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Antifungal Effectiveness Test of Cinnamon Extract (*Cinnamomum Burmannii*) Against *Candida Albicans*

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ABSTRACT

Background: *Candida albicans*, is a normal flora that lives on the oral mucosa, digestive tract and vagina. *Candida albicans* can cause infection such as oral candidiasis. A number of antifungal agents are widely used in the treatment of *Candida* infections, but some antifungal drugs sometimes have unpleasant side effects. This prompted researchers to examine the contents of natural ingredients that have antifungal activity, one of which is cinnamon. Cinnamon (*Cinnamomum burmannii*) contains several chemical compounds including cinnamaldehyde and eugenol which are components that have antifungal effects.

Objective: to analyze the effectiveness of cinnamon (*Cinnamomum burmannii*) antifungal against *Candida albicans*.

Materials and Methods: The research was performed as laboratory experiment that calculates the diameter of inhibition using the agar-well diffusion method. 25 samples tested were *Candida albicans* cultured in SDA (Saboroud Dextrose Agar) medium. Variations in treatment concentrations were cinnamon extract (*Cinnamomum burmannii*) 25%, 50%, 75%, nystatin oral suspension (positive control), and aquades (negative control).

Results: The average inhibition of 75% cinnamon extract (*Cinnamomum burmannii*) was 16.8 mm, whereas in the positive control nystatin oral suspension was 13.4 mm. The hypothesis test has a value of $p=0.000$ ($p<0.05$).

Conclusion: There is an antifungal effectiveness of cinnamon (*Cinnamomum burmannii*) against *Candida albicans* at a concentration of 75%.

KEYWORDS: Cinnamon, *Cinnamomum burmanni*, *Candida albicans*, Antifungi, Inhibition Zone.

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INTRODUCTION

Oral candidiasis is an opportunistic infection that most commonly affects the oral mucosa. In the majority of cases, the lesions are caused by *Candida albicans*.[1] A number of antifungal agents are widely used in the treatment of *Candida* infections. Some antifungal drugs such as nystatin, fluconazole, clotrimazole, ketoconazole can cause unpleasant side effects. This has encouraged researchers to examine the contents of various natural ingredients and try to find natural ingredients that can prevent the development of pathogenic fungi or Candida albicans.[2] Many traditional plants have strong antifungal activity, with low side effects, but are not known to well by society. One of them is cinnamon.[3] Cinnamon is one of the oldest known spices, one of which is *Cinnamomum burmannii*.[4] Few people know that cinnamon also functions as a fungicidal function because it has active substances in the form of cinnamaldehyde, eugenol, and linalool, so that Cinnamon can have a good effect on health.[2] Because of the effectiveness of cinnamon, research was carried out on testing the inhibitory power of cinnamon extract (*Cinnamomum burmannii*) on the growth of *Candida albicans*.

METHODS

Equipment

This research used, petri dishes, sterile oshe, autoclaves, measuring pipettes, micropipettes, bunsens, test tubes, hole makers, rulers and calipers.

Antifungal Effectiveness Test of Cinnamon Extract (Cinnamomum Burmannii) Against Candida Albicans

Material

The materials used in this research were cinnamon (*Cinnamomum burmannii*), 70% ethanol, 0.9% NaCl, Saboraud Dextrose Agar, sterile distilled water, nystatin oral suspension and the fungus *Candida albicans* ATTC 10231 obtained from the Trisakti University *MiCore* Lab.

Cinnamon Extract

This research uses the maceration method to extract cinnamon. 300 grams of dry cinnamon (*Cinnamomum burmannii*) is put into a crusher and given 1,000 mL of 70% ethanol, then mixed.

Maceration for 72 hours, then filtered with a buchner funnel, and the tissue filtrate was evaporated using a rotary evaporator. After evaporating, 10 grams of extract was obtained. This result shows 100% extract. The cinnamon extract was then dissolved in sterile distilled water to obtain extract solution concentrations of 25%, 50% and 75%.

Antifungal Test

This research uses the agar well diffusion method. The well method is carried out by making perpendicular holes in solid agar that has been inoculated with the test fungus. Then the hole was filled with cinnamon extract, positive control (oral nystatin suspension) and negative control (distilled water) using a micropipette. Then incubate for 24-48 hours and calculate the area of resistance around the hole (Figure 1).

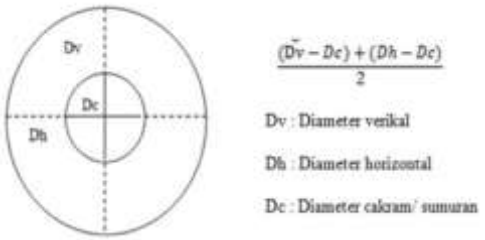

Figure 1. Inhibition zone diameter calculation formula.[5]

Data Analysis

This data analysis was carried out with the help of a computer-based statistical program. Before testing the hypothesis, each control group and treatment group were tested for normality using the *Shapiro-Wilk* test (because the samples used were < 50). Then it was analyzed using the *Kruskall-Wallis* test because the normality requirements were not met.

The results of the *Kruskall-Wallis* test show a significance value of less than 0.05 (0.000), meaning that there is at least 1 treatment that produces significant data. Therefore, it was continued with a post-hoc test using the *Mann-Whitney* test.

Based on the results of the *Mann-Whitney* test, because there is a diameter of *Candida albicans* inhibition in the cinnamon extract treatment that is significantly different from the control group, it can be concluded that there is antifungal effectiveness of cinnamon extract (*Cinnamomum burmannii*) on the growth of *Candida albicans* where the concentration is 75% provide a significant influence.

RESULT

Inhibition is indicated by the absence of growth of the Candida albicans fungus around the well in the form of a clear zone (Figure 2). The clear zone is measured with a caliper in millimeter.

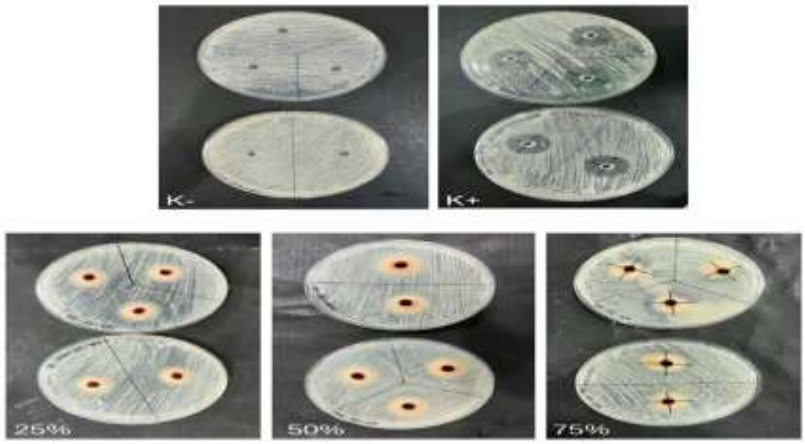

Figure 2. Test results of the effectiveness of cinnamon extract (Cinnamomum burmannii) against the Candida albicans fungus.

25 research samples consisting of 5 samples from each group of 25%, 50% and 75% cinnamon extract as well as positive control (oral nystatin suspension) and negative control (distilled water) were tested using agar well diffusion. From the research results, it is known that all samples of 75% cinnamon extract and the positive control (nystatin oral

suspension) showed that they had inhibitory activity with an average of 16.8 mm and 13.4 mm respectively, whereas for all samples of cinnamon extract it was 25%, 50% and the negative control group of distilled water had no inhibitory activity with an average of 0.0 mm (Table 1).

Table 1. Test results for the effectiveness of cinnamon extract (*Cinnamomum burmannii*) against the Candida albicans fungus.

Concentration	Repitition 1	Repitition 2	Repitition 3	Repitition 4	Repitition 5
25%	-	-	-	-	-
50%	-	-	-	-	-
75%	19	16	18	16	15
K(+)	13	14	14	13	13
K(-)	-	-	-	-	-

Table 2. Mean, median, standard deviation, minimum and maximum Diameter data for *Candida albicans* inhibition.

	Mean	Median	Standard Deviation	Minimum	Maximum
Cinnamon 25%	*0*	*0*	*0.0*	*0*	*0*
Cinnamon 50%	0	0	0.0	0	0
Cinnamon 75%	16.8	16	1.64	15	19
K+ (nystatin oral suspension)	13.4	13	0.54	13	14
K- (distilled water)	0	0	0,0	0	0

Based on table 2, it can be seen that the highest inhibitory diameter was in the 75% concentration *Cinnamomum burmannii* extract group with an average of 16.8 mm, median 16 mm, standard deviation 1.6 mm, minimum 15 mm and maximum 19 mm.

DISCUSSION

The results of the research showed that the antifungal effectiveness of cinnamon extract (*Cinnamomum burmannii*) on the growth of *Candida albicans* was indicated by the formation of a clear zone around the well. The treatment group that showed a clear zone was cinnamon extract with a concentration of 75% with an average diameter of 16.8 mm. Based on table 3, 75% cinnamon extract is classified as having moderate inhibitory power. However, concentrations of 25% and 50% did not show antifungal effectiveness against the growth of the *Candida albicans*, which was indicated by the absence of inhibition or clear zones around the wells.

Table 3. Classification of fungal growth inhibition power according to *Greenwood*.[6]

Inhibition Zone Diameter	Classifications
> 20	High
16 - 20	Moderate
10 -15	Weak
< 10	None

This study used two types of controls to compare research results, the negative control was distilled water and the positive control was nystatin oral suspension. In the negative control using distilled water, no inhibition zone was formed, this was due to the absence of active compounds contained in distilled water. Distilled water was used as a negative control because distilled water is the easiest solvent to obtain, cheap and is neutral and not dangerous.[7] Then in the positive control of nystatin oral suspension, an inhibition zone was obtained which was marked by a clear zone that formed around the well. Nystatin was used as a positive control because nystatin is an antifungal drug used to treat infections caused by fungi, nystatin is widely used in testing against Candida albicans strains.[8]

The extraction process used in this research is the maceration method. The maceration method was chosen because it has several advantages, such as being able to avoid damage to thermolabile compounds, but there are disadvantages to this maceration method, namely that it takes a lot of time, quite a lot of solvent is used, and there is a high possibility that some compounds will be lost.[9]

This research uses ethanol as a solvent for extracting cinnamon (*Cinnamomum burmanni*). Ethanol solvent was chosen as the solvent because ethanol solvent can extract eugenol well.[10] According to Dina & Hussein (2017) ethanol solvent extracts more secondary metabolite compounds in cinnamon bark because it can include polar compounds such as flavonoids.[11] The reason for choosing ethanol solvent is also the type solvents that are safe or non-toxic due to their

low level of toxicity. Flavonoid compounds are generally in the form of glycosides which are polar so they must be dissolved in a polar solvent, and ethanol is a polar solvent.[12] The use of solvents and the results in this research are different from research conducted by Nuryanti et al (2015) where the research used solvents. distilled water, hexane and ethanol as solvents. However, no inhibitory effect was found in the ethanol solvent. However, in this study, the hexane solvent obtained a greater percentage of inhibition of the growth of the *Candida albicans* when compared to the distilled water and ethanol solvents. The distilled water solvent has a very small ability to extract eugenol so that the percentage of inhibitory power obtained is also small, however, in the cinnamon extract media with ethanol solvent, not only the edges of the media but even the wells grow with fungus which means no inhibitory power presentation was found.[10] Differences in solvents in extraction can affect the total content of bioactive compounds. This is caused by the difference in polarity of the solvent. The extraction process is influenced by several factors, including the type of solvent, the ratio of solvent to extraction material, temperature, pressure and extraction time as well as plant bioactive components.[13]

Antifungal activity testing was carried out to determine the diameter of the inhibitory power. In this study, the agar well diffusion method was used. The agar well diffusion method was chosen because it has the advantage that it is easier to measure the area of the inhibition zone formed because the fungus is active not only on the top surface of the agar but also down to the bottom.[14] However, the agar well diffusion method has disadvantages where the results of previous research obtained less good results because influenced by the lack of sharpness of the tool used to make the wells so that there is a possibility that fragments will form in the media and cause the extract to spread to the bottom of the media resulting in poor results.[10] This can cause bias when calculating the resistance diameter around the wells (Figure 3).

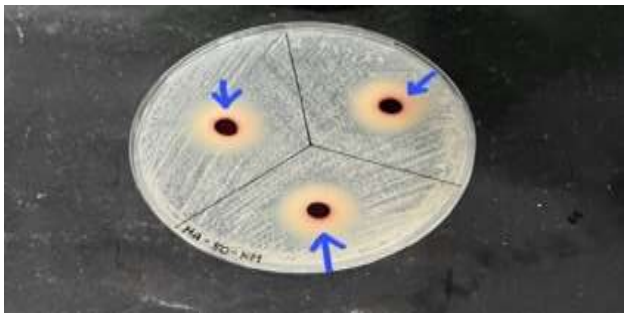

Figure 3. Formation of orange fragments at the bottom of the agar media.

Based on the results of previous research, cinnamon has an inhibitory effect on the growth of *Candida albicans*. This is because cinnamon contains the compound cinnamaldehyde, which belongs to the aldehyde group, which has the strongest antifungal effect compared to other components in cinnamon. Cinnamaldehyde is included in the flavonoids. As antifungals, flavonoids can inhibit fungal growth. Cinnamaldehyde, which acts as an antifungal, is a flavonoid whose mechanism of action interferes with the process of food diffusion into cells so that the growth of the Candida albicans fungus can stop or until the fungus dies. The inner organelles are destroyed and the cells burst after, the cell walls are damaged, the organelles are destroyed and most of the cytoplasm becomes empty bubbles.[15] Cinnamaldehyde can suppress the secretion of *Candida albicans* hydrolytic enzymes. Cinnamaldehyde can cause damage to yeast cell membranes by suppressing the ergosterol biosynthetic pathway and simultaneously interacting with the membrane by binding to ergosterol. Cinnamaldehyde has also been reported to have a strong inhibitory effect on the plasma membrane.[16]

Another active component is eugenol, which is a group of phenols, plays an important role in the activity of inhibiting *Candida albicans* colonies.[17] Eugenol is known to be lipophilic, which can penetrate between fatty acid chains and bilayer membrane layers by changing the permeability of cell membranes. If phenolic compounds interact with *Candida albicans* cell walls, protein denaturation will occur in *Candida albicans* cells. This interaction causes changes in the balance of protein molecules, resulting in changes in protein structure and triggering coagulation. Proteins that experience coagulation will lose their physiological activity so they cannot function properly. Changes in protein structure in *Candida albicans* will cause an increase in cell permeability, so that cell growth is inhibited and cells will die, so eugenol has the ability to reduce permeability and inhibit the metabolism of Candida albicans biofilms.[18]

CONCLUSION

There is antifungal effectiveness of cinnamon extract (*Cinnamomum burmannii*) against the *Candida albicans*.

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