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ANTIBACTERIAL EFFECTIVITY TEST OF ULIN BARK EXTRACT (*Eusideroxylon zwageri*) ON THE GROWTH OF *Porphyromonas gingivalis*

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ABSTRACT
Background*: Chronic periodontitis is an infectious disease caused by bacterial colonization of dental plaque. Bacteria that play a role in chronic periodontitis is Porphyromonas gingivalis. One of mouthwash that reduce the number of P.gingivalis colonies is chlorhexidine 0.2%. Long term use of chlorhexidine 0.2% can cause some side effects to the oral cavity, so we need an alternative mouthwash from natural ingredients that can reduce the side effects of chlorhexidine 0.2%. Ulin bark extract contains phenolic, flavonoid, tannin, alkaloid, terpenoid, and saponin which can be an alternative base for mouthwash besides chlorhexidine 0.2%.* ***Purpose:*** *It is to analyze antibacterial effectivity of ulin bark extract on the growth of Porphyromonas gingivalis.* ***Methods:*** *This study was using true experimental research and post-test only with control group design, that used 9 treatment groups with 4 replications, that were 5%, 10%, 20%, 40%, 60%, 80%, 100% concentrations, chlorhexidine 0.2%, and sterile aquadest against P. gingivalis. The total samples were as many as 36.* ***Result:*** *One Way ANOVA and Post-Hoc Games Howell tests showed that the average absorbance values has a significant difference, then Kruskall Wallis and Post Hoc Mann Whitney tests showed the number of colonies with significant differences. MIC in this study was at 5% concentration and MBC was at 20% concentration..* ***Conclusion:*** *Ulin bark extract with 40% concentration has an absorbance value equivalent to 0.2% chlorhexidine and 20% concentration has a bactericidal effects equivalent to 0.2% chlorhexidine against the growth of P.gingivalis.*

Keywords: *Antibacterial, Dilution method, Porphyromonas gingivalis, Ulin Bark Extract.*
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INTRODUCTION

According to (RISKESDAS) 2018 there were 57.6% of Indonesia's population experienced dental and oral diseases which were mostly caused by microbial infections, one of them was periodontal disease.[1] Based on these data the prevalence of periodontal disease reached 50%.[2] One of periodontal disease is chronic periodontitis. Chronic periodontitis is a multifactorial infectious disease caused by several specific microorganisms so that progressive damage occurs to the periodontal ligament and alveolar bone.[3] The main factor that can cause the chronic periodontitis is due to bacteria in subgingival plaque such as *Porphyromonas gingivalis*.[4]

Porphyromonas gingivalis is an anaerobic gram-negative bacteria that is pathogenic and cause chronic periodontitis.[4] *Poprhyromonas gingivalis* bacteria produces a variety of virulence factors that can cause chronic periodontitis.[5] Chronic periodontitis can be /prevented with scaling and root planning and also by using mouthwash. One of the mouthwash that becomes the gold standard to reduce the number of *Porphyromonas gingivalis* colonies is chlorhexidine 0.2%.[2] Excessive use of chlorhexidine 0.2% can cause brown color teeth, bad taste, unilateral and bilateral parotid swelling, increased the formation of supragingival calculus and can give less efficient side effects to the oral cavity. Now, alternative mouthwash from natural ingredients which is considered safer to reduce side effects from the use of the chlorhexidine's chemical is needed. [6]

One of natural ingredients needed as an alternative herbal mouthwash is ulin bark (*Eusideroxylon zwageri*) which is a typical plant from Borneo. Most people in Kalimantan use ulin wood as traditional medicine to treat toothache.[6] The ulin wood is proven to contain ingredients that can be used as antibacterial compounds.[8] Based on the result of phytochemicals screening that have

been done, ulin bark extract (*Eusideroxylon zwageri*) contains bioactive compounds such as phenolic, flavonoids, tannins, triterpenoids, saponins, and alkaloids. The largest content of ulin bark extract is flavonoids, tannins, and phenolics. The content of flavonoid compounds has levels of 30.48 mg/g and phenolic of 31.28 mg/g.[9] Research conducted by Wila et al (2018) states that ulin bark extract (*Eusideroxylon zwageri*) has been proven to has an effect to inhibit the growth of *E.Coli* and *S.thypi*.[10] Another study conducted by Darussalam (2016) found that ironwood extract (*Eusideroxylon zwageri Tet B*) is able to inhibit the growth of *Staphylococcus aureus* at concentrations of 20%, 40%, 60%, 80%, and 100%.[7]

Research on the antibacterial effectivity of ulin bark extract (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis* has never been done. Based on this state, it is necessary to conduct a research to analyze the effectiveness of the inhibitory and killing power of ulin bark extract (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis* which can cause chronic periodontitis.

MATERIALS AND METHODS

This research was conducted at the Basic Laboratory of the Mathematics and Natural Sciences Faculty, Lambung Mangkurat University, Industrial Research and Consultation Center Surabaya, and the Microbiology Laboratory Research Center Faculty of Dentistry, Airlangga University, Surabaya. The study has got the permission and ethical clearance from the Ethical Committee of Faculty of Dentistry Lambung Mangkurat University No. 009/KEPKG-FKGULM/EC/I/2020. The method that used in this study was true experimental with post-test only with control group design using 9 treatment groups for antibacterial test. The number of replication for each treatment was 4 times, this was determined by the Federer formula. The total samples size used were 36. Ulin bark extract was made using maceration method. 200 grams of ulin bark extract are cleaned and dried in an oven at 40ºC for 4 hours, then blended and sifted to get simplicia powder. Then the powder soaked in 96% ethanol solvent for 1x24 hours and stirred with the help of a shaker, repeated 4 times. The solvent was evaporated using a rotary evaporator at 60ºC and heated on a waterbath until the solvent completely evaporated to obtain 14 g of ulin bark extract with 100% concentration. The free ethanol test was done by adding a few drops of potassium dichromate ($K_2Cr_2O_7$).

Ulin bark extract was diluted to several concentrations of 5%, 10%, 20%, 40%, 60%, and 80%. The concentration obtained is according to the dilution formula as follows:

$$\mathbf{V_1 \times M_1 = V_2 \times M_2}$$

V_1 = Volume of solution to be diluted (ml)
M_1= ulin bark extract available concentration (%)
V_2= volume of solution (water and extract) desired (ml)
M_2= the desired concentration of ulin bark extract (%)

Ulin bark extract with a concentration of 5%, 10%, 20%, 40%, 60%, 80%, and 100%, chlorhexidine 0.2% and sterile aquades into the antibacterial test treatment group on *Porphyromonas gingivalis* from pure isolates placed on NA media, then incubated for 2x24 hours at 37ºC. After that, the dilution was done by adding sterile aquades and homogenized until the turbidity was equal to Mc Farland's standard ($1{,}5x10^8$).

After that, 1 ml of bacterial suspension that has been standardized with Mc Farland turbidity 0.5 (1.5x108) was inserted into each test tube containing 1 ml of extract with 7 different concentrations of 5%, 10%, 20 %, 40%, 60%, 80% and 100%, also chlorhexidine 0.2% and sterile aquades. The test tube was cultured to determine the effect of ulin bark extract (*Eusideroxylon zwageri*), chlorhexidine 0.2%, and sterile aquades on the growth of *Porphyromonas gingivalis* bacteria then the absorbance was measured with the Uv-Vis Biobase BKD-500 Spectrophotometer. The MIC results can be seen by the absorbance values reduction between each concentration and control (-). Then, MBC is calculated by the method of solid dilution through the agar media with manual calculation.

RESULTS

The results of the antibacterial effectivity test was done by measuring the MIC value with the Uv-Vis Biobase BKD-500 Spectrophotometer can be seen as follows.

Table 1. Results of Minimum Inhibitory Concentration Test (MIC) of ulin bark extract (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis*.

Group	Abs. negative control	After 24 hours incubation	Reduction
CHX 0,2%	1,823	0,021	-1,802
KBPU 100%	1,823	0,034	-1,789
KBPU 80%	1,823	0,042	-1,781
KBPU 60%	1,823	0,036	-1,787
KBPU 40%	1,823	0,023	-1,80
KBPU 20%	1,823	0,043	-1,78
KBPU 10%	1,823	0,578	-1,245
KBPU 5%	1,823	1,077	-0,746

Information:
AS : Sterile aquades (negative control)
CHX 0,2% : chlorhexidine 0.2%, (positive control)
KBPU : Ulin bark extract

Based on these results it is known that the ulin bark extract (*Eusideroxylon zwageri*) concentrations of 5%, 10%, 20%, 40%, 60%, 80% and 100% can inhibit the growth of *Porphyromonas gingivalis* bacteria indicated by negative mean reduction values which means all extract group has a bacteriostatic effect and for the MIC value is at a concentration of 5%.

Figure 1. The results of the measurement of the absorbance value to determine the MIC on ulin bark extract with Chlorhexidine 0.2% against *Porphyromonas gingivalis* bacteria.

MIC data obtained from each treatment was then tabulated and Saphiro-Wilk normality test was performed. The results of the Saphiro-Wilk normality test for the minimum inhibitory concentration (MIC) obtained p> 0.05 so it can be said that the data is normally distributed. Then the MIC data was carried out homogenity test using Levene's test.

The Levene's homogeneity test results obtained a value of 0.031, which means p <0.05, it can be concluded that the data between groups have different variants. However, it can be continued with parametric tests using the One-Way ANOVA hypothesis test.

The results of minimum bactericidal concentration (MBC) test of ulin bark extract (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis* as seen from the number of *Porphyromonas gingivalis* colonies on the plate can be seen as follows:.

Table 2. Results of Minimum Bactericidal Concentration Test (MBC) of ulin bark extract (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis*

Group	N	Average colony (CFU/µl) ± Standard Deviation
AS	4	134,25± 6,994
CHX 0,2%	4	0 ± 0,000
KBPU 100%	4	0 ± 0,000
KBPU 80%	4	0 ± 0,000
KBPU 60%	4	0 ± 0,000
KBPU 40%	4	0 ± 0,000
KBPU 20%	4	0 ± 0,000
KBPU 10%	4	11,25 ± 1,258
KBPU 5%	4	30,75 ± 3,304

The result of the minimum bactericidal concentration test of Ulin bark extract was the 5% concentration was unable to kill *Porphyromonas gingivalis*, marked by the growth of bacterial colonies as many as 30,75 CFU/µl. The 10% of Ulin bark extract was also still unable to kill *Porphyromonas gingivalis*, because of the presence of 11,25 CFU/µl colony growth. Ulin bark extract concentrations of 20%, 40%, 60%, 80% and 100% can killed *Porphyromonas gingivalis* and it is equal to the positive control of chlorhexidine 0.2% characterized by the absence of bacterial growth (0 CFU/µl). Negative control with sterile aquades cannot killed bacteria characterized by bacterial growth of 134.25 CFU/µl.

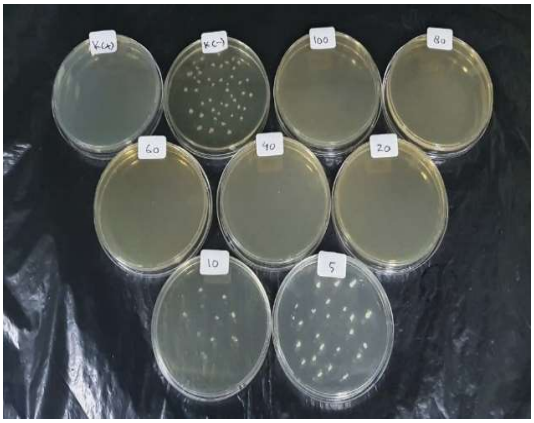

Figure 2. Measurement results for the number of *Porphyromonas gingivalis* colonies in all treatment groups.

The results in table 2 then tabulated and was done Shapiro Wilk normality test. The normality test result was p <0.05 so the data was not normally distributed. Then the data processing was continued with the non-parametric Kruskal Wallis test. Non parametric test kruskal Wallis results showed a significance value of p = 0,000 (p <0.05) which means that there was significant differences in the mean between each treatment group, then it was continued by Mann Whitney test.

In the table 4 the results of Post-Hoc Games Howell test stated the differences between the MIC test treatments of ulin bark extract (*Eusideroxylon zwageri*) 5%, 10%, 20%, 40%, 60%, 80% and 100% concentrations showed that the ulin bark extract concentration of 5% and 10% had a significant differences in inhibiting the growth of *Porphyromonas gingivalis* compared to all treatment groups. Ulin bark extract concentrations of 20%, 40%, 60%, 80%, and 100% had a significant differences when compared to the negative control, concentrations of 10% and 5% in inhibiting the growth of *Porphyromonas gingivalis*.

Based on the table 5 Mann Whitney test results explain the differences between groups from the MBC test of ulin bark extract (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis* bacteria. Ulin bark extract concentrations of 20%, 40%, 60%, 80%, and 100% compared to positive control had not significant differences (p> 0.05) and vice versa, whereas ulin bark extract concentrations of 5 %, 10%, and sterile aquades have a significant differences in reducing the number of *porphyromonas gingivalis* colonies compared to concentrations of 20%, 40%, 60%, 80%, 100%, positive control and vice versa.

Table 4. Post-Hoc Games Howell Test of Ulin Bark Extract (*Eusideroxylon zwageri*) MIC On The Growth of *Porphyromonas gingivalis* Bacteria.

Treatments	**K-**	**K+**	**KBPU 100%**	**KBPU 80%**	**KBPU 60%**	**KBPU 40%**	**KBPU 20%**	**KBPU 10%**	**KBPU 5%**
K-	-	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*
K+	.000*	-	0.907	0.371	0,670	1,00	0,260	.000*	.000*
KBPU 100%	.000*	0.907	-	0.990	1,00	0,913	0,960	.000*	.000*
KBPU 80%	.000*	0.371	0.990	-	0.992	0.266	1,00	.000*	.000*
KBPU 60%	.000*	0.670	1.00	0.992	-	0.589	0.943	.000*	.000*
KBPU 40%	.000*	1.00	0.913	0.266	0.589	-	0.103	.000*	.000*
KBPU 20%	.000*	0.260	0.960	1.00	0.943	0.103	-	.000*	.000*
KBPU 10%	.000*	.000*	.000*	.000*	.000*	.000*	.000*	-	.000*
KBPU 5%	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	-

* = significant differences (p<0,05)

Tabel 5. *Mann Whitney* Test of Ulin Bark Extract (*Eusideroxylon zwageri*) MBC On The Growth of *Porphyromonas gingivalis* Bacteria.

Treatments	K-	K+	KBPU 100%	KBPU 80%	KBPU 60%	KBPU 40%	KBPU 20%	KBPU 10%	KBPU 5%
K-	-	.014*	.014*	.014*	.014*	.014*	.014*	.020*	.021*
K+	.014*	-	1.00	1.00	1.00	1.00	1.00	.013*	.014*
KBPU 100%	.014*	1.00	-	1.00	1.00	1.00	1.00	.013*	.014*
KBPU 80%	.014*	1.00	1.00	-	1.00	1.00	1.00	.013*	.014*
KBPU 60%	.014*	1.00	1.00	1.00	-	1.00	1.00	.013*	.014*
KBPU 40%	.014*	1.00	1.00	1.00	1.00	-	1.00	.013*	.014*
KBPU 20%	.014*	1.00	1.00	1.00	1.00	1.00	-	.013*	.014*
KBPU 10%	.020*	.013*	.013*	.013*	.013*	.013*	.013*	-	.020*
KBPU 5%	.021*	.014*	.014*	.014*	.014*	.014*	.014*	.020*	-

* = significant differences ($p<0,05$

DISCUSSION

Based on research results of ulin bark extract (*Eusideroxylon zwageri*) at concentrations of 5%, 10%, 20%, 40%, 60%, 80%, 100%, and positive control has antibacterial effectiveness against *Porphyromonas gingivalis*. Concentrations of 5%, 10%, 20%, 40%, 60%, 80, and 100% have been shown to inhibit the growth of *Porphyromonas gingivalis* which is characterized by decreased absorbance after 24 hours incubation.

The results of this study are supported by another study conducted by Darussalam (2016) which concerned on ironwood extracts that can inhibit the growth of gram-positive bacteria, such as *Staphylococcus aureus*, which has peptidoglycan as a cell wall. If there is damage to the cell wall, it can cause lysis in bacterial cells. Meanwhile, *Porphyromonas gingivalis* is gram-negative bacteria that have a periplasmic peptidoglycan layer which includes the inner membrane and the outer membrane. Peptidoglycan in gram-negative bacteria has a thick layer which ranging from 3nm-5nm on the outer membrane.[7,12]

The results of the statistical data of Minimum Inhibitory Concentration (MIC) Test of the extract of ulin bark (*Eusideroxylon zwageri*) on the growth of *Porphyromonas gingivalis* is located at a concentration of 5% which can be seen by knowing the reduction in the mean absorbance data of each concentration compared to the negative control, then MIC value was obtained at the smallest concentration that decreased while compared to the negative control.[13] The mean absorbance value of the smallest concentration is 1,07775 which is smaller than the negative control value that as many as 1,82325. The MIC in this study can be seen at a concentration of 5%. Based on this, the results obtained are in accordance with the hypothesis in this study. This is in line with research conducted by Arifurrahman (2017) about the effect of pumpkin leaves ethanol extract on the growth of *Porphyromonas gingivalis*, that the compounds are almost the same as ulin bark extract. It also can inhibit *Porphyromonas gingivalis* at the smallest concentration, 5%, because the presence of dominant bioactive compounds in ulin bark extract is flavonoids at 31.28 mg GAE/g, phenolic at 31.28 mg GAE/g and tannins which have great effectiveness.[9,14]

The content of ulin bark extract can inhibit the growth of *Porphyromonas gingivalis*, like flavonoids as an antibacterial agent that can inhibit cell membrane function, energy metabolism and form complex compounds so it can damage the cell membrane of *Porphyromonas gingivalis*.[15] Alkaloids have a function to inhibit the synthesis of the components of the bacteria's cell wall. The cell wall is not fully formed because the peptidoglycan in *Porphyromonas gingivalis* bacteria is damaged and causes lysis of cells so that cells died.[16] Terpenoids have activities that can bind to lipids in membranes and cell carbohydrate proteins which can decreased membrane permeability and causes lysis of bacterial cells.[10]

Tannin as an antibacterial compound has the ability to interfere with the course of proteins in cells, causing bacterial cell death. Saponin as an antibacterial compound can reduce cell membrane permeability so that lysis occurs in bacterial

cells.[15] Phenolic is able to precipitate cell proteins and damage and penetrate the cell wall of *Porphyromonas gingivalis*.[17]

The positive control used in this study was Chlorhexidine 0,2%. Chlorhexidine is believed as a mouthwash that can reduce plaque formation and prevent periodontal disease. The mechanism of chlorhexidine has similarities with the ulin extract which containing flavonoid, terpenoids, tannins, and saponins that work together to cause changes in the permeability of bacterial cell membrane and eventually cause the death of these bacteria.[1]

Based on the results of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) statistical data in this study, the value is equal to the chlorhexidine 0,2%. That is detected at 20%, 40%, 60%, 80%, and 100% concentrations where there is no significant difference between them so that it can be interpreted that all concentrations of Ulin bark extracts have antibacterial activity equal to chlorhexidine 0,2%. This is consistent with the theory that said the higher concentration of antibacterial substances, the higher the antibacterial power.[14] In the result of absorbance reduction of 40%, the concentration has a reduction of value greater than the concentration of 60%, 80%, and 100%. This happens because the difference in the suspension of the bacterial cells that were grown on the media is not necessarily the same. The one factor that greatly influences the antimicrobial activity is the dose of the inoculum, the sensitivity of the organism is lower due to the greater bacterial inoculum. Smaller bacterial population will inhibit the growth of bacteria faster and more perfect than a large population.[18, 19]

The results of this study are supported by research conducted by Armedita (2018) about the effect of Angsana gum leaves ethanol extract which 50% concentration is the optimal concentration and 75% is not optimal due to the higher concentration. Because of it, the extract is difficult to work optimally into the medium so that it produces a decrease in the inhibitory of bacteria that makes the antibacterial performance unstable.[14]

In this study, ulin bark extract (*Eusideroxylon zwageri*) was investigated to be an alternative mouthwash to reduce the side effects of using chlorhexidine 0,2% and decrease the prevalence of chronic periodontitis. In conclusion, ulin bark extract (*Eusideroxylon zwageri*) shows MIC value at 5% concentration and MBC value at 20%. It means ulin bark extract (*Eusideroxylon zwageri*) has inhibitory and killing power equal to the chlorhexidine 0,2% as a positive control on the *Porphyromonas gingivalis* growth.

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