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Effectivity of gambir extract (uncaria gambir roxb.) on the growth of bacteria staphylococcus aureus

Hafsah Katu,[1] Amirah R. Syamsul,[1] Rehatta Yongki,[1]
Harun Achmad,[2] Egita A. Sukarno[1*]

Abstract

Objective: This study aims to see the Minimum Inhibitory Concentration (MIC), minimum contact time of gambir extract, and the effectiveness of gambir extract (Uncaria gambir Roxb.) on the inhibition of growth of Staphylococcus aureus bacteria.

Material and Method: This research is an experimental laboratory research. The research design used was the pre-test post-test control group. The samples used in the study were Staphylococcus aureus smear, gambir extract with the concentration of 2%, 4%, 6%, positive control (Chresophene), and negative control (aqubidest). Wilcoxon test and Mann Whitney test was used as the data analysis.

Results: The study showed that the Minimum Inhibitory Concentration of gambir extract at the concentration of 2% and the inhibitory of gambir extract at the concentration of 2%, 4%, 6%, and aquabides against the growth of Staphylococcus aureus bacteria with a diameter of the inhibitory zone formed around the disc paper was 6.46mm, 6.64mm, 7.16mm, and 5.60mm within 24 hours while 6.57mm, 6.95mm, 7.54mm, and 5.60mm was formed within 48 hours.

Conclusion: The MIC of gambir extract to Staphylococcus aureus is 2%, minimum contact time is 24 hours, and moderate inhibitory to Staphylococcus aureus bacteria

Keywords : Gambir extract (Uncaria gambir Roxb.), Inhibitory, Root canal infection, Staphylococcus aureus
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Introduction

The most common dental and oral health problems is dental caries. The prevalence of caries in Indonesia is still high, reaching 8.1% and continues to increase. Generally, caries are caused by the interaction of bacteria.[1] Caries bacteria that have reached the pulp can enter the root canal, and can cause pulp necrosis. Microroganism that play a role in root canal infections are Streptococcus, Staphylococcus, Bacillus spp, Lactobacillus acidophilus, Actinomyces, Porphyromonas, Candida albicans and Entrococcus Faecalis.[2] Based on research by Irfan (2014) on bacteria identified from necrotic root canals, seven types of bacteria were identified including Staphylococcus aureus.

The presence of Staphylococcus species as components of the oral flora is controversial. Staphylococcus aureus are a component of flora in the mouth, a study shows that most infections that occur in the oral cavity are caused by the bacteria staphylococcus aureus.[3] However, there are several studies on the spread of disease by Staphylococcus in the oral cavity such as endodontic infection.[4]

In the case of root canal infection, treatment of choice is root canal treatment which includes removing soft tissue of tooth and disinfection of the root canal following by disinfected root canal system is then applied with an irrigation solution to prevent entry and development of microbes.[5]

Application of herbal ingredients for traditional medication is widely practiced by Indonesian people and might potential as an alternative to the main medicinal ingredients.[6] Raw materials for traditional medication easier to obtain and relative inexpensive. Herbal medicine has less side effects compared to modern medicine.

One of the herbal ingredients that can be used as antibacterial is gambir extract. Gambir leaf extract contains catechin as the main compound as well as several other compounds such as catechu tannic acid, querce-tin, red catechu, uorescent gambir, fat and wax.[7]

Based on several studies, gambir relation to the antibacterial with a Minimal Inhibitory Concentration (MIC) 1% with a MIC of 2.5% can inhibit growth of Streptococcus mutans. A study published that gambir extract with a concentration of 0.781% can inhibit the growth of the Candida albicans. It was published that the anti-pain properties of gambir extract are smaller than eugenol.[8,9]

This study aimed to investigate potential gambir extract as an antibacterial in endodontic to inhibit S. auerus in root canal infections. Gambir extract is a product of the gambir plant (Uncaria gambir roxb) containing functional compounds included polyphenol compound, especially catechins that have the potential to be antioxidants and antibacterial.[8]

[1]Department of Conservation Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar Indonesia
[2]Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar Indonesia

*Correspondence to:
Egita A. Sukarno, Department of Conservation Dentistry, Faculty of Dentistry, Hasanuddin University, Makassar Indonesia
egitaaprllia18@gmail.com

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Table 1. The average measurement results of the inhibition zone of Staphylococcus aureus

Concentration	N	24 Hours	48 Hours	Mean Diferences±SD
			Mean±SD	
Gambir extract 2%	5	6.46±0.27	6.57±0.26	0.11±0.15
Gambir extract 4%	5	6.64±0.20	6.95±0.44	0.31±0.43
Gambir extract 6%	5	7.16±0.18	7.54±0.622	0.38±0.70
Chresopene	5	41.48±1.17	38.74±3.41	2.74±2.24
Aquades	5	5.60±0.00	5.60±0.00	

Table 2. Statistical test results of the Staphylococcus aureus inhibition zone

Concentration	N	24 Hours	48 Hours	Comparison test
			Normality test*	
Gambir extract 2%	5	0.980*	0.435*	0.180
Gambir extract 4%	5	0.023	0.699*	0.225
Gambir extract 6%	5	0.254*	0.142*	0.285
Chresopene	5	0.017	0.142*	0.042***
Aquades	5	0.435	0.013	1.000

Table 3. Post hoc Mann-Whitney test results of Staphylococcus aureus inhibition zone

Group (i)	Differences (j)	Observation	
		24 Hours	48 Hours
Gambir extract 2%	Gambir extract 4%	0.421	0.151
	Gambir extract 6%	0.008*	0.008*
	Chresophene	0.008*	0.008*
	Aquades	0.008*	0.008*
Gambir extract 4%	Gambir extract 6%	0.016*	0.142
	Chresophene	0.008*	0.008*
	Aquades	0.008*	0.008*
Gambir extract 6%	Chresophene	0.008*	0.008*
	Aquabides	0.008*	0.008*
Chresophene	Aquabides	0.008*	0.008*

Material and Methods

The research method used is experimental laboratory with pre-test & post-test and control group design. This research was conducted at the Biopharmaca Laboratory of the Hasanuddin University Faculty of Pharmacy and the Microbiology Laboratory of the Hasanuddin University Faculty of Medicine in March - May 2019. The ethical number of this research was obtained from the health research ethics committee of Hasanuddin University Dental and Oral Hospital with the number UH17120182.

Gambir extraction

Gambir extract was made using maceration and rotary evaporator methods. The gambir obtained from the market was made into small pieces then weighed as much as 300 g. The next step is round bottom flask was washed and sterilized with ethanol, then 300 g of gambir was put into a round bottom flask and added 1 L of 70% ethanol before it stored for a week with continuous stirring twice a day. After filtering twice the end solution is 1500 mL of the gambir extraction. The macerated gambir subsequently filtered and then rotated at a temperature of 60o C for ± 1 hour until the thick extract was obtained. After that, let the extract dry for 2 hours.

Gambir extraction in different concentrations.

The gambir extract was weighed using analytical scales and each weighed 0.1 g; 0.2 g; 0.3 g obtained from previous studies with the dilution. The weighed gambir extract is then dissolved with 10 mL of 10% DMSO solution to obtain a 2%; 4%; 6% concentration. Then the gambir extract concentration was put into a vial bottle and labelled based on each concentration.

Preparation of Staphylococcus aureus suspension.

Pure culture of S. aureus was obtained from the Microbiology Laboratory of Faculty of Medicine Unhas. The bacterial suspension was made in a test tube containing 9% NaCL by equalizing the turbidity with Mc. Farlands 0.5 - 0.65 using a densi check turbidity equalizer.

Determination of Minimal Inhibitory Levels (MIC) which can inhibit the growth of Staphylococcus aureus bacteria.

A total of five screw tubes were prepared and filled with 5 mL BHIB medium. Then 0.2 mL of Staphylococcus aureus bacteria were inserted into each tube. After that, 5 mL of 2%, 4% and 6% gambir extract were added to the first five tubes, 5 mL positive control (Cresophene) was inserted in the next tube, and another tube filled with sterile distilled water as negative control. All tubes were incubated at 37°C for 24 hours and 48 hours, the examination was carried out for the presence or absence of Staphylococcus aureus growth which was indicated by the occurrence of turbidity in the tube. The Minimum Inhibitory Concentration is determined by observing the first concentration which appears to be clear. The tube that looks cloudy indicates that there is still bacterial growth. After determining the MIC, it is ready to test the inhibition in a petri dish using a paper disc.

Antibacterial effectivity test of gambir extract to the growth of Staphylococcus aureus.

This test was carried out using the concentration of gambir extract which was also used in the determination of MIC. Five petri dishes are sterilized in autoclave for ± 2 hours. The five petri dishes were filled with Mueller-Hinton Agar medium. Dip the cotton swab in the bacteria culture then the cotton is pressed against the side of the tube so that the water drains. Then a cotton swab was evenly applied to the entire surface of the petri dish containing the medium. Several pieces of paper disc were immersed in each sample and then placed on the surface of the medium. Five for each sample concentration of gambier extract, one for negative control (sterile

distilled water), and one for positive control (Cresophene). Pressed with tweezers so that the paper disc is completely attached to the medium. Then the petri dishes are incubated at 37°C for 2x24 hours. After 24 hours and 48 hours the inhibition zone was measured using a calliper. This procedure was carried out five times (five replications) on five different petri dishes to reduce bias.

Inhibition zone measurement.

Inhibition is defined based on diameter measurement of the inhibition zone (clear zone or clear area without microorganisms) that forms around the paper disc. These measurements is in millimeter using calipper.

Results

Minimal Inhibitory Concentration (MIC) test using concentration of 2%, 4%, 6%, and sterile distilled water as a negative control, and chresophene as a positive control, after incubation process between gambir extract (Uncaria gambir Roxb.) toward S. aureus for 1 x 24 hours and 2 x 24 hours. The results showed that at a concentration of 2% the growth of S. aureus began to look clear, indicating that an inhibition zone had begun to form. The average inhibition zone in the study was obtained with the following data:

Based on table 1, it can be seen that the gambir extract (Uncaria gambir Roxb.) with a concentration of 6% has the largest inhibition zone of 7.54 ± 0.622 at 48 hours when compared to all concentrations of gambir extract. The inhibition zone in the negative control group using Aquades had the smallest zone of inhibition with 5.60 ± 0.00. The inhibition zone in the positive control group using Chresopene had the largest zone of inhibition of 41.48 ± 1.17 at 24 hours observation and decreased to 38.74 ± 3.41 at 48 hours observation.

Based on the Shaphiro-Wilk statistical test to determine the normality value and some data obtained p value <0.05, which means that the data is not normally distributed so the test is continued with a non-parametric test, namely Kruskall Wallis table 2. Based on the Kruskall Wallis statistical test, it was found a significance value of 0.000 (p <0.05), which means that there were significant differences between treatment groups. Then the test was continued to Wilcoxon to determine the difference between 24 hours and 48 hours in each group. In the gambir extract group of 2%, 4%, 6% and distilled water control there was no significant difference between 24 hours and 48 hours of observation, while the chresophene control group had a significance value of p <0.05, which means that there was a significant difference between 24 hours and 48 hours. Furthermore, the post hoc test was carried out using the Mann Whitney test to determine the differences in each group.

The results of the Post Hoc Mann-Whitney test of the inhibition zone between groups of S. aureus showed a significant value (p <0.05). At 24 hours and 48 hours of observation, the 2% concentration of gambir extract compared to the 6% concentration of gambir extract and control using Chresopene and distilled water had a p value <0.05, which means that there is a significant difference or has a different effect. While the 2% concentration of gambir extract compared to the 4% concentration of gambir extract has a p value> 0.05 which means there is no significant difference or does not have a different effect.

Discussion

As the observation results of the Minimum Inhibitory Concentration (MIC) and the inhibitory test were carried out 5 times to get valid results. Minimal Inhibitory Concentration (MIC) was seen from the minimum concentration of experimental materials that were able to inhibit bacteria after incubation for 24 and 48 hours and did not show any macroscopic bacterial growth which could be seen from the culture results in the tube which began to turn clear using the dilution method. From the observations, it shows that at a concentration of 2% the growth of S. aureus begins to look clear, which indicates that an inhibition zone has begun to form.

That gambir extract have a Minimal Inhibitory Concentration (MIC) of 1% to Entrococcus faecalis. Research conducted by Agung 2016 (unpublished) that gambir extract with a concentration of 0.781% can inhibit the growth Candida albicans.

The difference occurs because the bacteria used by the researchers are different, as well as the gambir used came from Aceh-North Sumatra meanwhile in this study the gambir came from Pakpak Bharat-North Sumatra and the extract solvent in the previous researcher used 96% ethanol solution meanwhile this study used a 70% ethanol solution.

In the study of inhibition test using gambir extract (Uncaria gambir Roxb.) on the growth of Staphylococcus aureus found that the formed inhibition zone increased in as well as the increasing of extract concentration. This is because the active substances contained in gambir are catechins, polyphenol compounds that have the potential to be antibacterial and antifungal. These catechins are flavonoids that can be found in green tea, black tea, gambir, grapes and other food plants such as fruits and cocoa.[9]

The results obtained from this study are

gambir extract with the concentration of 2%, 4%, and 6% indicates a clear zone. At the concentration of 2% shown a clear zone but with the smallest diameter, this means that the gambier extract with a concentration of 2% already has inhibitory power but is not significant enough to be used in inhibiting the growth of S. aureus, compared to the concentration of 4% and 6% shown a clear zone with a larger diameter. Gambir extract with the concentration of 6% has the highest inhibition zone, so it can be said that the leaf concentration is the most effective in inhibiting the growth of Staphylococcus aureus, with an average diameter of the clear zone of 7.4 mm at 24 hours and 8.6 mm at 48 hours. (the biggest inhibition zone means that it has the highest antibacterial power). Among several concentrations that have been tested, it can be seen that the higher the concentration of gambir extract has the higher the antibacterial inhibition of the gambir extract.

It can be seen that the response to growth inhibition of gambir extract has a moderate resistance response to S. aureus. It can be seen that each concentration of gambir extract has an inhibition zone diameter ranging from 6.1-7.4 mm at 24 hours and 6.3-8.6 mm at 48 hours. The results of this research on 3% leaf extract of flavonoid gambir, produced an inhibition zone diameter of 8 mm of S. aureus which is classified as moderate category. In the ethanol extract of 12.5% curry leaves, the S. aureus inhibition zone diameter was 5.2 mm, and is classified as moderate. Different finding to the research on 2% gambir extract at 24 hours, the inhibition zone diameter of enterococcus faecalis was 17.83 mm, whereas at 48 hours the inhibition zone diameter was 18.13 mm, and both diameters were classified as strong. Ethanol extract of 5% gambier leaves forms an inhibition zone of 100-1000 ppm of Staphylococcus aureus, of which 0.001-0.01 mm is classified as weak.

This study proves that the extract of gambir (Uncaria gambir Roxb.) has antibacterial power to S. aureus with a Minimal Inhibitory Concentration (MIC) of 2% with an average diameter of the inhibition zone formed of 6.46 ± 0.27 mm at 24 hours. Whereas at 48 hours the diameter of the inhibition zone formed was 6.57 ± 0.26 mm and the largest inhibition zone was formed in the extract of gambir (Uncaria gambir Roxb.) with a concentration of 6% with an average diameter of 7.54 ± 0.62 mm. At this concentration, it was able to inhibit bacterial growth but was lower than the positive control. The concentration of gambir extract increased in diameter as well as the time was increased, while the positive control experienced a decrease in diameter. Thus the research hypothesis is not accepted.

Conclusion

Based on the results of the research that has been done, it can be concluded that the Minimum Inhibitory Concentration (MIC) is 2% with an average diameter of the inhibition zone formed of 6.46 mm at 24 hours while at 48 hours the diameter of the inhibition zone formed is 6.57 mm and the the biggest inhibition zone was formed by gambir extract (Uncaria gambir Roxb.) with the concentration of 6% has an average diameter of 7.54 mm. Gambir extract (Uncaria gambir Roxb.) has an inhibitory power to the growth of bacteria that cause root canal infections (Staphylococcus aureus). It is necessary to carry out further research of gambir extract (Uncaria gambir Roxb.) more carefully and using different methods and higher concentrations so that the inhibition zone is achieved at the standard values to Staphylococcus aureus and other bacterias.

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Conflict of Interest

The authors report no conflict of interest

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