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COMPARISON OF THE INHIBITORY EFFECT BETWEEN ULIN (*Eusideroxylon zwageri)* BARK EXTRACT AND *Chlorhexidine gluconate 0.2%* AGAINST *Streptococcus sanguinis*

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ABSTRACT
Background: *Gingivitis is caused by a build up of plaque bacteria, one of which is Streptococcus sanguinis. Streptococcus sanguinis act as an anchor for the attachment of oral organisms that colonize the surface of the teeth, forming plaque so that it becomes the development of gingivitis. Currently, many natural ingredients, as alternatives, have antibacterial effects. One of which is Eusideroxylon zwageri, because it contains flavonoids, tannins, phenolics, saponins, alkaloids, and terpenoids.* ***Purpose:*** *to compare the inhibitory effect between ulin bark extract and Chlorhexidine gluconate 0.2% against Streptococcus sanguinis.* ***Method:*** *True experimental research with post test only with control group design using ulin bark extract concentrations of 20%, 40%, 60%, 80%, and 100% and Chlorhexidine gluconate 0.2% as the K(+). The maceration method was used to extract ulin bark while the inhibitory test was using the diffusion method with 6 treatment groups and 4 replications, so that there were 24 samples. All treatment groups were incubated 24 hours at 37^{o}C then measurement of inhibition zones was using callipers.* ***Results:*** *The results of the test showed that ulin bark extract concentrations of 20%, 40%, 60%, 80%, 100%, and Chlorhexidine gluconate 0.2% obtained an average inhibition zone with diameter of 7.18 mm, 9.06 mm, 11.08 mm, 13 mm, 15.09 mm, and 18.14 mm. Analysis of One Way ANOVA and Post Hoc Games-Howell data showed a significant difference between treatment groups.* ***Conclusion:*** *Ulin bark extract can inhibit the growth of Streptococcus sanguinis but has not been able to equal to Chlorhexidine gluconate 0.2%.*

Keywords**:** *Chlorhexidine gluconate 0,2%, gingivitis, inhibitory activity, ulin bark extract, Streptococcus sanguinis.*
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INTRODUCTION

South Kalimantan has the second highest dental and mouth problems in Indonesia with 36,1% and increased in 2018 with almost 60%. Dental and mouth problems in Banjarmasin is quite high, that is 38,2%.[1] The most occurence of dental and mouth problems are periodontal disease and caries.[2] Periodontal disease is an oral cavity disease that often experienced by Indonesian society.[3] Periodontal disease was caused by interaction between bacteria and host itself. Plaque bacteria and calculus that accumulated on teeth surface is a local factor and the main cause of periodontal disease. Gingivitis and periodontitis are periodontal diseases that often can be found. Gingivitis is divided by several kinds, the most common is the type of gingivitis that caused by plaque induced. The said type of gingivitis was caused by acummulated plaque bacteria which is *Streptococcus sanguinis*.[4,5]

Streptococcus sanguinis is included in facultative anaerobic bacteria if categorized by its oxygen need, because oxygen utilized to produce energy with respiration.[6] *Streptococcus sanguinis* is a gram-positive bacteria that has a role as an anchor to oral cavity microorganisms adhesion and will colonize on teeth surface, so that plaque formed will become gingivitis development.[7] Mouthwash will be used for daily plaque controlling. The most common used mouthwash is *Chlorhexidine gluconate* 0,2%. *Chlorhexidine gluconate* 0,2% is a kind of disinfectant and antiseptic that is bacteriocidal and bacteriostatic agent against gram-

negative and gram-positive bacteria.[8] *Chlorhexidine gluconate* 0,2% usage in a long period of time or more than 2 weeks will cause side effects, so that alternative substance will be needed, that can be used and will not causing any side effect.[7]

Herbal or natural substance has been developed right now, so it can be an alternative substance that has antibacterial effect, one of which is ulin. Ulin or *Eusideroxylon zwageri* is a unique plant from Kalimantan. Partly of Kalimantan society usually using ulin marinade to cure toothache.[9] Phytochemicals test result by Wila (2018) showed that stem bark of ulin is contained flavonoid, tannin, phenolic, saponin, alkaloid, and terpenoid. The most compounds are flavonoid, tannin, and phenolic.[10] Flavonoid content on ulin stem bark extract is 30,48 mg CE/g, meanwhile phenolic content on ulin stem bark extract is 31,28 mg GAE/g.[11]

The research result by Darussalam (2016) showed that ulin waste extract has antibacterial activity against *Staphylococcus aureus* which is a gram-positive bacteria with the average inhibition zone diameter at concentration 20% was 8,8 mm, at a concentration 40% was 10,3 mm, and was increasing at a concentration 100% with 14,8 mm.[12] Based on description above, it is known that there is inhibitory activity of ulin extract with 20%, 40%, 60%, 80%, and 100% concentrations on *Staphylococcus aureus*, but there is no research on inhibitory effect of ulin bark extract to *Streptococcus sanguinis*, so the researcher is interested in doing a research using 20%, 40%, 60%, 80%, and 100% concentrations of ulin bark extract to know the comparison of the inhibitory effect of ulin bark and *Chlorhexidine gluconate* 0,2% against *Streptococcus sanguinis*.

MATERIALS AND METHODS

This research was done in Basic Laboratory of Mathematics and Science Faculty, Lambung Mangkurat University Banjarbaru, Research and Industry Consultation Center Surabaya, and Microbiology Laboratory Research Center of Faculty of Dentistry Airlangga University Surabaya. This research started after ethical clearence No. 011/KEPKG-FKGULM/EC/I/2020 obtained, which was issued by Faculty of Dentistry Lambung Mangkurat University ethical commitee. This research was a true experimental with post test only with control group design. The sample of this research was groups of ulin bark concentrated in 20%, 40%, 60%, 80%, and 100% and *Chlorhexidine gluconate* 0,2% as positive control. After being calculated using Federer formula, all 6 groups were repeated 4 times, so total samples were 24.

The first procedure performed was the taking of ulin bark at Muara Kintap, Tanah Laut Regency, South Kalimantan. Later the inside of ulin bark that brownish red colored was taken as much as 2 kg using knife without harming the cambium. Ulin bark was cleaned from foreign matter (moss and dirt) and the outside bark was sundried until dried and cutted with ± 2 cm size. Ulin bark that has been cut was made into powder using hammer mill and filtered using mesh screen. Maceration process was done with ulin powder with 200 grams weight put into the extractor tool and 100 ml of 96% etanol were added (ratio 1:5). This process was done for 24 hours while stirring it with the help of shaker. The obtained extract was filtered, then, using rotary vacuum evaporator filtrated filter, will be evaporized with 59-60ºC temperature until concentrated extract was obtained. Then, it heated up on waterbath until all of the solvent evaporized, so 14 g of 100% concentrated brown colored residual of the liquid has been obtained. Ulin bark extract was etanol-free tested with few drops of potassium dichromate ($K_2Cr_2O_7$) that have been added to ulin bark extract and observed. If there was no changing in color of the extract then there was no etanol contained in the ulin bark extract.

The making of ulin bark extract use solution dilution formula which is:[13]

$$\mathbf{V_1 \,.\, N_1 = V_2 \,.\, N_2}$$

V_1 : volume of the solution that available (ml)
N_1 : ulin bark extract concentration that available (%)
V_2 : volume of the solution to be made (ml)
N_2 : ulin bark extract concentration to be made (%)

The *Streptococcus sanguinis* was taken from pure isolate in Microbiology Laboratory Research Center of Faculty of Dentistry Airlangga University Surabaya. Then, into 5 ml of liquid BHI, the bacteria were inoculated and incubated at 37ºC temperature for 2x24 hours into the anaerob incubator. The said suspension was diluted with liquid BHI media until the turbidity proportioned with 0,5 *Mc Farland* standard or total $1{,}5x10^8$ CFU. *Streptococcus sanguinis* that has been 0,5 *Mc Farland* standarized rubbed with sterile cotton swab on Mueller Hinton Agar (MHA) media, then paper disk was marinated on 20%, 40%, 60%, 80%, and 100% concentrated ulin bark and *Chlorhexidine gluconate* 0,2% for 30 minutes. The marinated paper disk attached to bacteria-filled MHA media using tweezer. Then MHA media incubated for 24 hours with 37ºC temperature. *Streptococcus sanguinis* bacteria growth inhibiton zone was measured using callipers.

RESULT

The inhibitory activity test of ulin (*Eusideroxylon zwageri*) bark extract and *Chlorhexidine gluconate* 0,2% against *Streptococcus sanguinis* was carried out by the diffusion method to obtain the amount of inhibition zone by measuring the clear area around the paper disk using calliper in mm.

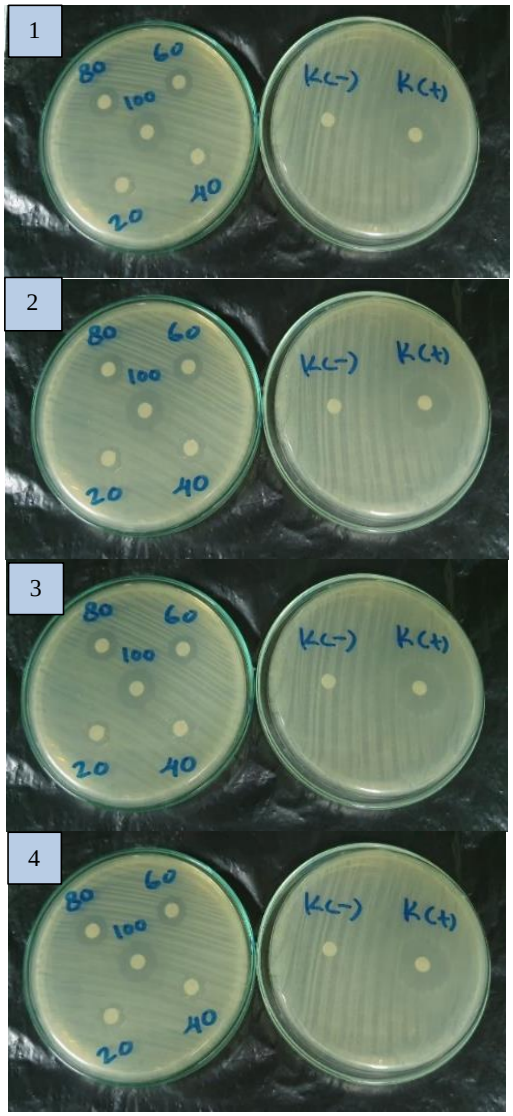

Figure 1. Inhibition zone of ulin bark extract and *Chlorhexidine gluconate* 0,2% on *Streptococcus sanguinis* with 4 replications.

Result of inhibitory activity test of ulin (*Eusideroxylon zwageri*) bark extract and *Chlorhexidine gluconate* 0,2% against *Streptococcus sanguinis* next were analyzed using IBM SPSS Statistics 26 for Windows and can be seen on table 1.

Tabel 1. The average inhibiton zone diameter of ulin bark extract and *Chlorhexidine gluconate* 0,2% on *Streptococcus sanguinis*.

Treatment	N	Mean	Deviation Std.
EKBU 20%	4	7,18	0,06
EKBU 40%	4	9,06	0,49
EKBU 60%	4	11,08	0,12
EKBU 80%	4	13,00	0,14
EKBU 100%	4	15,09	0,10
CHX 0,**2%**	4	18,14	0,09

Information:
EKBU 20% : 20% Ulin bark extract
EKBU 40% : 40% Ulin bark extract
EKBU 60% : 60% Ulin bark extract
EKBU 80% : 80% Ulin bark extract
EKBU 100% : 100% Ulin bark extract
CHX 0,2% : *Chlorhexidine gluconate* 0,2%

Table 1. shows that the higher the concentration of ulin bark extract that has been given, the wider the inhibition zone. The result of this research is known that 100% ulin bark extract has wider inhibition zone diameter average compared to 80%, 60%, 40% and 20% concentration with 15,09 mm. However, 100% concentrated ulin bark extract has narrower inhibition zone average compared to 0,2% *Chlorhexidine gluconate* inhibition zone average, which is 18,14 mm.

The obtained data from every treatment then tabulated and normality tested using Shapiro-wilk with p>0,05 data requirement. After that, the data showed that sig. value of every treatment was more than 0,05 (p>0,05) which meant that the data was normally distributed. Ulin bark extract and 0,2% *Chlorhexidine gluconate* to *Streptococcus sanguinis* data homogeneity were tested using Levene's Test, resulted 0,036 (p<0,05) significance value which meant that every group variant was not the same, so the homogeneity assumption in this research was not fulfilled.

The data of this research that was normal distributed then analyzed using One Way Anova parametric analysis test with 95% confidence level. The result of One Way Anova parametric analysis test was p value=0,000 (p<0,05), which showed that there was a treatment that give different influence, then it can be continued to *Post Hoc Games-Howell* test to see group that give significance difference.

Table 2. The result of *Post Hoc Games-Howell* inhibition zone diameter of ulin bark extract and 0,2% *Chlorhexidine gluconate* on *Streptococcus sanguinis*.

Treatment	CHX 0,2%	EKBU 100%	EKBU 80%	EKBU 60%	EKBU 40%	EKBU 20%
CHX 0,2%		0,000*	0,000*	0,000*	0,000*	0,000*
EKBU 100%			0,000*	0,000*	0,000*	0,000*
EKBU 80%				0,000*	0,001*	0,000*
EKBU 60%					0,014*	0,000*
EKBU 40%						0,020*
EKBU 20%						

*Significance (p<0,05)

Table 2. shows that p<0,05 value meant every treatment has significant difference. Based on that matter, every group that has been given with ulin bark extract and 0,2% *Chlorhexidine gluconate* treatment has statistically different inhibition zone.

DISCUSSION

The research of comparison in inhibitory activity of ulin (*Eusideroxylon zwageri*) bark extract and *Chlorhexidine gluconate 0,2%* against *Streptococcus sanguinis* proven that ulin bark extract with 20%, 40%, 60%, 80% and 100% concentrations are capable to inhibit *Streptococcus sanguinis* bacteria growth. This is supported by previous research by Darussalam (2016) which stated that ulin extract can resist *Staphylococcus aureus* bacteria growth that included in gram-positive bacteria with 20%, 40%, 60%, 80% and 100% concentrations.[12]

Based on the inhibition zone diameter average result on every concentration, it shows that the higher the concentration of ulin bark extract, the wider the formed inhibition zone. This corresponds with the research by Darussalam (2016) which stated that every different concentration extract will give different effect to *Staphylococcus aureus* bacteria because the higher the concentration, the more active substance contained in test solution.[12] This also supported by Qomar et al statement (2018) that the different levels of content on each extract concentration can have effect to form the inhibition zone.[14] Based on qualitatively phytochemical test by Wila et al (2018), it showed that ulin extract contains flavonoid, phenolic, and tannin on high level, meanwhile saponin, alkaloid and terpenoid are on medium level. Some research results stated that flavonoid, tannin, phenolic, saponin, terpenoid and alkaloid have a role as antibacterial. This shows that every type of bioactive compound contained in ulin bark extract has antibacterial potential.[10]

According to Jawetz et al (2017), there are some ways of antibacterial compound mechanism on resisting bacteria growth. Those are resisting cell membrane function, resisting cell wall synthesis, resisting nucleic acid synthesis, and resisting protein synthesis.[15] Based on antibacterial compound mechanism on resisting bacteria growth, the possibility occured in *Streptococcus sanguinis* is cell wall synthesis and cell membrane function are resisted. Cell wall contains peptidoglycan consisting of polypeptide and polysaccharides layers. *Streptococcus sanguinis* included in gram-positive that has very thick peptidoglycan-contained cell wall for mantaining cell's integrity with its rigidity. If the cell wall gets damaged or experiencing obstacles in its forming, then it can cause bacterial cell lysis and lose its ability to form colonies and bacterial death will occur. This happens because there are flavonoids and tannins contained in ulin bark exract.[12,16]

Flavonoid and tannin are phenol derivative that can interact with enzym, lipid, and protein from bacteria cell so it can change bacteria cell's permeability and caused proton, ion and bacteria cell's macromolecules detachment. This can change bacteria cell's surface hydrophobicity. Furthermore, flavonoid can deactivate bacteria adhesin that can affect the ability of its attachment. Phenolic compound is capable of causing heavy damage to cell membrane. Bacteria membrane damage can cause bacteria deattachment from the biofilm. This can happen because the attachment ability of *Streptococcus sanguinis* depends on saliva protein with philia binded, especially amylase so it can contribute to biofilm forming on saliva-layered surface. *Streptococcus sanguinis* is the early bacterial colonization that will connect to other bacteria with pellicle on teeth surface. *Streptococcus sanguinis* lysis can caused other bacteria can not attach to the pellicle, which next can detach from pellicle so the biofilm-forming process will be disturbed.[17,18,19]

Saponin on ulin bark extract also has antibacterial character. Saponin has a role as strong surfactant agent because saponin can reduce inter cells surface tension. Saponin that absorbed on the cell surface can cause bacteria cell damage with increasing membrane permeability which will relieve essential substances such as enzymes and protein inside of bacteria and reduce *Streptococcus sanguinis* surface cell attachment. *Streptococcus sanguinis* is one of the bacteria that can be found on plaque. At the beginning of plaque-forming, a non specific category of bacteria adhesion occurs through hydrophobic characterized interaction.

Hydrophobicity of bacterial cell's surface is an important factor of bacterial attachment on teeth surface. *Streptococcus sanguinis* is known to has cell component with hydrophobic domain which contains non-polar amino acids. The existence of these components causes an increased ability of interaction between the tooth surface with the hydrophobic bacterial cells so as to allow the adhesion of bacterial cells.[17]

Antibacterial compound mechanism that contained in ulin bark extract such as tannin, phenolic, flavonoid, saponin, terpenoid and alkaloid can cause complex damage on *Streptococcus sanguinis* bacterial structure which can cause bacterial lysis. The death of *Streptococcus sanguinis* as pioneer bacteria in plaque-forming can reduce the ability of plaque accumulation in oral cavity so events of gingivitis can be reduced.[20]

Based on research by Amalia et al (2018), antibacterial effect criteria according to Davis and Stout is the scale of formed inhibition zone diameter. If inhibition zone diameter is ≤ 5 mm then will be categorized as a weak antibacterial. If inhibition zone diameter is 5-10 mm then it will be categorized as medium antibacterial. If inhibition zone diameter is 10-19, then it will be categorized as a strong antibacterial, and if the inhibition zone diameter is ≥ 20 mm then it will be categorized as very strong antibacterial.[21] Based on these criteria, then the inhibitory activity of 20% and 40% concentrations of ulin bark extract give medium antibacterial effect because the inhibition zone diameter average is on the range of 5-10 mm, meanwhile 60%, 80% 100% concentrations and *Chlorhexidine gluconate 0,2%* give strong antibacterial effect because inhibition zone diameter average is on the range of 10-19 mm.

Ulin bark extract concentrations that give strong effect due to the extract potential as antibacterial agent, but it is still not strong enough if compared to *0,2% Chlorhexidine gluconate*. Based on statement of Nuryani et al (2017), it is mentioned that *0,2% Chlorhexidine gluconate* is a gold standart mouthwash and a bis-biguanide derivative that has large spectrum, quick kill ability, reducing 80% of oral cavity's microbe and has low toxicity.[22] Furthermore, *0,2% Chlorhexidine gluconate* is the most effective active agent to reduce and resist plaque accumulation and can kill gram-positive and gram-negative bacteria.[23] *0,2% Chlorhexidine gluconate* also blocks acids and saliva glycoprotein, then reduce absorption of protein on the teeth surface so bacteria adhesion can not be attach which can cause resisting of bacteria attachment.[24]

Post Hoc Games-Howell statistic test result (Table 2) shows every concentration treatment of ulin bark extract gave significant difference to *Chlorhexidine gluconate 0,2%* which meant inhibitory activity of 20%, 40%, 60%, 80% and 100% concentrations of ulin bark extract is still not be able to equal with inhibitory activity of *Chlorhexidine gluconate 0,2%*, although 60%, 80% and 100% concentrations are categorized as a strong inhibition effect. This might because no research was done on quantitatively phytochemical tests so it is not known how many levels of each compound of ulin bark extract that used in this research. Furthermore, the possibility of minimum inhibition level from ulin bark extract to *Streptococcus sanguinis* that still has not be known yet, so the scale of inhibition zone that has been formed is still can not be equal with the inhibition zone scale of *Chlorhexidine gluconate 0,2%* as positive control. The used bacteria in this research is pure isolate bacteria that has been bred, this is also suspected to cause formed ulin bark extract inhibition zone still can not be equal with *Chlorhexidine gluconate 0,2%* inhibition zone.

Based on this research, it can be concluded that ulin bark extract is capable to resist *Streptococcus sanguinis* growth based on formed inhibition zone, so it can be an herbal mouthwash alternative. The 20%, 40%, 60%, 80% and 100% concentrations of ulin bark extract have smaller inhibition power compared to *Chlorhexidine gluconate 0,2%* against *Streptococcus sanguinis*. Based on data analyis, each concentration treatment of ulin bark extract has significant difference because each has p value<0,05, so the inhibitory activity of 20%, 40%, 60%, 80% and 100% concentrations of the extract is still can not be equal with *Chlorhexidine gluconate* 0,2% against *Streptococcus sanguinis*.

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