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The Inhibition of Betel Leaf Infusion (*Piper betle linn*) against the Growth of *Candida Albicans*

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ABSTRACT

One of plants commonly used by the community for herbal medicine is betel (*Piper betle Linn*). The reason is that betel contains antiseptic substances that can kill germs. Betel nutritious or some infection caused by bacteria and fungi. One of fungi that infects humans is *Candida Albicans*. This study was conducted to find out the effectiveness of some concentration of inhibitory power of green leaf infusion to the growth of *Candida Albicans*. The experiment was conducted between May and August 2017 at Laboratory Centre of Poltekkes Kemenkes (Polytechnic of Health) in Medan, Indonesia. Different concentrations were made with 7 types of boiling water of green betel leaves using sizes of 0.6 grams, 1.0 grams, 1.4 grams, 1.8 grams, 2.2 grams, 2.6 grams and 3 grams. Furthermore, various concentrations of green betel infuse were installed by using the diffusion disc method. One way anova was employed for analysing normal distributed data and post-hoc test was also used to determine the difference of inhibitory power for each concentration. Results show that infusion of betel leaf from various concentrations proved in inhibiting the growth of *Candida Albicans* with p-value = 0.00 (<0.05). The mean of inhibitory zone values of each concentration were 12.10 mm (0.6 grams), 14.10 mm (1.0 gram), 15.80 mm (1.4 grams), 17.40 mm (1.8 grams), 20.40 mm (2.2 grams), 22.50 mm (2.6 grams) and 24.50 mm (3.0 grams). While the drag zone on positive control with ketoconazole were at 36.20 mm. This study also explain that the more number of leaves used in the manufacture of infuse, the more width of the inhibitory zone to *Candida Albicans* growth. The results on post-hoc test show that there is no different inhibitory effect of each green betel infusion concentration to the growth of *Candida Albicans* with p-value = 0.00 (<0.05).

KEYWORDS : *Inhibitory, Infuse of betel, Herbal Medicine, Candida Albicans, Antiseptic*

INTRODUCTION

As one of tropical countries, Indonesia has 143 million hectare of tropical forest. The country is the third largest in the world on the possession of tropical forest. Having diverse on biodiversity, Indonesia has approximately 30,000 species of plants, where 1,260 of them contain medicine (Siswadi, 2006). The trend on back to nature is an option for medical treatment in order to remedy diseases, especially degenerative diseases. Herbs can be natural medicine by utilizing natural plants. Medical treatment through natural medicine is relatively cheap since herbs or plants containing medicine can be found easily in the community. They can also be grown in gardens. Moreover, currently there are special shops selling medicinal plants and herbs. In addition, compared to chemical medicine, natural remedies do not cause side effects. Thus, it is safe to be consumed as long as following instructions or experts' suggestion in consuming natural medicine⁸.

One of plants commonly used as herbal medicine is betel (*Piper Betle L.*). Betel contains antiseptic substances which are believed to kill germs. The leaves, sap plant fluid and oil of betel are important part to be used as medicine. Essential oil of betel contains *sesquiterpene*, starch, diastase, sugar and *chavicol* which have inhibitory effect on growth of germs, antioxidants and fungicides^{12,6,8}. Thus, betel works effectively to overcome infections caused by bacteria and fungi. One of the fungi infecting humans is a species, called *Candida Albicans*.

Candida Albicans is an oval (3-5 µm) yeast cell with *blastoconidia* and *pseudohyphae*. In human beings, under normal conditions, *C. Albicans* lodges into mouth, digestive tract and vagina without causing symptoms. When vaginal acidity changes or changes in hormonal balance, *C. Albicans* will develop excessively, causing symptoms with thick vaginal secretions like cheese, burned and/or itchy in the area around the vagina, affecting the most vaginal discharge¹¹.

Leucorrhoea is a form of infection in vagina that commonly happens to women all around the world. Women are predicted to suffer from vaginal discharge at least once in their lives. *Candida Albicans* itself is the most frequent cause of vaginal discharge at 40% and the fluid flowed is usually thick, white like milk, smelly and accompanied by intense itching on the genitals. This will cause some serious diseases including infection in pelvis resulting infertility¹¹.

Women who live in rural areas experience more symptoms of leucorrhoea due to poor health treatment and behavior in preventing vaginal discharge than those in urban areas. 19.8% of women in rural areas experience the symptom, and 14.1% of women in urban areas experience the symptom. In addition, younger women with less education and living in country sides know less about the symptoms of vaginal discharge. The SKRTRI's result in 2007 found that 71% of women, 51.9% of senior secondary and 78.8% of women living in rural areas did not know the symptoms. They also did not have prevention efforts and vaginal discharge treatment.

Actions of health workers who still underestimate patients' complaints on *Leucorrhoea* have an impact on the behavior of adolescents. Patients tend to carry out their own treatment before going to the doctor health worker. There is a habit of people to use traditional herbs in treating vaginal discharge. People believe that vaginal complaints are normal and can be cured without going to doctors or health services. A research conducted by Wiraguna and Dwarsa (1999) in Bali, found that there were 1834 patients or around 56.9% had done their own treatment before going to doctors¹.

One of traditional medicines known as a way to self-treatment in overcoming *Leucorrhoea* is betel leaf (*Piper Betle L.*), which is believed to have inhibitory effect on the growth of germs, antioxidants and fungicides, including *Candida Albicans* which causes most in vaginal discharge. Thus, this study was conducted on the inhibition test of Piper Betle Linn on the growth of *Candida Albicans*.

MATERIALS AND METHODS

126 gram of betel leaves (*Piper Betle Linn*) were used as sample. This type of research is experimental research with disc diffusion technique to see the effect of green betel leaf infusion (*Piper Betle L.*) on the growth of *Candida Albicans*. The method used in collecting data is an experiment in a laboratory. The test method used was disc diffusion, where at the initial stage the betel leaf infusion was made and then tested for the inhibitory power of the fungus of *Candida Albicans*.

Making Betel Leaf Infuse

The method used in making betel leaf infusion was the decoction method. The techniques and materials to make it can be explained as follows; preparing 126 grams of betel leaves, washing them out thoroughly with sterile aquadest. Next was separating into 7 different sizes such as with each this composition: 0.6 grams, 1.0 grams, 1.4 grams, 1.8 grams, 2.2 grams, 2.6 grams, and 3 grams. The next step was to place each sample into 7 erlenmeyer flasks, and adding 500 ml of aquadest into each flask. The next step was boiling them for around 15 minutes. After liquid was cold, the stew was filtered and stored in a sterile bottle which was tightly closed. Then, each of the 10 µl pieces of betel leaf were put into infuses from to each blank disk and then letting them dried. After drying, the paper disk was ready to be planted into the *Sabaroud Dextrose Agar* (SDA) that had been planted with *Candida Albicans* fungi.

a. Making Mushroom Suspensions⁸

The steps are; first is to add 10 ml of 0.9% Nacl into a sterile tube, then taking the strain of fungi using the ose ring and putting it in the tube and homogenizing it. After that, there is the need to adjust the turbidity of the fungus through Mc Farland standards. If the color is not suitable, there is still the need to add the suspension of the fungi until similar color obtained.

b. Inhibitory Testing⁸

There are several steps on inhibitory testing which can be explained as follows:

First is to put fungi suspension on the surface of *Sabouroud Glucose Agar* using sterile sticky cotton until even. Second is to take disk paper that has been dripped with infusion: 3 sheets, 5 sheets, 7 sheets, 9 sheets, 11 sheets, 13 sheets and 15 sheets, with negative control (positive disk) and positive control (Ketoconazole). Then place them into the surface of the media that has been planted with *Candida Albicans* fungi. Third is to incubate for 1 to 2 times 24 hours at 37 ° C. Fourth is to measure the diameter of the inhibition using a micrometer.

Data Analysis

From data, it can be analyzed whether there are significant differences in the effectiveness of inhibition of each test disc, containing negative controls of various concentrations of the extract of betel leaf and positive control (*ketoconazole*) in inhibiting the growth of fungus of *Candida Albicans*.

This study used a comparative numeric hypothesis of more than 2 unpaired groups, so that the statistical test used was One Way Anova with normal distribution. However, if data is not normally distributed, the non-parametric test, namely the Kruskal-Wallis test was used to determine which concentration has significance; then Poc-Hoc analysis was performed using the Mann-Whitney test.

RESULT

The results of laboratory tests on the inhibition test of green betel leaf (*Piper betle L.*) on the growth of *Candida Albicans* can be seen in the table below:

Weight of Leave (Gram)	The wide range of protected zone (mm)										Mean (mm)	SD
	Repetition											
	1	2	3	4	5	6	7	8	9	10		
0,6	12	13	12	11	12	13	13	13	11	11	12,10	0,876
1,0	14	15	14	14	13	15	14	14	14	14	14,10	0,568
1,4	16	16	15	16	15	17	16	16	15	16	15,80	0,632
1,8	18	17	17	18	17	18	17	18	17	17	17,40	0,516
2,2	20	20	20	21	21	20	21	20	20	21	20,40	0,516
2,6	22	23	22	23	23	22	23	22	23	22	22,50	0,527
3,0	25	24	25	24	24	25	24	26	24	24	24,50	0,707
Control + (ketoconazole)	35	35	36	37	37	35	37	36	37	37	36,20	0,919
Control -	0	0	0	0	0	0	0	0	0	0	0	0

From the table above, it can be seen that the boiled water of betel leaves has inhibitory effect on the growth of *Candida Albicans* fungi. The average value of the inhibition zone produced is 21.76 mm with a standard deviation at 7.45 mm. In the positive control group, the average inhibition zone formed was 36.20 mm with a standard deviation at 0.919 and a negative zone was not formed in the negative control. The green betel leaves infuse has been shown to be strong in inhibiting the growth of *Candida Albicans* fungi. This result is in line with a study conducted by Stefani Gunawan (2010), which also proved that red betel leaf extract and green betel leaf can inhibit the growth of *Candida Albicans* fungi at a concentration at 25%.

Another study with the result of the inhibition of betel leaf to the growth of *Candida Albicans* was also carried out by Kusumaningtyas et al (2008) who tested the inhibitory power of extract and cream of betel leaf extract (*Piper betle Linn*) against *Candida Albicans* and *Trichophyton Menagrophytes*. Thus, it can be said that the green betel leaf (*Piper betle Linn*) has the ability to inhibit the growth of *Candida Albicans* and can be an option for alternative medicine or natural remedy to treat vaginal discharge caused by *Candida Albicans*.

CONCLUSION

This study did experiment at the laboratory on the effect of green betel leaf infuse (*Piper betle Linn*) on the growth of *Candida Albicans*. This study concludes that at all concentrations of leaves with disc diffusion

method significantly inhibits the growth of *Candida Albicans*. Thus, findings of this study strengthen previous studies in the effect of *Piper betle* *Lim* to the growth of *Candida Albicans*.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest in this research.

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