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EFFECT OF DIFFRENTS CONCENTRATION CINNAMON EXTRACTS *(CINNAMOMUM BURMANNII)* IN GROWTH *LACTOBACILLUS ACIDOPHILUS* (*IN VITRO)*

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ABSTRACT

Background: One of the dental and oral health problem that really need attention is caries. Lactobacillus acidophilus is one of the bacteria that play a role in the process of further caries in dentin. We can control it by using materials like antibacterial. Cinnamon barks *(Cinnamomum burmannii)* is a plant from Indonesia that contains antibacterial compounds such as sinamaldehid, flavonoid, tannins, alkaloids, and saponins. **Purpose:** To know the effect of various concentrations of cinnamon barks extracts (*Cinamomum burmannii*) on the growth of *Lactobacillus acidophilus* in vitro. **Methods:** True experimental study with the post test control group design has 25 samples. Cinnamon barks was extracted by the soxhletation method then phytochemical tests were performed to determine the antibacterial compounds. Antibacterial tested using the diffusion method to determine the diameter of the inhibitory zone. Statistical tests using *Kruskal-Wallis* and *Man-Whitney*. **Results:** The mean inhibition zone diameters at concentrations of 6.25%, 12.5%, 25%, 50%, and positive controls were 4.80 mm, 10.99 mm, 16.83 mm, 19.14 mm, 29, 80 mm. *Kruskal Wallis* test showed significant differences in inhibition zone diameter in inhibiting the growth of Lactobacillus acidophilus p = 0,000321 (p <0.05). The *Man Whitnney* test showed a significant difference between the concentration of 6.25% with concentrations of 25% and 50% and concentrations of 12.5% with concentrations of 25% and 50%. There were no significant differences between the concentrations of 6.25% with 12.5% and 25 % with 50%. **Conclusion:** There is an effect of cinnamon bark extracts from various concentrations on the growth of *Lactobacillus acidophillus* as assessed by the formation of inhibition zone diameters. The 25% concentration is the minimum concentration that can significantly inhibit bacteria
Keywords: *Extract of cinnamon, Antibacterial, Lactobacillus acidophilus*

BACKGROUND

Dental and oral health problems that occur in Indonesia are increasing. Based on Riset Kesehatan Dasar (Riskesdas) in 2013 and 2018, dental and oral health problems increased from 25.9% until 57.6%. One of the dental and oral health problems that really need attention is caries. The prevalence of caries in Indonesia currently reaches 44.6% which occurs to children and adults.[1] The impacts will be experienced by someone with dental problems such as caries include limited dental function, pain when chewing and psychological discomfort.[2]

Lactobacillus sp is one of the bacteria that plays a role in the process of further caries occurrence, especially in dentin. *Lactobacillus* testing has been used to identify susceptible caries. It is proven that the bacteria can be found in the carious area, dental plaque and saliva of> 100,000 / ml of saliva in high caries activity.[3] *Lactobacillus acidophilus* is found in root in adults as well as children with severe caries.[4,5] Previous studies have shown that, there are 24 *Lactobacillus acidophilus* colonies found in saliva of children with dental caries.[6] *Lactobacillus acidophilus* plays a secondary role in the caries process because it belongs to the category of acidogenic and asiduric bacteria, which means it is resistant to acid conditions.[7]

Lactobacillus acidophilus can be controlled by antibacterial material. The use of antibacterial agents are still the main choices because bacterial caries plays an important role in here. However, antibacterial agents contain chemicals that have a detrimental effect if we used it for a long time. There are changes in taste and staining sensation of teeth and tongue that limit their use and resistance to antibacterial.[8] Antibacterial substances can be obtained from natural ingredients. One of the herbs that can be used as an antibacterial to improve oral health is cinnamon bark.

Cinnamon bark (*Cinnamomum burmannii*) is aromatic spice that easily found in Indonesia. Antibacterial compounds that play a role are sinamaldehihd, flavonoid, tannins, alkaloids and saponins. Sinamaldehihd has an antibacterial mechanism by inhibiting the synthesis of proteins from bacterial cell walls while flavonoids and tannins are able to inhibit nuclei acid synthesis, energy metabolism and cytoplasmic membrane function, and

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damage the bacterial cell membranes due to toxicity. Alkaloids and saponins have a mechanism that interferes with the integrity of the components of the cell wall peptidoglycan and increases the permeability of the cell membrane.[9]

Based on this background, the researchers conducted a study to determine the effect of cinnamon bark extracts *(Cinnamomum burmannii)* from concentrations of 6.25%, 12.5%, 25% and 50% on the growth of *Lactobacillus acidophilus* in vitro.

METHOD

This is true experimental research with the post test control group design. This study used a sample of cinnamon barks with *Cinnamomun burmanni* then extracted using the soxhletation method. The number of samples of this study were 25 pieces divided into 5 groups. The treatment group consisted of 4 kinds of extract concentrations namely 6.25%, 12.5%, 25% and 50%. The control group consisted of 0.2% clorhexidine as a positive control and sterile aquades as a negative control. The diffusion antibacterial test method was carried out of each treatment group inserted into a petri dish which was washed with Lactobacillus acidophilus bacteria as much as 100 µl. After that the petri dish was incubated at 37^{o}C for 48 hours. Inhibition zones diameter measurements were carried out using a digital calipers.

This study used Saphiro Wilk normality test because the sample size are <50 subjects. Homogenity tested was done by using the lavene's test to see the data variance. The data obtained an abnormal distribution and not homogeneous, then a non-parametric Kruskall Wallis test is performed to analyze the differences between groups, then proceed with the Mann Whitney U test.

RESULT

The results of the normality test using Saphiro-Wilk showed that there were 3 data that were normally distributed, that were positive control of chlorhexidine with value p = 0.238 (p> 0.05), a concentration of 25% with value p = 0.904 (p> 0.05), and a concentration of 50% with value p = 0.725 (p> 0.05). Two data are not normal were 6.25% concentration has a median value of 0.00 and a concentration of 12.5% has a median value of 13.42. It can be concluded that the treatment group of cinnamon extracts data distribution is not normal (Table 1). Then the homogeneity test was continued to get the value of p = 0.019 (p <0.05) so that the data was declared not homogeneous. The data in this study were not normal and not homogeneous, so we did the Kruskal Wallis test. Kruskal Wallis test results obtained with p = 0,000321 (p <0.05), which means that there were significant differences in inhibiting the growth of *Lactobacillus acidophilus* bacteria between the 5 treatment groups (Table 2).

Tabel 1. Test Results of Normality Diameter of Inhibitory Zone of Cinnamon Bark Extracts Against *Lactobacillus acidophilus*

Treatment Group	N	Mean ± SD	Median (Minimum-Maxsimum)	*p*
K+	5	29,81 ± 2,10	28,97 (28,11 – 33,12)	0,238*
Concentration 6,25%	5	4,80 ± 6,61	0,00 (0,00 – 12,90)	0,017
Concentration 12,5%	5	10,99 ± 6,38	13,42 (0,00 – 15,32)	0,042
Concentration 25%	5	16,83 ± 2,51	17,05 (14,06 – 20,96)	0,904*
Concentration 50%	5	19,14 ± 2,35	18,83 (16,46 – 22,08)	0,725*

Note : *Significant *p* > 0,05

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Tabel 2. Tests Result of *Kruskall Wallis* test ofInhibiting Zone Diameters of Cinnamon Bark Extracts Against *Lactobacillus acidophilus*

Treatment Group	N	*p*
K+	5	
Concentration 6,25%	5	
Concentration 12,5%	5	0,000321*
Concentration 25%	5	
Concentration 50%	5	

Note : *Significant $p < 0,05$

The statistical test using the Mann Whitney test found that there was a significant difference in inhibition zone diametesr between positive control and all concentrations of cinnamon bark extract (p <0.05). Significant differences in inhibition zone diameter were found at concentrations of 6.25% with concentrations of 25% and 50% (p <0.05) and between concentrations of 12.5% with concentrations of 25% and 50% (p <0.05). There was no significant difference between the concentration of 6.25% with 12.5% (p> 0.05) and the concentration of 25% with 50% (p> 0.05) (Table 3).

Tabel 3. Result *Mann Whitney* U Test Inhibitory Zones of Cinnamon Bark Extracts Againts *Lactobacillus acidophilus*

Group	Nilai *p* diameter zona hambat			
	Concentration 6,25%	**Concentration 12,5%**	**Concentration 25%**	**Concentration 50%**
K+	*0,008**	*0,009**	*0,009**	*0,009**
Concentration 6,25%	-	*0,131*	*0,008**	*0,008**
Concentration 12,5%	*0,131*	-	*0,028**	*0,009**
Concentration 25%	*0,008**	*0,028**	-	*0,209*
Concentration 50%	*0,008**	*0,009**	*0,209*	-

Note : *Significant $p < 0,05$

DISCUSSION

An experimental in vitro study with *Lactobacillus acidophillus* as a sample of Gram positive bacteria treated with cinnamon bark extracts at a concentration of 6.25%, 12.5%, 25%, 50% and positive control in the form of clorhexidin 0.2% and negative control in the form of sterile aquades. Cinnamon bark with *Cinnamomum burmanni* has been carried out a plant determination test at the FSM UNDIP Ecology and Biosystematic Laboratory. Making cinnamon bark extract in the Herbal Medicine Laboratory and has been tested qualitatively phytochemicals with positive results from saponins, flavonoids, tannins and negative for alkaloids Research on the effect of various cinnamon bark extracts on the growth of Lactobacillus acidophillus was conducted at the Microbiology Laboratory of the Faculty of Medicine, UNDIP.

The observations made on five petri dishes showed inhibition zone diameters formed around which were given various concentrations of cinnamon bark extract. The mean inhibition zone diameter produced by cinnamon bark extract at a concentration of 6.25%, 12.5%, 25%, 50%, and positive control were 4.80 mm, 10.99 mm, 16.83 mm, 19 , 14 mm, 29.80 mm. That were given sterile aquades did not show the diameter of the inhibition zone. the increase in extract concentration will form a larger inhibition zone diameter. This shows an increase in concentration followed by an increase in antibacterial strength

Inhibition zone assessment based on the classification of Davis and Stout (1970) is classified as weak if inhibition zone is less than 5 mm, medium if 5-10 mm inhibition zone, strong if inhibition zone 10-20 mm and very strong if inhibition zone> 20 mm.[11] Based on the assessment of the bacterial

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growth inhibition zone, the cinnamon clit extract from a concentration of 6.25% has a weak inhibition while the concentrations of 12.5%, 25% and 50% have a strong inhibitory power. Positive control in the form of 0.2% clorhexidin showed very strong inhibition.

These results are consistent with Puspita's research (2014) which states cinnamon bark extract can inhibit bacterial growth and the greater the concentration of cinnamon bark extract, the greater the diameter of the inhibitory.[10] The results of the study concur with Parisa (2019) which says that cinnamon bark extract can inhibit the growth of Gram positive bacteria S.aureus compared to Gram negative bacteria E. coli. The results of the study also found that the extract concentration of cinnamon bark 10% to 40% had a strong inhibition against Gram-positive bacteria.[12]

The result of Kruskal Walis Test obtained p = 0,000321 (p <0.05), which means that there are significant differences in inhibition zone diameter at various concentrations of cinnamon bark extract. This is consistent with the research hypothesis which states that there are effects of various concentrations of cinnamon extract from the growth of *Lactobacillus acidophilus* as seen from the diameter of two inhibitory zones.

The ability of cinnamon bark extract to inhibit *Lactobacillus acidophilus* bacteria is obtained by the content of antibacterial compounds. There are cinnamaldehyde, tannin, flavonoids, and saponins.. Sinamaldehid and saponin affect the integrity of the membrane. Damage the cell permeability can cause increased permeability of cell membranes so that the membrane becomes unstable and results in cell hemolysis such as electrolyte leakage, nucleic acids, and proteins which in turn will cause bacteria to experience cell death.[13,14]

Tannins are also able to interfere with the integrity of the constituent components of peptidoglycan in bacterial cells. Peptidoglycan is a component of bacterial cell walls so that the interference will cause the cell wall layer to not be formed intact and cause cell death. Other antibacterial compounds, namely flavonoids from polar phenol groups, have 3 anti-bacterial mechanisms that inhibit cell membrane function, inhibit nucleic acid synthesis, and inhibit energy metabolism so that it will affect the activity of metabolite absorption and biosynthesis of bacterial macromolecules[15,16,17]

The results of the *Man Whitney* test there is a difference between positive control and all concentrations. This shows that the highest concentration of cinnamon bark extract at 50% still has a difference in positive control (clorhexidine 0.2%). The positive control (clorhexidine 0.2%) had the largest inhibitory zone diameter and showed very strong inhibition against *Lactobacillus acidophilus*. That is because clorhexidine contains chlorine, phenol and bisbiguanida which have been known to have broad-spectrum antibacterial activity against the growth of aerobic and anaerobic bacteria, both Gram-positive and Gram-negative.[18] The antibacterial mechanism of clorhexidine is causing damage to cell walls followed by an increase in membrane permeability, both Gram-positive and Gram-negative. thus causing cell leakage and ultimately intracellular coagulation and precipitation such as proteins and nucleic acids. Precipitation will block the activity of sugar transport and inhibit the production of acid in cariogenic bacteria.[19] Factors that cause cinnamon extract 50% concentration have inhibitory zones smaller than positive control if there is absence of alkaloid content in extracts that play a role in inhibiting bacterial DNA replication and damaging bacterial cell wall. Loss of alkaloid compounds can be caused by the decomposition of these compounds by heat and the presence of oxygen during or after the extraction process.[20]

In addition, structure and composition of bacterial cells also have an important role in the antibacterial mechanism. *Lactobacillus acidophilus* is a gram-positive bacterium that has a cell wall structure with more lipid peptidoglycan and contains polysaccharides (theatricic acid). Theatricic acid is a water-soluble polymer that functions as a way to exit and enter ions from and into bacterial cells. It is this water solubility which shows that the cell wall of gram-positive bacteria is more polar. The incoming antibacterial compounds will result in greater osmotic pressure in the cell, causing lysis.[21]

This study also showed that the concentration of 6.25% was not significantly different from the concentration of 12.5% and the concentration of 25% was not significantly different from the concentration of 50%. There were no significant differences indicating that between the groups had the same average inhibitory power against Lactobacillus acidophilus and had the same amount of active substances.There is a significant difference in inhibition zone diameter at

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concentrations of 6.25% with concentrations of 25% and 50% and between concentrations of 12.5% with concentrations of 25% and 50%. This shows that the concentration of 25% is the minimum concentration that can significantly inhibit bacteria.

CONCLUSION

There are an effect of cinnamon bark extracts of various concentrations on the growth of *Lactobacillus acidophillus* as assessed by the formation of inhibition zone diameters. There was a significant difference in inhibition zone diameter at concentrations of 6.25% and 12.5% with concentrations of 25% and 50%. There was no significant difference between the concentrations of 6.25% with 12.5% and 25% with 50%. Concentration of 25% is the minimum concentration that can significantly inhibit bacteria.

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