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THE ANTIBACTERIAL EFFECT OF KASTURI LEAF EXTRACT (*Mangifera casturi*) AGAINST THE GROWTH OF *Streptococcus mutans*

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ABSTRACT

Background: Dental caries is a common problem of teeth and mouth. Dental caries is caused by a complex biological interaction between acidogenic bacteria, fermented carbohydrates and host factors, such as teeth and saliva. *Streptococcus mutans* is known as one of the main bacteria that causes dental caries. Kasturi leaf extract (*Mangifera casturi*) has secondary metabolite compounds, such as alkaloids, terpenoids, flavonoids, phenolics and saponins which are efficacious as antibacterial. **Objective:** The aim of this study was to analyzing the effectiveness of antibacterial kasturi leaf extract (*Mangifera casturi*) against the growth of *Streptococcus mutans* bacteria. **Method:** This study was true experimental research with randomized pretest-posttest with control group design with 8 treatment groups consisted of kasturi leaves extract (concentration with 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml and 45 mg/ml), positive control (chlorhexidine 0.2%) and negative control (aquades). Each treatment was repeated three times. Testing was done by liquid dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The data were analyzed using One Way Anova 95% (α=0.05) followed by Post hoc LSD for MIC data and Dunnet T3 for MBC data. **Results:** Based on the results of the analysis, it was known that Kasturi leaf extract had antibacterial effectiveness against the growth of *Streptococcus mutans* bacteria with MIC obtained at a concentration of 20 mg/ml and MBC obtained at 40 mg/ml. **Conclusion:** Kasturi leaf extract has an antibacterial effect against the growth of *Streptococcus mutans*.

Key words: Antibacterial, kasturi leaf extract (*Mangifera casturi*), MIC, MBC, *Streptococcus mutans*

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INTRODUCTION

Oral and dental health are often a problem that is less noticed for some people, even though the oral cavity is the gateway to the entry of germs and bacteria which will then spread to other organs. Dental caries is a common problem of teeth and mouth that is often complained by both children and adults. Based on the 2013 and 2018 RISKESDAS, there was an increase in the percentage of residents who had complaints about their teeth and mouth, from 25.9% to 57.6%. Dental caries is caused by a complex biological interaction between acidogenic bacteria, fermented carbohydrates and host factors, such as teeth and saliva.[1] *Streptococcus mutans* is known as one of the main bacteria that causes dental caries. The main habitat of this bacterium is mouth, pharynx and intestines. The *Streptococcus mutans* and *Lactobcillus sp* bacteria are strong acid producers that can cause an acidic environment, which is a risk of becoming dental caries.[2] *Streptococcus mutans* is able to attach to other enamel and plaque bacteria. The presence of microorganisms attached to dental plaque, low salivary pH, lack of fluorine and an imbalance in food intake which tends to consume more sweet products, can cause the appearance of dental caries.[2,3]

The use of antibacterial antiseptics can prevent the growth of cariogenic bacteria completely. Antiseptics are usually packaged in the form of mouthwash, which aims to reduce the growth of pathogenic bacteria, reduce the occurrence of plaque, so that dental caries can also be reduced.[4] One of the antibacterial mouthwash that is often used to prevent dental caries is chlorhexidine. This mouthwash is known to reduce plaque growth, effective to reduce the growth of *Streptococcus mutans* bacteria and prevent dental caries.[5] However, the use of chlorhexidine for a long time is known to have a detrimental effect on its users.[6]

The use of natural ingredients can be an alternative treatment to prevent the growth of

Streptococcus mutans bacteria that cause dental caries. Indonesia is a tropical country that has various types of plants in it. Many plants have the potential for medicine and are commonly used in the community. One of them is the Kasturi plant (*Mangifera casturi*). Kasturi leaf is known to contain alkaloids, terpenoids, flavonoids, phenolics and saponins so that they have antibacterial properties.[7] The secondary metabolite content possessed by Kasturi leaf can inhibit and stop the growth of *Streptococcus mutans* by denaturing bacterial cell proteins, damaging cell membranes, activating essential enzymes both in bacterial cells and by causing bacterial cell leakage.[8]

From the research by Marliani et al. (2016), it was found that the highest phenolic and flavonoids content was possessed by the Kasturi leaf compared to its fruit rind and stem bark, but researches on Kasturi leaf extract which is able to inhibit bacterial growth in the oral cavity was rarely found. This caused the researchers to be interested in conducting research on the antibacterial effect of kasturi leaf extract (*Mangifera casturi*) against the growth of *Streptococcus mutans* that causing dental caries.[9]

MATERIAL AND METHOD

This research began with the making of the research permits and ethical clearance issued by the Faculty of Dentistry, Lambung Mangkurat University No. 097/KEPKG-FKGULM/EC/XII/2018. This was a true experimental research with randomized pretest-posttest with control group design with 8 treatment groups which were Kasturi leaf extract (concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml and 45 mg/ml), positive control (0.2% Chlorhexidine) and negative control (Aquades). Each treatment was repeated three times. Testing was done by liquid dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Sterilization of Tools

The tools in this research were dried first in an oven at 170^0C, then were sterilized by using *autoclave* at 121^0C for 15 minutes.

Determination Test of Kasturi Plant

Kasturi leaves were obtained from Munggu Raya Village, Astambul District, Banjar Regency, South Kalimantan. The determination test of kasturi leaf was carried out in Basic Laboratory of FMIPA at Lambung Mangkurat University.

The Making of Kasturi Leaf Extract

Kasturi leaf extract was made by maceration method. 2 kg of Kasturi leaves were washed and dried by using an oven at 40^OC for 4 hours. After being dried, Kasturi leaves were then blended and obtained as much as 900 grams of kasturi leaf powder. After that, Kasturi leaf powder was soaked with solvent in the form of 70% ethanol. Soaking was done for 3 days. Furthermore, the immersion results were filtered by using filter paper 3 times or until the filtrate got clear. All filtrates were put together and then evaporated by using a *Rotary Evaporator* to become thick and form an extract. Kasturi leaf extract was diluted with aquadest solution, so that some concentrations obtained were 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml and 45 mg/ml.

Antibacterial Test

The suspension of *Streptococcus mutans* bacteria that had been synchronized with *Mc Farland* 0.5 solution was transferred into each vacuum tube of 1 ml. Then kasturi leaf extract was added as it had been diluted according to the concentration made in which each was 1 ml, for positive control, 0,2% chlorhexidine was added as much as 1 ml while the negative control was added with 1 ml sterile aquadest. The vacuum tube was covered with sterile cotton and homogenized with a vortex mixer. Then it was measured by a UV-Vis spectrophotometer with a wavelength of 600 nm to measure the absorbance value before the incubation. Then it was being incubated for 24 hours at 37^0C. The absorbance results after 24 hours incubation were measured again using a UV-Vis spectrophotometer with a wavelength of 600 nm. MIC results were seen from the difference in absorbance results before and after 24-hour incubation. After that, samples with the concentration of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml 40 mg/ml, 45 mg/ml, control (+) and control (-) were transferred as much as 5µL to NA media by using a micropipette that had been sterilized with 96% alcohol and then got flattened. The sample was then incubated for 24 hours at 37^0C. After the incubation, the results for MBC calculation were able to read. The calculation was performed by using a colony counter.

Data analysis

The data obtained in this research were collected and then performed a statistical test with *Saphiro-Wilks* normality test and homogeneity test by using *Levene's test*. Then it was followed by analysis test in the form of *One Way Anova* 95% ($\alpha = 0.05$). The data of Minimum Inhibitory Concentration (MIC) were known to be normally distributed and homogeneous, so that it was followed by *Post hoc LSD* (*Least Significant Differences*) test while for MBC data, that were normally distributed, had heterogeneous data variant that used *Post-hoc* Dunnet T3 test.

RESULT

This research was conducted by measuring Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of kasturi leaf extract (*Mangifera casturi*) against the growth of *Streptococcus mutans* bacteria. MIC was

measured by using UV-Vis spectrophotometer with wavelength of 600 nm. The measurements were made before and after incubation to determine whether there was a decrease in the average absorbance value which showed the amount of *Streptococcus mutans* growth while MBC was calculated using the Colony counter. The measurement results for the Minimum Inhibitory Concentration can be seen in table 1.

Tabel 1. The Measurement Result of Minimum Inhibitory Concentration of Kasturi Leaf Extract (*Mangifera casturi*) against the Growth of *Streptococcus mutans* Bacteria

EDK	N	Before Incubation (0 jam)	After Incubation (24 jam)	Difference	Standard Deviation	Description
20 mg/ml	3	2,521	0,938	-1,583	0,064	Decreased
25 mg/ml	3	2,586	1,045	-1,541	0,054	Decreased
30 mg/ml	3	2,778	0,858	-1,920	0,016	Decreased
35 mg/ml	3	2,685	0,585	-2,100	0,052	Decreased
40 mg/ml	3	2714	0,549	-2,165	0,078	Decreased
45 mg/ml	3	3000	0,628	-2,372	0,044	Decreased
K (+)	3	2,003	0,485	-1,518	0,013	Decreased
K (-)	3	0,084	0,567	+0,483	0,012	Increased

Description: The "-" sign indicated a decrease in the number of populations of *Streptococcus mutans* bacteria while the "+" sign showed an increase in the population of *Streptococcus mutans* bacteria. "Decreased" showed the absorbance value after incubation ≤ absorbance value before incubation which meant that bacterial growth was inhibited. "Increased" showed the absorbance value after incubation> absorbance value before incubation, which meant that there was bacterial growth.

Table 1 showed the mean of absorbance value of Minimum Inhibitory Concentration (MIC) after conducted measurement by using spectrophotometer before and after the incubation. In the Kasturi leaf extract concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, 45 mg/ml, and 0.2% chlorhexidine, there was a decrease of the absorbance mean value that showed the decrease of the amount of *Streptococcus mutans* bacteria. In other word, the growth of the bacteria was inhibited. Whereas, in aquades, the negative control, there was an increase of absorbance mean value which revealed that there was an increase of *Streptococcus mutans* amount. The Kasturi leaf extract concentration of 20 mg/ml showed the decrease of absorbance mean value, so this concentration was established as Minimum Inhibitory Concentration (MIC) of Kasturi leaf extract against the growth of *Streptococcus mutans* bacteria. The result of the test obtained the significance value of each $p>0.05$ which showed that the data were normally distributed. Then, the data were tested its homogeneity by using *Levene's test* and obtained the significance value of 0.158 ($p>0.05$), so it can be concluded that the variance of the data between the groups were homogeneous. The result of One Way Anova test on the extract of Kasturi leaf with the concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, and 45 mg/ml obtained the significance level $p = 0.000$ which meant that $p<0.05$ and it showed that there was a significant difference between the treatments. This showed that the extract of Kasturi leaf inhibited the growth of *Streptococcus mutans* bacteria. Based on the result of *Post Hoc* LSD test, it was known that each experimental group was statistically different. The research was then continued with the calculation of Minimum Bactericidal Concentration (MBC) by using Colony Counter. The results of the calculation can be seen in Table 2.

Table 2. The Calculation Result of Minimum Bactericidal Concentration of Kasturi *(Mangifera casturi)* Leaf Extract against the Growth of *Streptococcus mutans* Bacteria

EDK	N	Mean	Std. Deviation
20 mg/ml	3	250	34,078
25 mg/ml	3	118	14,731
30 mg/ml	3	44	14,295
35 mg/ml	3	62	11,269
40 mg/ml	3	0	0,000
45 mg/ml	3	0	0,000
K(+)	3	12	10,017
K(-)	3	1671	194,086

Based on Table 2, it was known that the extract of Kasturi leaf with the concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, and 35 mg/ml were unable to kill the *Streptococcus mutans* bacteria and it could be seen that the Minimum Bactericidal Concentration of Kasturi leaf extract was obtained in the concentration of 40 mg/ml in which the growth of *Streptococcus mutans* bacteria was not found.

The result of the normality test obtained that the significance value of $p>0.05$ on each experimental group so the data were normally distributed. Then, homogeneity test was carried out by using *Levene's test* and obtained significance value of 0.011 so it did not meet the requirement of $p>0.05$. Therefore, it can be concluded that the variance between the groups was not homogeneous. The result of One Way Anova test on the extract of Kasturi leaf on the concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, and 45 mg/ml obtained significance value of $p=0.000$ which meant that $p<0.05$ and showed that there was a significant difference between the treatments. Meanwhile, the results of the Post hoc Dunnet T3 test showed that the treatment group was significantly different, while the other treatment groups were not statistically different.

DISCUSSION

The research result with the concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ml, 40 mg/ml, and 45 mg/ml obtained the Minimum Inhibitory Concentration in 20 mg/ml concentration which was shown by the decrease of absorbance mean value as much as 1.583. Whereas, The Minimum Bactericidal Concentration obtained from the extract of Kasturi leaf in 40 mg/ml concentration showed that there were no *Streptococcus mutans* bacteria grew in this concentration.

In the Kasturi leaf extract with the concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 40 mg/ml, and 45 mg/ml, it was known that there was an antibacterial activity which marked by the decrease of absorbance mean value after the bacteria were given the extract of Kasturi leaf and showed the decrease amount of *Streptococcus mutans* bacteria population. This happened because the extract of Kasturi leaf had some secondary metabolite compound contents, such as alkaloids, terpenoids, flavonoids, and saponins, so it is effective as an antibacterial.[7] The secondary metabolite content of Kasturi leaf inhibit the growth and kill *Streptococcus mutans* bacteria through protein denaturation of bacterial cells, damaged cells' membrane, deactivated essential enzymes in bacterial cells or by causing bacterial cell leakage.[10]

Minimum Inhibitory Concentration (MIC) was obtained on the concentration of 20 mg/ml. According to Jawetz *et.al* in Rosyidah *et. al* (2010), an extract inhibited the growth of bacteria because of the inhibition against the bacterial protein synthesis. The extract of Kasturi leaf contained saponins and terpenoids that inhibited the growth of *Streptococcus mutans* bacteria by inhibiting its protein synthesis, so the constituent components of bacterial cells undergone changes.[11] Saponins contained within the extract of Kasturi leaf is known to be able to give protection from a potent pathogen. This compound disturb the surface tension of bacterial cell walls and release the protein and enzyme from the bacterial cell.[12] Saponins interact with cholesterol in bacterial cell membranes which then cause lipid modification in bacterial cell membranes, so that causing bacteria are difficult to interact with these membranes.[13] Disruption of bacterial interactions with cell membranes causes the antibacterial substances enter the cells more easily, so that bacterial metabolism is disrupted and then cause the bacterial death.[14] Terpenoids are known to react with porin of the outer membranes of the bacterial cell wall by forming strong polymeric bonds which then damage the porin, so that the permeability of the bacterial cell wall is disrupted and cause the bacteria experienced lack of nutrients, so its growth is inhibited or dead.[8,14] Kasturi leaf extract is also known to have alkaloids compounds which has antibacterial ability by damaging bacterial cell wall.[15]

In this research, kasturi leaf extract with concentrations of 20 mg/ml, 25 mg/ml, 30 mg/ml, 35 mg/ ml 40 mg/ml and 45 mg/ml had a greater decrease in the mean value of the solution absorbance compared to the 0.2% chlorhexidine which was a positive control. This was able to be correlated to the secondary metabolite compounds possessed by kasturi leaf extract. 0.2% chlorhexidine is known to have bacteriostatic and bactericidal properties in a variety of bacteria. The performance mechanism of chlorhexidine is by depositing cytoplasmic acid proteins from bacteria, so that it causes the changes in permeability of bacterial cell membranes and leakage in the bacterial cell membranes from various directions.[16] Chlorhexidine is known to have a positive charge (cation) and part of the bacterial molecules charge is negative (anion). This causes chlorhexidine to able to strongly attached to bacterial cell membranes.[9] Meanwhile, kasturi leaf extract is known to contain phenolic compounds which antibacterial mechanism is similar to chlorhexidine which causes bacterial cell leakage by forming protein complexes through hydrogen bonds by attacking phosphate groups. Leakage of bacterial cells causes substances, such as organic ions enzymes, amino acids and nutrients in the bacterial cells to come out of cells and caused the bacteria became lysis.[17] Phenolic also able to damage the constituent components of peptidoglycan which are constituent components of cell walls in gram-positive bacteria. This occurs because the integrity of the bacterial cell is disrupted, so that the bacterial cell layer is not formed intact.[17,18]

Kasturi leaf extract also contained tannins compounds known to be able to pass through cell membranes because these compounds are capable of precipitation on proteins. Tannins can suppress the number of enzymes including glucosyltransferase enzyme which is an enzyme which catalyst results are able to produce glucan product that can help attach *Streptococcus mutans* bacteria to the surface of the teeth.[19,20,21]

In the journal by Azzahra and Hayati (2018) it was known that tannins are able to bind to lipoteichoic on the surface of the cells of *Streptococcus mutans* bacteria. Lipoteichoic acid is one of the teichoic acids which became the entrance and exit of ions from and into the bacterial cells. This lipoteichoic acid is in peptidoglycan on gram-positive bacterial cell walls. This causes the kasturi leaf extract inhibited the growth of *Streptococcus mutans* bacteria more easily which is a gram-positive bacterium. The flavonoids contained in kasturi leaf extract is also able to inactivate proteins in bacterial cell membranes. This leads to unstable bacterial cell walls because of the reaction between flavonoids compounds and phospholipids on bacterial cells, so that the protein structure is damaged. The longer, the bacterial cell lost its shape and then it experiences lysis.[12,22,23]

Minimum Bactericidal Concentration (MBC) was obtained at a concentration of 40 mg/ml. At this concentration, no growth of *Streptococcus mutans* bacteria found any longer. According to Pelczar et al., in the journal by Rosyidah et al., (2010), to be able to kill bacterial growth, an antibacterial material must be able to enter bacterial cells through the cell wall. *Streptococcus mutans* bacteria are gram-positive bacteria that has a cell wall structure with fewer lipids compare to gram-negative bacteria. The terpenoids compounds contain in kasturi leaf extract has soluble properties in lipids, so that these compounds are easier to penetrate the bacterial cell wall and cause the kasturi leaf extract is able to kill the *Streptococcus mutans* bacteria. Kasturi leaf extract also contained flavonoids which cause the death of bacterial cells. Rings A and B of flavonoids has important roles in the process of binding hydrogen by accumulating nucleic acid bases which then cause the inhibition of the formation of DNA and RNA in the bacteria. This compound causes the stability of bacterial cell membranes decrease, damage the cell membranes and inhibite the process of respiration, so that the energy of metabolic processes possessed by bacteria are disrupted. This reduces the energy availability of the bacteria and then causes death in bacterial cells after the kasturi leaf extract was given.[10,13] It can be concluded that there is an antibacterial effect of kasturi leaf extract against the growth of *Streptococcus mutans* bacteria.

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