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Test of The Inhibitory Power of Lemongrass Extract Against The Growth of *Candida albicans* On Natural Resources Medium

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ABSTRACT

Candidiasis is an acute or chronic inflammation that causes disorders of the body's system caused by the fungus *Candida albicans*. *Candida albicans* is a common cause that does not cause disease in a person's normal immune system but can attack a person's poor system. Candidiasis can be treated with antifungal drugs, but prolonged and extensive use of antifungal drugs causes side effects. So alternative drugs are needed that can reduce these side effects. Lemongrass (*Cymbopogon citratus*) has pharmacological activity as an antifungal. Research Objectives: To determine the inhibition of lemongrass extract on the growth of *Candida albicans* in an SDA medium with concentrations of 0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%. The study used a descriptive research design. The method of testing the resistance is carried out by the good diffusion method. Lemongrass extract can inhibit the growth of *Candida albicans* fungus by forming an inhibition zone. At a concentration of 0% lemongrass extract, no inhibition zone was formed, a concentration of 0.2% formed an inhibitory zone of 10.5 mm, a concentration of 0.4% formed an inhibition of 6.1 mm, a concentration of 0.6% formed an inhibition zone of 14 mm, the concentration 0.8% formed an inhibition zone of 10.8 mm, a concentration of 15 formed an inhibitory zone of 27.6 mm.

KEYWORDS

Candida albicans; lemongrass; well diffusion

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Introduction

Candidiasis is an acute and chronic inflammation that causes disorders of the body system, (generally on the skin and membranes) caused by the fungus *Candida albicans*. *Candida albicans* is generally a normal flora in the human body and does not cause disease in the condition of a person's normal immune system, but can attack a person's bad body system. Based on ICU adult patient data from Hasan Sadikin Hospital Bandung from June 2016 - July 2017, the ICU inpatient population was 1,434, after blood culture was obtained from 313 patients, as many as 11 patients were positive for invasive candidiasis (Indriani et al., 2018). In 2010, data from the Ministry of Health of the Republic of Indonesia showed the occurrence of candidiasis infection by 25% - 50%, and in 2011 - 2013 it was found that candidiasis vulvovaginitis with a prevalence of 30% - 35% (Lusiana, N, Yuswantina, R, Retno, 2015). Prevention can be overcome by the administration of antifungal drugs such as ketoconazole, nystatin, miconazole, and trichome. The drug works to damage the cell membranes of fungi and inhibits the synthesis of proteins and RNA (Masloman, AP, Pangemanan, DHC, Anindita, 2016). Treatment of candidiasis is necessary with antifungal therapy, but the use of antifungal drugs can cause side effects (Maulana, HR, Sumard, U, Koesoemadinata, 2019). Long-term and widespread use of the drug is the main cause of the effects caused (Mutammima, 2017). Prevention can be overcome by the administration of antifungal drugs such as ketoconazole, nystatin, miconazole, and trichome. The drug works to damage the cell membranes of fungi and inhibits the synthesis of proteins and RNA (Mutiawati. Vivi, n.d.). Treatment of candidiasis is necessary with antifungal therapy, but the use of antifungal drugs can cause side effects (Oktasila, D, Nurhamidah, Handayani, 2019). Long-term and widespread use of the drug is the main cause of the effects caused (Pujawati, RS, Rahmat, M, Djuminar, A, Rahayu, 2019). One of the plants used as alternative medicine is the lemongrass plant (*Cymbopogon citratus*). Lemongrass plants are easy to find in Indonesia because they are widely grown with sufficient soil fertility and do not require special care. Lemongrass (*Cymbopogon citratus*) is a herbal plant that contains antifungal and antibacterial compounds (12). Lemongrass plants have chemical content in them, such as tannins, phenols, steroids, alkaloids, saponins, essential oils, and flavonoids. Research conducted by Pujawati (2019) with kitchen lemongrass extract (*Cymbopogon citratus*) against the growth of *Candida albicans* macmicrovillusthod showed that the lowest concentration of kitchen lemongrass extract in inhibiting the growth of *Candida albicans* was at an extract concentration of 0.4% and the effective contact time in inhibiting the growth of *Candida albicans* was at a contact time of 36 hours (Oktasila, D, Nurhamidah, Handayani, 2019). The purpose of this study was to measure the inhibitory power of lemongrass extract (*Cymbopogon citratus*) against the growth of *Candida albicans* in Sabouraud

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Dextrose Agar (SDA) medium by well diffusion method. Its particular purpose is to identify the inhibitory zone of lemongrass extract (*Cymbopogon citratus*) with concentrations of 0%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0% in use scientific writing according to the rules fungi.

Method

The research was conducted at the Bacteriology and Parasitology Laboratory, Faculty of Health, Rajawali Health Institute Bandung. This research is a descriptive study. The population in this study was an isolate of Candida albicans. The tools used include disc paper, autoclave, laminar air flow, petri dish, oven, incubator, and ruler (mm). The ingredients used are SDA medium, alcohol, spirits, lemongrass extract, aqueous, and DMSO 10%. The samples used in this study were colonies of Candida albicans isolates. The collected data is then processed by going through the labeling stage. After conducting laboratory research on the material tested by the inhibitory power test, then observation of the inhibitory zone formed around the well was carried out. The measurement of the diameter of the inhibition zone is presented in the form of a table which is then calculated on average. The diameter of the formed inhibition zone is measured using a ruler and calculated by the formula: (Sri Rezeki, Santi Chismirina, 2017)

$$\textbf{Block zone diameter} = \frac{(\boldsymbol{D1} + \boldsymbol{D2}) - \boldsymbol{Ds}}{\mathbf{2}}$$

Results

This research was carried out by preparing lemongrass that has been cut into small parts, then mashed using a blender and processed by maceration until a dry extract is obtained. The following is the data on the weighing results of lemongrass extract in table 1.

Table 1. Data on Weighing Results of Lemongrass Extract

Weight of lemongrass stalks (kg)	Weight of dried lemongrass stalks (grams)	The volume of ethanol is 96% (L)	Weight of dry extract (grams)
1	450	6,7	24

The purpose of observing the morphology of *Candida albicans* mushroom colonies is to determine the purity of the fungi tested. Macroscopic observations can be seen in table 2.

Table 2. Macroscopic Observations of *Candida albicans* on SDA Medium

Colony Observation	Results
Colony color	Yellowish white
Colony Surface	Slick
Exudate drop	exist
Radial furrow	none
Zoning	exist
Mucoid colony	Mucoid
Color behind	Create
Growth zone	exist
Colony Edge	flat
Breeding age	48 hour
Shape	Round

Microscopic observations were carried out with gram staining and LPCB staining in fungi until the result data were prepared in table 3.

Table 3. Microscopic observations of *Candida albicans*

Microscopic observation	Coloring Observation Results	
	Grams	LPCB
blastospore	Exist	Exist
Spore shape	Round	Round
Pseudohyphae	Exist	not visible

Table 4. Phytochemical Test Results

Identified Compounds	Interpretation of Results	Description
Alkaloids (Mayer)	Positive	The results showed the presence of a white precipitate.
Alkaloids (Dragendorf)	Positive	There is a brownish-orange precipitate, positive for alkaloid compounds.
Tannins	Positive	The solution is purple, positive for tannins.
Saponins	Positive	There is a foam that lasts for 1 minute.
Flavonoids	Positive	The reaction product is pink.

Steroids/terpenoids	Negative	The result shows a yellow color which indicates steroid/terpenoid negative

Table 5. Research Test Result Data

Sample code	Concentration	Repetition				Diameter average (mm)	Category
		1	2	3	4		
1	Positive control					36,0	Very strong
2	Negative control					0	Weak
3	0%	0	0	0	0	0	Weak
4	0,2%	11,0	11,0	10,0	10,0	10,5	Strong
5	0,4%	10,5	0	0	14,0	6,1	Currently
6	0,6%	8,5	10,0	27,5	10,0	14,0	Strong
7	0,8%	11,0	11,5	10,5	10,5	10,8	Strong
8	1%	30,0	21,5	30,0	29,0	27,6	Very strong

The diameter of the inhibition zone can be seen in the Picture 1.

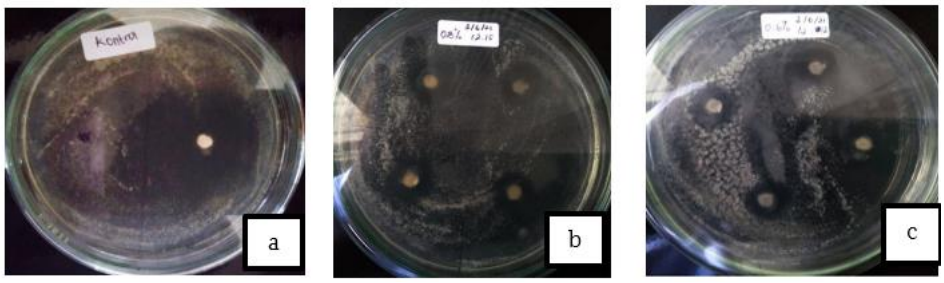

Figure 1. Inhibition Zone Diameter a) positive control; b) Concentration 0,8%; c) Concetration 0,6%

Discussion

The research was conducted from April to June 2021, at the Bacteriology and Parasitology Laboratory of the Faculty of Health, Rajawali Health Institute Bandung. The objective of the study is to determine the inhibitory power of lemongrass extract (*Cymbopogon citratus*) against the growth of *Candida albicans* in the natural resources medium. Testing is carried out by the method of diffusion of wells. The plant used, namely lemongrass (*Cymbopogon citratus*) is cut into small pieces. Lemongrass is dried to a moisture content of <10% (simplistic). Lemongrass Simplicia was added with a 96% ethanol solvent of 6.7 L, then allowed to stand for 24 hours. The solution is filtered using filter paper which is then evaporated with a rotary evaporator until a viscous extract is obtained. The manufacture of the extract is carried out by the maceration method. The viscous extract is processed by lyophilization to obtain a pure dry extract. Lemongrass was used for as much as 1 kg, then a dry extract of 450 g was obtained with a volume of 96% ethanol as much as 6.7 L. The dry extract obtained was 24 g (Table 1).

The results of macroscopic observations of *Candida albicans* are shown (Table 2). *Candida albicans* were cultured on Sabaoroud Dextrose Agar (SDA) medium for 48 hours at room temperature to observe the macroscopical morphology of the fungus *Candida albicans*. Identification of *Candida albicans* grown on a solid medium so that the natural SDA temperature of 25˚C after 24-48 hours candida colonies grew with a rounded, protruding shape, smooth, slippery surface, and a yellowish-white colony color. This is by the research conducted which shows the results (Pangesti et al., 2017).

Figure 2. Macroscopic *Candida albicans* on SDA medium

Microscopic examination of *Candida albicans* is performed to see the structural or morphological shape of *Candida albicans* by doing LPCB staining and gram staining. The results of the LPCB staining research that has been

carried out were found to be a blue round spore shape with observations on a 100x magnification microscope in Figure 3.

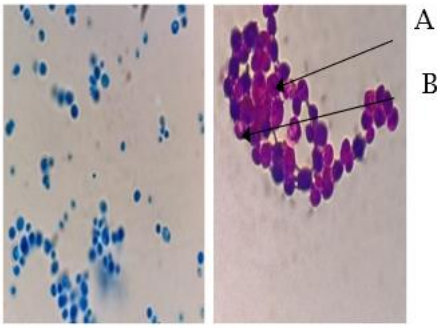

Figure 3. Coloring *Candida albicans* (A) Blastospore (b) Pseudohifa

Gram staining in fungi is the same as bacterial staining. Observations of *Candida albicans* preparations on Gram staining were carried out with an enlargement of 100x so that it was found that the morphology of purple fungi with a spherical spore shape was thick and some were thin and had hyphae between the spores. On gram, staining is visible clusters of fungi in the form of blastospores, hyphae, and pseudohyphae (EN., 2012). The results of the study are the same as the results of the research that has been carried out, where the results of the study found spores with a round shape of blastospores and there are also pseudo-hyphae or adjacent blastospores can be seen in Figure 2. Identification of compounds in lemongrass extract is carried out by phytochemical tests, which is a qualitative test of secondary metabolites (Trianingsih, 2019). Phytochemical testing is carried out by taking a small sample of lemongrass extract and then reacting it with a reagent of the compound to be tested.

Based on the results of the identification of phytochemical tests in Table 4, it can be seen that the active compounds contained in lemongrass extract are saponins, tannins, flavonoids, and alkaloids. These results are in line with the study that pujawati (Pujawati, RS, Rahmat, M, Djuminar, A, Rahayu, 2019) did which contained alkaloid compounds, flavonoids, terpenoids, saponins, and tannins in kitchen lemongrass extract.

The clear zone formed indicates the presence of inhibition of the growth of *Candida albicans* by secondary metabolite compounds produced by lemongrass. Tannin compounds function to change the structure of the cell wall of *Candida albicans*. Cell destruction is carried out using the denaturation of proteins that disrupt the metabolism and processes of absorption of fungal nutrients (Masloman, AP, Pangemanan, DHC, Anindita, 2016). Alkaloid compounds are capable of inhibiting esterase, cell respiration, DNA, and RNA polymerase. Saponins have the function of interfering with permeability, this is done by lowering the surface tension of the sterols and cell walls of *Candida albicans* so that there is an increase in permeability which causes more concentrated intracellular fluids to come out of the cell so that, metabolic substances, nutrients, enzymes, and proteins in the cell come out which results in the fungus experiencing death. Flavonoids work by inhibiting the growth of fungi resulting in impaired permeability of fungal cell membranes. Flavonoid compounds have hydroxyl groups that can change organic components and nutrient transport so that the growth of fungal cells is inhibited and subject to death (Wahyuni, S, Sri, SD, Wildiani, 2018). Antifungal activity is calculated based on the area of the clear zone by observing the zone on the medium. The weak inhibitory power category has a diameter of <5 mm, the medium inhibitory power category has a diameter of 5-10 mm, the strong inhibitory power category with a diameter of 10-20 mm, and the very strong inhibitory power category with a diameter of >20 mm (Gunawan et al., 2018). Based on the results of the inhibitory power test in Table 4.5 with a concentration of 0% lemongrass extract, no inhibition zone is formed against the growth of *Candida albicans* on sabaoroud Dextrose Agar medium (SDA). At a concentration of lemongrass extract of 0.2% an inhibitory zone is formed with an average of 10.5 mm (strong category), a concentration of 0.4% is formed an inhibitory zone with an average of 6.1 mm (medium category), a concentration of 0.6% is formed an inhibitory zone with an average of 14 mm (strong category), a concentration of 0.8% is formed an inhibitory zone with an average of 10.8 mm (strong category), a concentration of 1.0% is formed an inhibitory zone with an average of 27.6 mm (very strong category).

At higher concentrations, different average inhibition zones are obtained. This can be seen in the average diameter of the inhibition zone concentrations of 0.2%, 0.4%, and 0.8%. In this case, the average value of the inhibitory zone produced by the concentration of 0.8% is greater than the concentrations of 0.2% and 0.4%. while the inhibitory zone of 0.2% concentration is greater than 0.4%. Several factors cause differences in the diameter of the inhibitory zone, namely the turbidity of the mushroom suspension. A less turbid mushroom suspension has a large inhibitory zone, while if the mushroom suspension is more cloudy the diameter of the inhibitory zone will be smaller. The turbidity of the suspension should be measured with a nephelometer to produce accurate turbidity when compared to McFarland's turbidity of 0.5 (Kusdarwati et al., 2013). In this study, the turbidity levels of the suspension and McFarland were measured using a spectrophotometer to see the absorbance. The thickness of the substrate order affects the inhibition zone of fungal growth. The effective agar thickness is 4 mm, and the agar thickness that is less than 4 mm causes faster diffusion of the extract. Whereas if more than 4 mm diffusion of the extract becomes slow (Amanah et al., 2018). In this study, the measurement of the thickness of the media was not carried out. The incubation temperature can also affect the diameter of the inhibition zone of fungal growth. Plates less than 37˚C have a larger inhibitory zone diameter. This happens due to plate buildup during incubation. The middle plate temperature is less than 37˚C so the diffusion of the extract becomes less good (Afrina et al., 2017).

Another factor that favors the occurrence of differences in the area of the inhibitory zone at each concentration is due to the small content of antifungal active substances contained in lemongrass extract, the speed of antifungal substances in the medium, the pH of the environment, the metabolic activity of microorganisms, the

reaction of the active substance to the medium and the incubation temperature, as well as the incubation time (Trianingsih, 2019). Based on the results of the study, show the difference in the inhibition zone between the concentration of lemongrass extract and the extent of the inhibition zone. This is due to the lack of turbidity of the fungal suspension, and several other factors that cause the wide difference in the growth inhibition zone of *Candida albicans*.

Conclusion

Based on the studies that have been carried out, it can be concluded that the concentration of lemongrass extract (*Cymbopogon citratus*) of 0% does not form an inhibitory zone against the growth of *Candida albicans.* A concentration of lemongrass extract (*Cymbopogon citratus*) of 0.2% formed an inhibitory zone with an average of 10.5 mm against the growth of *Candida albicans*. A concentration of lemongrass extract (*Cymbopogon citratus*) of 0.4% formed an inhibitory zone with an average of 6.1 mm against the growth of *Candida albicans*. Acknowledgments The concentration of lemongrass extract (*Cymbopogon citratus)* 0.6% formed an inhibitory zone with an average of 14 mm against the growth of *Candida albicans*. The concentration of lemongrass extract (*Cymbopogon citratus*) 0.8% formed an inhibitory zone with an average of 10.8 mm against the growth of *Candida albicans*. The concentration of lemongrass extract (*Cymbopogon citratus*) of 1.0% formed an inhibitory zone with an average of 27.6 mm against the growth of *Candida albicans*.

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