) because ketoconazole is an antifungal drug that is often used in the treatment of dermatophytosis. Ketoconazole was the first oral azole to be used clinically. Azole itself is a synthetic compound that is classified into imidazole or triazole (Lely et al., 2017).

Table 1. Antimicrobial Activity Test Result of Telang Flower Extract against *Candida albicans* Fungus

Concentration treatment	Results/Replication (mm)					Mean of Whole Replication (mm) ± SD
	1	2	3	4	5	
Telang extract flower 25%	0	0	0	0	0	0 ± 0
Telang extract flower 50%	0	0	0	0	0	0 ± 0
Telang extract flower 100%	0	0	0	0	0	0 ± 0
Positive control (Ketocenazole)	16	15,4	15	16	15,5	15,5 ± 0,4
Negative control (DMSO 10%)	0	0	0	0	0	0 ± 0

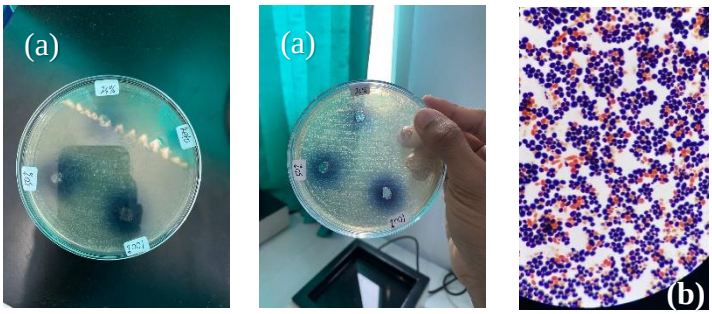

Figure 1. Macroscopic (a) and Microscopic (b) Antimicrobial Activity Test of Telang Flower Extract against *Candida albicans* Fungus

According to research conducted by (Rezaldi, Agustiansyah, et al., 2022) showed that the concentration of 40% in probiotic shampoo preparations made from fermented kombucha solution of telang flower was the best concentration in inhibiting fungus *C. albicans*, *A. fumigatus*, and *P. ovale*. This is because the higher the concentration, the potential ability as an antifungal will be high. However, this study is in contrast to previous studies, because the inhibitory zone is only formed in the positive control group (C+) and not formed in the treatment group 1, treatment 2, and treatment 3.

Telang flower plant (*Clitoria ternatea L.*) is a pod plant that belongs to the *Fabeaceae* family (Marpaung, 2020). This plant is a multipurpose plant because besides being able to be used as decoration it also contains bioactive compounds that are used for treatment (Styawan & Rohmanti, 2020).

Telang flowers contain various secondary metabolite compounds such as saponins, flavonoids, tannins, and terpenoids (Purwaniati et al., 2020). Flavonoid and tannin compounds act as antioxidants, antimicrobials, and antifungals (Sumi et al., 2020). Saponins play a role in anti-inflammatory, antifungal, antibacterial, antiparasitic, anticancer, and antiviral activities (Mugford & Osbourn, 2013), while the role of terpenoids is as anticancer (Cox-Georgian et al., 2019).

Various important ingredients in Telang flowers can be used as an alternative as an antifungal drug. Based on research conducted by (Jayadi et al., 2022) flavonoids can be used as antifungals because they are able to inhibit the process of fungal cell proliferation. The compound will take up microtubule proteins in the cell and interfere with the spindle mitosis process, so that fungal growth will be inhibited (Nguyen et al., 2021). Although from the research conducted did not form inhibitory zones in the treatment group using Telang flower extract.

This study used mushrooms *C. albicans*, where *C. albicans* are fungi that have an oblong shape, whose mode of reproduction is sprouting and producing pseudomycelium both in culture and in tissues and exudate. *C. albicans* is a normal flora of the mucous membranes of the respiratory tract, gastrointestinal tract and female genital organs. In these 3 places if there is dominance by fungi *C. albicans* would then be associated with a pathogenic state (Kalista et al., 2017).

The results showed that the inhibitory zone formed in the positive control (C+) was very large at 15.6 mm, when compared with treatment 1, treatment 2, and treatment 3 using Telang flower extract. This very significant difference indicates if the effect of the antifungal possessed by ketoconazole against fungi *C. albicans* is better than with telang flower extract. This is likely due to the unknown concentration of active compounds in Telang flowers that are responsible for giving antifungal effects, so that the average higher inhibition zone formed in the control group of Positive (C+) antifungal ketoconazole.

CONCLUSION

From the results of ethanol extract test 70% Telang flowers showed no antimicrobial activity against the growth of *C. albicans*. This is because it is not yet known the concentration of active compounds of Telang flowers that have an active role as an antifungal.

RECOMMENDATION

It is recommended to use several other telang flower extraction methods so that it is expected to obtain maximum antimicrobial compounds that can be used as antimicrobials against *C. albicans*.

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