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**INHIBITORY ACTIVITY OF ULIN BARK (*Eusideroxylon zwageri*) EXTRACT
 TO *Lactobacillus acidophilus***

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ABSTRACT

Background: *Lactobacillus acidophilus* is a bacterium which plays a role in dental caries. It is believed as a pioneering bacterium in advanced caries and much likely to be isolated in dentin caries zone, resulting in the needed for tooth restoration. The use of 2% Chlorhexidine digluconate as cavity cleanser is recommended as an effort to prevent secondary caries but can cause side effects. One of the natural materials that can be used as a cavity cleanser is ulin bark extract (*Eusideroxylon zwageri*), a traditional medicine originally from Kalimantan, because it contains phenolic, flavonoid, tannin, alkaloid, saponin and terpenoid. **Purpose:** To discover the inhibitory activity of ulin bark extract on *Lactobacillus acidophilus* growth. **Methods:** This was a true experimental laboratory and post test only with control group design, that used 20%, 40%, 60%, 80%, 100% concentrations of ulin bark extracts and K(+) 2% Chlorhexidine digluconate. Difussion method was used to test inhibitory activity with 6 treatment groups and 4 replications, comprising a total of 24 samples. All groups were incubated for 24 hours at 37°C temperature. The inhibition zone was measured using calipers. **Results:** The 20%, 40%, 60%, 80% and 100% concentration of ulin bark extracts and 2% Chlorhexidine digluconate had an average inhibition zone of 7.17 mm, 9.02 mm, 11.14 mm, 13.06 mm, 15.17 mm and 19.22 mm. One Way ANOVA and Post Hoc Bonferroni tests showed significant difference between all groups. **Conclusion:** Ulin bark extract can inhibit *Lactobacillus acidophilus* growth starting from 20%, 40%, 60%, 80% and 100% concentration.

Keywords: 2% Chlorhexidine digluconate, inhibitory activity, *Lactobacillus acidophilus*, ulin bark extract.

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INTRODUCTION

Dental caries is a disease of dental and oral health that is marked by the damage of tooth hard tissue which includes enamel, dentin and cement.¹ Dental caries is generally affected by cariogenic bacteria, one of which is *Lactobacillus acidophilus* that usually can be found in dentin caries zone.^{2,3} *Lactobacillus acidophilus* is a positive-gram bacteria that plays a role in the development and the progression of caries. Initially, White Spot will be observed on the surface of tooth enamel which continues to develop and can cause an ongoing damage in dentin area which, if abandoned, will infect other tissue so that tooth restoration is needed.⁴ The use of cavity cleanser is highly recommended to eliminate residual bacteria as an effort in preventing secondary caries.⁵

Cavity cleanser is a material for cavity cleaning after preparation. One of the cavity cleanser materials is 2% *Chlorhexidine digluconate* that can be said as the gold standard.^{5,6} The use of *Chlorhexidine* demonstrates cytotoxic effect related to the exposure duration and the used concentration.⁷ To minimize the side effect, ulin (*Eusideroxylon zwageri*) can be utilized as an alternative of cavity cleanser.

Ulin is a wood that is originally from Kalimantan. It can be formulated into traditional medicine since it is believed to contain several compounds that can be used in the pharmaceutical world. Kalimantan society utilized ulin soaking as a medication for toothache empirically due their belief in the presence of antimicrobial analgesic compound in it.^{8,9,10} Based on phytochemical test,

the ulin bark contains phenol, alkaloid, flavonoid, terpenoid, tannin and saponin. The most content in ulin bark extract is 31,28 mg GAE/g of phenol, 30,48 mg CE/g of flavonoid, and tannin.^{10,11}

Based on the research by Wila *et al* (2018), it is stated that the ulin bark extract has inhibitory activity to *E. coli* and *S. typhi* bacteria with a strong inhibitory response.¹⁰ Other research by Darussalam (2016) claimed that the ironwood stem bark extract is capable to inhibit *Staphylococcus* bacteria growth.⁸ Evaluating the description above, the researcher is interested in conducting the research of ulin bark extract to discover its inhibitory activity to *Lactobacillus acidophilus* bacteria growth.

MATERIALS AND METHODS

This research was conducted at Basic Laboratory of Mathematics and Science Faculty Lambung Mangkurat University Banjarbaru, Research and Industrial Consultation Hall Surabaya, and Microbiology Laboratory Research Center Dentistry Faculty Airlangga University Surabaya. Before the research was conducted, ethical clearance was already submitted to Dentistry Faculty Lambung Mangkurat University that was ethically approved based on ethical clearance letter No. 010/KEPKG-FKGULM/EC/1/2020.

This research used the true experimental method with post test only and control group design. The treatment groups in this research are 20%, 40%, 60%, 80% and 100% concentration of ulin bark extract and 2% *Chlorhexidine digluconate* as positive control. Based on Federer formula, it was obtained that the amount of repetition for each group was four times.

The initial procedure of this research was the collection of 2 kg brownish red colored inner part of ulin bark that was taken without harming its cambium. The bark was further cleaned, dried, and also cut small, down to ± 2 cm size. The ulin bark was pulverized into powder using *hammer mill* and filtered using *mesh screen*. To extract the ironwood stem bark using the maceration method, two hundred grams of ulin bark were inserted into extractor tool and soaked in 1000 ml of 96% ethanol. The filtrate was mixed every 24 hours using a *shaker*. The residues were collected from the filtrate and the extraction process was continued for 4 times. The extract was evaporized using a *rotary evaporator* at 59-60°C temperature and continued to be heated on the water bath, resulting in 14 gram of 100% brownish colored liquid residual. The thick extract was progressed

with ethanol free testing by adding potassium dichromate ($K_2Cr_2O_7$) to find out if there is ethanol in the extract or not.

For the preparation of bacterial testing, *Lactobacillus acidophilus* bacteria were obtained from pure isolate and later inoculated into 0.5 ml of liquid BHI and incubated for 2x24 hours at 37°C temperature. The suspension was further diluted until its turbidity corresponds to 0.5 McFarland standard or 1.5×10^8 CFU/ml of bacteria. *Lactobacillus acidophilus* bacteria were smeared onto Mueller Hinton Agar (MHA) medium, then the paper disk was soaked into 20%, 40%, 60%, 80% and 100% concentration of ulin bark extract and 2% *Chlorhexidine digluconate* for 30 minutes. Paper disk was put onto bacteria-grown MHA medium and incubated for 1x24 hours at 37°C temperature. Then, the measurement of inhibition zone was conducted using calipers.

RESULTS

The average inhibitor zone of *Lactobacillus acidophilus* after given ulin bark extract and 2% *Chlorhexidine digluconate* as positive control can be seen in Table 1.

Table 1. Ulin Bark Extract and 2% *Chlorhexidine Digluconate* Inhibition Zone

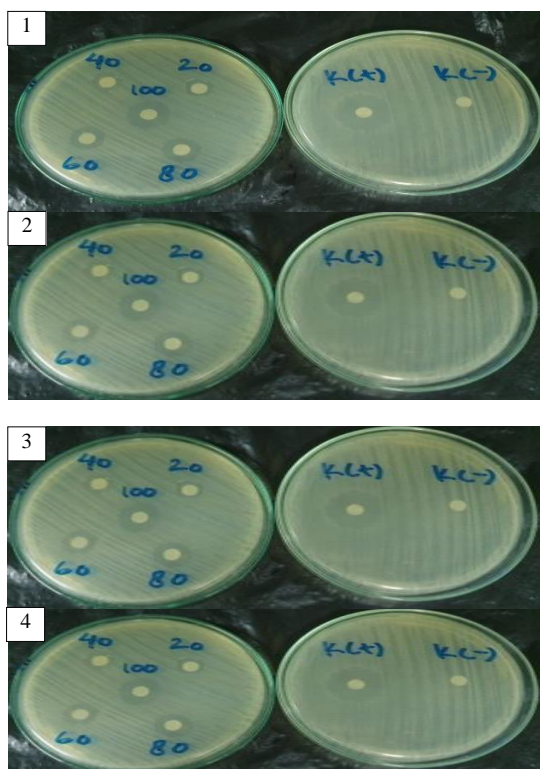
Treatment Group	N	Mean	Std.Deviation
EKBU 20%	4	7.17	0.06
EKBU 40%	4	9.02	0.17
EKBU 60%	4	11.14	0.09
EKBU 80%	4	13.06	0.10
EKBU 100%	4	15.17	0.24
CHX 2%	4	19.22	0.09

Explanation:

EKBU = Ulin Bark Extract

CHX 2% = 2% *Chlorhexidine digluconate*

It is known from Table 1 that 100% concentration of ulin bark extract has the largest inhibition zone diameter, yet it is smaller when compared to 2% *Chlorhexidine digluconate*. Meanwhile, the lowest inhibition zone can be found at 20% concentration of ulin bark extract. This shows that the higher the concentration of ulin stem bark extract the bigger the inhibition zone diameter.



Picture 1. The Inhibition Zone of Ulin Bark Extract and 2% Chlorhexidine digluconate to *Lactobacillus acidophilus* growth with 4 times repetition

The obtained data for each treatment was continued to be analyzed for normality test using *Shapiro Wilk*. From the normality test, the 20%, 40%, 60%, 80% and 100% concentration of ulin bark extract and 2% *Chlorhexidine digluconate* show p value > 0.05 which means the data were normally distributed. The analysis was then followed by homogeneity test using *Levene's Test*. The obtained result shows that the variant of each group is the same variant because the significance value was 0.141 which means $p \geq 0.05$, so it was proceeded to *One Way Anova* parametric test.

Based on *One Way Anova* parametric analysis test, the significance value was 0.000 which means $p < 0.05$ which shows that there was a difference between each treatment group. Because of the difference between each treatment group, the data were further investigated using *Post Hoc test* with *Bonferroni* method to identify the group that contributes to the significant difference as presented in Table 3.

Table 2. The *Post Hoc Bonferroni Test* Result of Ulin Bark Extract and 2% *Chlorhexidine digluconate* Inhibition Zone to *Lactobacillus acidophilus* bacteria growth

Treatment Group	EK BU 20%	EK BU 40%	EK BU 60%	EKB U 80%	EKB U 100%	CH X 2%
EKBU 20%	-	0.000*	0.000*	0.000*	0.000*	0.000*
EKBU 40%		-	0.000*	0.000*	0.000*	0.000*
EKBU 60%			-	0.000*	0.000*	0.000*
EKBU 80%				-	0.000*	0.000*
EKBU 100%					-	0.000*
CHX 2%						-

* : significant differences ($p < 0.05$)

The result for *Post Hoc Bonferroni* showed p value = 0.000 for each group, thus it is concluded that all treatment groups showed significant difference in the average inhibition zone diameter of *Lactobacillus acidophilus* due to the administration of ulin bark extract and 2% *Chlorhexidine digluconate*.

DISCUSSION

The result of this research shows that ulin bark extract (*Eusideroxylon zwageri*) has inhibitory activity to *Lactobacillus acidophilus* bacteria growth. This research used diffusion method by conducting formed inhibition zone observation. The measurement of the inhibition zone was performed using mm-units caliper.¹² The measurement show that the highest inhibition zone was observed at the 100% concentration of ulin bark extract with an average inhibition zone of 15.17 mm, then followed by 80% concentration of ulin bark with an average of 13.06 mm, 60% concentration of ulin bark with an average of 11.14 mm, 40% concentration of ulin bark with an average of 9.02 mm, and 20% concentration of ulin bark with an average of 7.17 mm. The minimum rate of inhibitory zone is in 20% concentration of ulin bark. The result of inhibitory activity test corresponds with the research by Masyithah *et al* (2015) claiming that if the formed inhibition zone diameter is bigger than 6 mm, then it can be said that the used material has antibacterial activity.¹³

According to Davis and Stout in Ashif *et al* (2019), the response of inhibition zone diameter are divided into 4 categories that are the category of very strong response (20 mm or more)

inhibition zone diameter, the category of strong response (10-20 mm), the category of moderate response (10 mm) and the category of weak response (5 mm or less).¹⁴ Based on those categories, then the inhibitory activity of ulin bark extract to *Lactobacillus acidophilus* bacteria growth at 20% and 40% concentration are classified in moderate response, while the 60%, 80%, and 100% concentration are included in strong response category.

This research used 2% *Chlorhexidine digluconate* as positive control which shows a meaningful difference with various concentrations of ulin bark extract based on the result of *Post Hoc Bonferroni* statistic test because it produces inhibitory activity with the biggest zone diameter for *Lactobacillus acidophilus* bacteria that is 19.22 mm. According to Sajjan *et al* (2016), *Chlorhexidine digluconate* at 2% concentration is a cavity cleanser that has a wide-spectrum antibacterial effects, resulting in effective property in inhibiting bacterial growth of gram-positive or gram-negative bacteria.¹⁵ The research by Celik *et al* (2016) also mentioned that 2% *Chlorhexidine digluconate* that used as cavity cleanser has high antibacterial effects and effective towards cariogenic bacteria, one of which is *Lactobacillus acidophilus* bacteria.¹⁶

The 2% *Chlorhexidine digluconate* is a synthetic material that has chemical compounds consisted of 2 groups of *bisguanide* and 4 rings of *chlorophenyl* that are connected by *hexamethylene* chain.¹⁷ The mechanism of action of 2% *Chlorhexidine digluconate* are by damaging bacteria cell wall that cause changes in permeability of cytoplasm membrane, changes in the stability of cellular osmotic, disturbance in bacteria metabolism. Such changes will consequently induce bacterial cell lysis and cause the death of bacteria cell.^{17,18}

The inhibitory activity of ulin bark extract against *Lactobacillus acidophilus* has been proven in this research which is associated with the content of ulin bark extract namely phenol, alkaloid, flavonoid, terpenoid, tannin and saponin. Based on the research by Kusuma *et al* (2018), the most dominant content of ulin bark extract is 31,28 mg GAE/g of phenol, 30,48 mg CE/g of flavonoid, and tannin.¹¹

Phenol is a compound that owns one or more *hydroxyl* (OH) group that affects the antibacterial activity in inhibiting bacteria. This compound works by interacting with bacterial cell through absorption process that involves hydrogen binding.¹⁹ Phenol will denature protein and damage cell cytoplasm membrane. Instability of cell wall and bacteria cytoplasm membrane cause membrane cell permeability function disturbed.²

The mechanism of flavonoid as antibacterial substance of ulin bark extract is by suppressing nucleic acid synthesis, that results in the disturbance of cell wall permeability and the capability to inhibit energy metabolism. Flavonoids form complex compound with extracellular matrix that dissolve into the bacterial cell so it may cause damage to the cell membrane and the leak of intracellular compound will be inevitable.²¹ Tannin works by impeding reverse transcriptase enzyme and topoisomerase DNA, resulting in arrest of bacterial cell formation, deactivation of cell adhesion, disruption in cell protein transport that further cause underdeveloped cell wall.² The said matter is the cause of *Lactobacillus acidophilus* bacteria growth inhibition.

The inhibitory activity of ulin bark extract on *Lactobacillus acidophilus* bacteria growth, aside from the inhibition property of antibacterial content in the extract, is also affected by the characteristics of bacterial cell wall. *Lactobacillus acidophilus* is gram-positive bacteria that possess less complex cell wall composition. This facilitates the entry of the antibacterial compound into the cell and assist the mechanism of antibacterial activity within.⁷ According to Indrawati and Rizki (2017), antibacterial compound activity depends on the structure of bacterial cell wall.¹⁴

The research of ulin bark extract was conducted so that it can be applied as an alternative for cavity cleanser on the effort to prevent secondary caries. The use of ulin bark extract as cavity cleanser is also expected to increase societal interest to maintain the cultivation of ulin, therefore it may prevent the extinction of the species. Based on the matter above, it can be concluded that the ulin bark extract is capable to inhibit *Lactobacillus acidophilus* bacteria growth starting from 20%, 40%, 60%, 80% and 100% concentrations, but the inhibitory activity of ulin bark extract is not yet equally capable with 2% *Chlorhexidine digluconate*. The 100% concentration of ulin bark extract has the biggest inhibition zone, so it can effectively become an alternative material for cavity cleanser.

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