ANTIBACTERIAL EFFECTIVITY OF KASTURI LEAF EXTRACT (Mangifera casturi) AGAINST THE GROWTH OF Streptococcus sanguinis BACTERIA

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Submission date: 13-Jun-2023 02:10PM (UTC+0700)
Submission ID: 2115082557
File name: 7._Jurnal_Nasional_8118-19505-1-SM.pdf (468.29K)
Word count: 3525
Character count: 17975

ANTIBACTERIAL EFFECTIVITY OF KASTURI LEAF EXTRACT *(Mangifera casturi)* AGAINST THE GROWTH OF *Streptococcus sanguinis* BACTERIA

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Dental and oral diseases are able to affect all groups of age, which have progressive and accumulative character. The most common type of dental and oral disease in Indonesia are caries and periodontal disease.[1] According to the survey by RISKESDAS 2013, the highest prevalence of dental and oral problem in Indonesia provinces were South Sulawesi (36.2%) followed by South Kalimantan (36.1%), and Central Sulawesi (35.6%). This result made South Kalimantan as one of three provinces in Indonesia that had oral and dental problems with high prevalence (>35%). The proportion of dental and oral health problems according to RISKESDAS 2018 showed that Indonesia had 45.3% of cavities problem, and 46.9% was found in South Kalimantan.[2,3] The prevalence and incidence of dental caries within the population was influenced by a number of risk factors such as age, socioeconomic status, gender, diet pattern, and habits in maintaining dental and oral hygiene.[4]

Caries is formed due to the calcified food debris that attach to the teeth. The impacts are calcification, decay, and fracture.[5] At the early stages, caries is characterized by the damage on the tooth surface caused by acid resulting from metabolism of carbohydrates produced by bacteria that caused tooth cavities. The main cause of caries and other dental diseases is the presence of bacterial aggregation that causes plaques and normal bacterial flora of oral cavity that is on the tooth surface. One of the *Streptococcus* bacteria which causes decay is *Streptococcus sanguinis*. It refers to one of the types of bacteria whose presence is dominant in dental plaques, and *Streptococcus sanguinis* is also the first microorganism to colonize newly erupted teeth.

After the teeth eruption in oral cavity, *Streptococcus sanguinis* will immediately colonize the surface. *S. sanguinis* colonization on the surface of tooth begins at the age of nine months in infants

and their population has been increased as they grow. It attaches to the surface of hydroxyapatite (HA) which become a major component of tooth enamel through interaction with salivary glycoprotein in the pellicle, then *Streptococcus sanguinis* initiates the adhesion of other oral bacteria.[8,9]

Various ways had been applied to prevent caries, one of them was by using an antiseptic mouthwash that was able to reduce the amount of pathogenic bacteria colonies in oral cavity, the occurrence of plaque, and caries.[10] One of various types of mouthwash often used today is chlorhexidine. Chlorhexidine is defined as a mouthwash that reduces and inhibits the occurrence of plaque even prevent the occurrence of periodontal diseases.[11] However, Chlorhexidine has various side effects, the most common are discoloration in the oral cavity, the increase of calculus forming, causes problems of decrease or change in taste, and discoloration in teeth. The more serious effect causes canker sores, white spots or sores in the mouth or on the lips, swollen salivary glands, the appearance an allergic reaction sign like difficulty breathing, or swollen face, lips, tongue and throat.[12]

Nowadays, high interest in oral care product was combined with plants extract medicine was because it was known to have lower toxicity.[13] One of the alternatives natural ingredients that can be used is Kasturi leaf. Kasturi as it is an endemic plant of Borneo that is interesting to be studied because its existence is currently threatened with extinction. The preliminary phytochemical test of Kasturi indicated that Kasturi's trunk contained compound of terpenoid, steroid, and saponin. Kasturi was known to be able to be used as traditional medicine because of flavonoid and phenolic compounds were ithin it.[14,15] According to the research done by Marliani et al., (2016), Kasturi's leaf contained phenolat as much as 18.44%, and flavonoid as much as 9.27%, which was higher than phenolat and flavonoid contents of Kasturi's trunk skin and fruit skin.[14]

In this research, the bacteria activity on Kasturi's *(Mangifera casturi)* leaf extract in the form of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) toward the growth of *Streptococcus sanguine* bacteria was carried out. This research is intended to find out the effectiveness of antibacterial content of Kasturi *(Mangifera casturi)* leaf extract toward the growth of *Streptococcus sanguine* bacteria.

MATERIALS AND METHODS

This research has obtained research ethics of No.5759/KEPKG-FKGULM/EC/X/2019 issued by ethical committee of Faculty of Dentistry, Lambung Mangkurat University. This research belonged to true experimental research category, and the research design was *randomized pre-test and post test with control group design* by using 5 experimental groups; group 1: colonies of *Streptococcus sanguinis* bacteria which was given 20 mg/ml of Kasturi leaf extract, group 2: colonies of *Streptococcus sanguinis* bacteria which was given 25 mg/ml of Kasturi leaf extract, group 3: colonies of *Streptococcus sanguinis* bacteria which was given 30 mg/ml of Kasturi leaf extract, group 4 (control +): colonies of *Streptococcus sanguinis* bacteria which was given chlorhexidine gluconate 0.2%, and group 5 (control -): colonies of *Streptococcus sanguinis* bacteria which was given aquadest.

The population of this research was bacterial isolates of *Streptococcus sanguinis* ATCC 10556. Each treatment was repeated 5 times obtained from the results of the calculation by using Federer formula, and the sampling technique was done by using *simple random sampling*. The samples were then tested with Minimum Inhibitory Concentration (MIC) by using broth dilution method with UV-Vis spectrophotometer and Minimum Bactericidal Concentration (MBC) by using agar dilution method with *colony counter*.

The tools used in this research were blender, petri dish, mask, handscoon, sterile ose, hot plate, pipette and micropipette, analytical scales, incubator, Bunsen and spirits, test tubes, *vortex mixer*, label paper, measuring cup, *rotary evaporator*, filter paper, UV-Vis spectrophotometer, erlenmeyer, beaker glass, stirring rod, horn spoon, and *autoclave*. The materials used in this research were Kasturi leaf extract, bacterial isolates of *Streptococcus sanguinis*, chlorhexidine 0.2% as the positive control, aquadest as the negative control, ethanol 70%, *Brain Heart Infusion-Broth* (BHI-B), Nutrient Agar media, and Mc. Farland solution (3×10^8 CFU/mL). The tools were washed cleanly and then dried, after that they were sterilized by using autoclave at 121^{o}C for 15 minutes. Kasturi leaf was washed by using flowing water.

Preparation of Bacteria

The colonies of *Streptococcus sanguinis* bacteria that had been stored in agar media was taken by using sterile ose needle, and then it was mixed with 30 ml of BHI-B solution in the test tube, then it was incubated for 24 hours at 37^{o}C. The suspension was then equalized its turbidity with 0.5 Mc.Farland standard solution.

The Making of Kasturi Leaf Extract using Maceration Method

As much as 2 kg of kasturi leaf were washed by flowing water and then drained. After that, kasturi leaf was dried by using an oven for 4 hours at 40°C until it turned into brown. Next, the dried leaf was blended until they became powder and 900 grams of powder were obtained, the kasturi leaf powder was then soaked by using 4.5 L of ethanol solution 70%, then was stirred until it was homogeneous then let it stand for 3 days. In addition, the immersion result was filtered by using

filter paper for 3 times or until the filtrate was clear and the liquid extract obtained. The liquid extract then was evaporated the solvent by using a rotary evaporator at 60°C until a thick extract was acquired with fixed weight. Kasturi leaf extract was then diluted according to the required concentrations of 20 mg/ml, 25 mg/ml and 30 mg/ml.

The Making of Tested Media

The media used in this research were Brain Heart Infusion Broth (BHIB) and Nutrient Agar (NA). BHIB media was weighed as much as 12 grams and put into a beaker glass, 30 ml aquadest was added, then heated by using hot plate until it got boiling while being homogenized by using magnetic stirrer. After that, BHIB media was poured into Erlenmeyer and covered by cotton, then it was put into the *autoclave* for 30 minutes at 121°C, after 100 ml of aquadest was added to the Erlenmeyer, blue and yellow tips were prepared inside the beaker glass. BHIB media was then transferred to 30 ml of sterile test tube and then stored. In the making of NA media, 1.11 grams of Nutrient Agar powder was taken and put it into the beaker glass, 600 ml of aquadest was added, then heat it by using hot plate until it got boiling and was homogenized by using a magnetic stirrer, next step was to pour it into the Erlenmeyer and covered it by using cotton tightly. NA media was then put into the autoclave and sterilized for 30 minutes at 121°C, then NA media was put into each petri disposible cup as much as 20 ml, after being frozen, NA media was stored in the storage refrigerator.

Antibacterial Test of Kasturi Leaf Extract

The determination of MIC and MBC carried out in this research was done by using broth dilution method. First step was making the main solution, 1 gram ofkasturi leaf extract was taken and diluted by using ethanol 96%as much as 10 ml, then being homogenized by using vortex mixer, it obtained the main solution with 100 mg/ml as its concentration. Furthermore, the main solution of kasturi extract was diluted according to the dilution formula covering $V_1C_1.V_2C_2$ to obtain the desired concentration in which it included the concentrations of 20 mg/ml, 25 mg/ml and 30 mg/ml. As many as 25 sterile test tubes were prepared, each group had 5 tubes for each repetition, then each group of 20 mg/ml, 25 mg/ml and 30 mg/ml concentrations, positive control and negative control was labeled. Each tube was then filled with 1 ml of *Streptococcus sanguinis* bacterial colony which was equivalent to McFarland's turbidity standard. In group 1, 1 ml of kasturi leaf extract with the concentration of 20 mg/ml was added; in group 2, 1 ml of kasturi leaf extract with the concentration of 25 mg/ml was added; in group 3, 1 ml of kasturi leaf extract with the concentration of 30 mg/ml was added; for group 4, 1 ml of chlorhexidine gluconate as positive control was added and in group 5, 1 ml of aquadest as negative control was added. The tubes were then covered by using sterile cotton, then was homogenized by using a vortex mixer, next the absorbance was measured by using UV-Vis spectrophotometer (λ = 600 nm) to obtain the initial absorbance value before the incubation, then put it into the incubator and was incubated for 24 hours at 37°C in anaerobic condition. After being incubation, the test tube was then removed and the absorbance was measured again by using UV-Vis spectrophotometer (λ = 600 nm) so that the final absorbance value was acquired. If the final absorbance value (after incubation) of each tube was smaller than the initial absorbance value (before incubation), it means that the decrease in bacterial growth occurred. On the other hand, if the final absorbance value was greater than the initial absorbance value, it means that there was still bacterial growth. This was the Minimum Inhibitory Concentration *(MIC)* determined from the smallest concentration of extract in the treatment tube which began to inhibit the growth of *Streptococcus sanguinis* bacteria. After obtaining the value of the inhibitory level, a further test was carried out to determine Minimum Bactericidal Concentration *(MBC)* value, the sample solution with the concentrations of 20 mg/ml, 25 mg/ml and 30 mg/ml, chlorhexidine gluconate and 5µl of aquadest was then planted on Nutrient Agar media by spreading it by using the tip of the sterilized micropipette, then was incubated for 24 hours at 37°C. After that, the number of growing bacterial colonies was calculated by using *colony counter*, if there was no bacterial growth, the result of the Minimum Bactericidal Concentration (MBC) was obtained.

RESULTS

The research entitled "Antibacterial Effectivity of Kasturi Leaf Extract *(Mangifera casturi)* against The Growth of *Streptococcus sanguins* Bacteria" was carried out by using 5 treatments (the concentrations of 20 mg/ml, 25 mg/ml and 30 mg/ml) covering kasturi leaf extract, control positive of chlorhexidine gluconate and negative control of aquadest. Each treatment was tested by using dilution method in 5 repetitions. The results of the mean absorbance values of MIC from each treatment on the *Streptococcus sanguins* can be seen on the Table 1.

Table 1. MIC Result of Kasturi Leaf Extract *(Mangifera casturi)* against *Streptococcus sanguinis*

Sample	Before 24 hours	After 24 hours	Deviation	Desc
EDK 20	2.461	1.509	-0.952	Decreased
EDK 25	2.560	1.092	-1.468	Decreased
EDK 30	2.761	0.633	-2.128	Decreased
CHX 0,2	1.079	0.882	-0.197	Decreased
AQ	0.064	0.919	0.855	Increased

Notes:
EDK 20 : Kasturi Leaf Extract of 20 mg/ml
EDK 25 : Kasturi Leaf Extract of 25 mg/ml
EDK 30 : Kasturi Leaf Extract of 30 mg/ml
CHX 0.2 : Chlorhexidine gluconate 0.2%
AQ : Aquadest

Table 1 showed the mean difference of absorbance values before and after the incubation of each treatment of kasturi leaf extract. "Decreased" indicated a decrease in absorbance value after incubation compared to the absorbance value before incubation, which means the bacterial growth was inhibited; while "Increase" revealed an increase in absorbance value after incubation compared to the absorbance value before incubation, which means that bacterial growth increased and was not inhibited. Kasturi leaf extract obtained with the concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, and positive control of chlorhexidine gluconate 0.2% inhibited the growth of *Streptococcus sanguinis* bacteria, while there was no inhibition of the growth of *Streptococcus sanguinis* bacteria on negative control of aquadest. The results of MBC measurement of each treatment on *Streptococcus sanguinis* can be seen on the Table 2.

Table 2. MBC Result of Kasturi Leaf Extract *(Mangifera casturi)* against *Streptococcus sanguinis*

Sample	N	Mean
EDK 20	5	109
EDK 25	5	15
EDK 30	5	0
CHX 0,2	5	0.4
AQ	5	2064.4

Notes:
EDK 20 : Kasturi Leaf Extract of 20 mg/ml
EDK 25 : Kasturi Leaf Extract of 25 mg/ml
EDK 30 : Kasturi Leaf Extract of 30 mg/ml
CHX 0,2 : Chlorhexidine gluconate 0.2%
AQ : Aquadest
N : Number of Repetitions

Table 2 showed the results of MBC testing of kasturi leaf extract on the growth of *Streptococcus sanguinis* in the tested media. The result of 30 mg/ml kasturi leaf extract became the smallest concentration that was able to kill the growth of *Streptococcus sanguinis* bacteria as there was no growth of bacterial colonies on petri dishes (0 CFU/μl). Kasturi leaf extract with the concentration of 30mg/ml was still more effective in killing *Streptococcus sanguinis* bacteria compared to the positive control, which was chlorhexidine gluconate, since it was indicated by the presence bacterial growth as much as 0.4 CFU/μl. Aquadest as a negative control was not able to kill the growth of *Streptococcus sanguinis* bacteria since the growth bacterial colonies reached 2064.4 CFU/μl.

Each datum of the research results was tested by *Saphiro-Wilk* normality test because the amount of data were less than 50. The results of MIC normality test obtained p> 0.05 so that it could be said that the data were normally distributed. Then the homogeneity test was performed through *Levene's test* with p> 0.05 and it obtained p=0.050 (p> 0.05) so that the data between groups in this research were not homogeneous and did not have the same variance. The results of MBC normality test on the concentrations of 20 mg/ml, 25 mg/ml, and aquadest obtained p> 0.05, at the concentration of 30 mg/ml the data were not able to be read because it had constant value (0), but the data obtained in chlorhexidine gluconate 0.2% was 0.006 (p <0.05), it can be said that the data were not normally distributed.

The MIC test statistic was performed by using *One Way ANOVA* with a confidence level of 95% (α=0.05). The result of *One Way ANOVA* test obtained p=0,000 (p <0.05), which means that there was significant difference between treatment groups. The results of MIC data were continued by *Dunnet T3 Post Hoc* test and value of p <0.05 for each treatment group were obtained, so it can be interpreted that each treatment group had significant difference. MBC statistical test was carried out through *Kruskal-Wallis* non-parametric test and p value=0,000 (p <0.05) was acquired, it means that there were significant differences between the treatment groups. Data were continued with *Mann-Whitney Post Hoc* test which showed that there was only 1 treatment that was not significant, it was the treatment of kasturi leaf extract on the concentration of 30mg/ml with chlorhexidine gluconate 0.2%.

DISCUSSION

The results of the research on antibacterial effectiveness of kasturi leaf extract (*Mangifera casturi*) on the growth of *Streptococcus sanguinis* bacteria obtained minimum inhibitory concentration (MIC) at the concentration of 20 mg/ml and

Minimum Bactericidal Concentration (MBC) obtained at the concentration of 30 mg/ml.

. Kasturi leaf extract with the concentrations of 20 mg/ml, 25 mg/ml, and 30 mg/ml inhibited the growth of *Streptococcus sanguinis* bacteria which was seen from the decrease in absorbance value before and after the incubation. While kasturi leaf extract with the concentration of 30 mg/ml was the minimum concentration that was able to kill the growth of *Streptococcus sanguinis*, pointed by the absence of growth of bacterial colonies on the tested media. It is related to the content of secondary metabolites found in kasturi leaf extract such as flavonoid, phenol, triterpenoid, saponin, tannin, and alkaloid which has antibacterial functions.[13]

The metabolites contains in kasturi leaf extract has antibacterial properties with different mechanisms. Flavonoid is able to inactivate proteins so that they interfers bacterial cell metabolism, and causes damage to permeability in cell wall, microsome, and bacterial lysosome due to the interactions between flavonoid and bacterial DNA.[9,16] Phenol compound at low level makes complex formation of protein-phenol with weak bond and it will be decomposed and the penetration of this compound is done into the cells and forms precipitation and protein denaturation. While at high concentration, phenol is acquired in protein coagulation and cell membrane lysis.[17] Triterpenoid compound causes the changes in the composition of cell membranes, so that cell membranes are damaged. This compound is able to interact with membrane proteins that causes lysis or rupture of the contents of the cell so that all material in the cell come and the cells do not function or die. Saponin disturbs the stability of bacterial cell membranes, which causes lysis of bacterial cells and damages the cell membranes and releases the proteins, nucleic acid, and nucleotide from bacterial cell so that bacterial cell does not grow and develop.[18] Tannin compound has antibacterial activity as it deactivates the adhesion of microbial cell, enzymes, and workes on polypeptides cell wall. The presence of tannin on the cell wall is to lyse bacteria cell so that it dies. Alkaloid interfers the constituent components of peptidoglycan in bacterial cell, so that the layer of cell wall does not form as a whole and causes the cell death.[19]

According to Rastina et al (2015), the ability of an antimicrobial material to negate the ability of microorganisms to live depended on the concentration of microbial material itself.[20] Kasturi leaf extract with the concentration of 20 mg/ml was the minimum concentration that inhibited the growth of *Streptococcus sanguinis* bacteria and kasturi leaf extract with the concentrations of 25 mg/ml and 30 mg/ml had higher inhibition indicated by greater decrease in absorbance value. Based on this research, it can be concluded that the higher concentration of kasturi leaf extract was, the greater effect on bacterial growth would be, it was due to the increasing number of antibacterial content at higher concentrations.

This research used chlorhexidine gluconate 0.2% as a positive control. Chlorhexidine gluconate 0.2% had inhibitory power as pointed by a decrease in absorbance value, but it had no bactericidal effect because there was still growth of *Streptococcus sanguinis* bacteria. Negative control in this research used aquadest. The test results showed that aquadest did not have inhibitory effect and bactericidal effect on the growth of *Streptococcus sanguinis* which was showed by an increase in the absorbance value. The increase in this value indicates that the growth of *Streptococcus sanguinis* bacteria increase because aquadest does not have any ability to inhibit or kill the growth of this bacteria.

It can be concluded that kasturi leaf extract possess antibacterial effectiveness on the growth of *Streptococcus sanguinis*. Minimum Inhibitory Concentration (MIC) is obtained at the concentration of 20 mg/ml and Minimum Bactericidal Concentration (MBC) is acquired at the concentration of 30 mg/ml.

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