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Inhibitory Concentration and Minimum Contact Time Gambir Extract (Uncaria gambier Roxb) Against Bacterial Growth Enterococcus faecalis

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Abstract

Enterococcus faecalis (E.Faecalis) gram-positive bacteria commonly found in endodontic retreatment cases. Extract Gambir, a type of dried sap from the leaves and young stems of plants gambier (Uncaria gambier Roxb) contains catechins, which are potent antibacterial and anti fungal with minimal side effects. This study aimed to determine the inhibitory concentrations and minimal contact time Gambir extract on the growth of the bacteria E. faecalis. Research applied a design with lab experiments, conducted on May 30-June 16, 2011 at the Laboratory of Pharmacognosy-Phytochemicals Faculty of Pharmacy and Laboratory of Microbiology, Faculty of Medicine, University of Hasanuddin. Materials and methods; 600 gr Gambir which has been crushed, extracted with reflux and rotary method. Minimal Inhibitory Concentration is determined with 5 ml of 5.25% NaOCl as a positive control and sterile distilled water negative control.

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Antibacterial activity is determined based on the time zone diameter hambat.Analisis contact and SPSS version 16.0 for windows with One-way ANOVA test and LSD on ($p < 0.05$). Results indicated that Statistical analysis showed a 1% concentration and contact time of 24 hours, effectively inhibits the growth of bacteria E.faecalis. in conclusion, Gambir extract effectively inhibits the growth of bacteria E. faecalis.

Keywords: Minimal Inhibitory Concentration; Time minimal contact; Extract Gambir; E. Faecalis.

1. Introduction

Enterococcus faecalis (E.Faecalis) is the largest and most frequent organism found in endodontics retreatment case, in addition to candida albicans, because its ability to adapt of the environment change [1]. In the field of dentistry, especially in the treatment of endodontics, which is discovery E.faecalis Species closely associated with the failure of root canal treatment, it is characterized by the presence of periapical lesions were found based on objective examination and radiological then called chronic apical periodondontis (CAP) [2,3]. Today the field of health research in general and in particular the field of dentistry is more focused on medicinal plants which are popular with the concept of "back to nature" as an effort to use herbal medicines with minimal side effects. In Indonesia there are many medicinal plants, one of it is gambier (Uncaria gambier Roxb). Gambier is dried sap from the leaves and young stems of plants gambier, containing catechin polyphenol compounds as the active component. Empirically, Gambier can relieve pain when applied to cavities that are accompanied by pain. The use of Gambier traditionally for thousands of years as a mixture of chewing piper beetle which is trust it can overcome thrush and relieve sore throats. In addition, as a cure for various kinds of diseases, for example, headaches, diarrhea, dysentery and burns. Gambier modern usage is as a raw material for medicine in pharmaceutical industry, astringent in the field of cosmetics and other industrial fields. Various studies indicate that extracts of gambier efficacious as anti-bacterial and anti fungal [4,15, 5,6,7]. Based on the above research it is conducted in order to determine the inhibitory concentration and the minimal contact time of extracts of gambier (Uncaria gambier Roxb) on the growth of the bacteria Enterococcus faecalis.

2. Materials and Methods

The design of this research was laboratory experimental study, conducted at the Laboratory of Pharmacognosy-Phytochemicals Faculty of Pharmacy and Laboratory of Microbiology, Faculty of Medicine, University of Hasanuddin Makassar on May 30th - June 16th, 2011. The independent variable is Extract of Gambier, the variable due to the growth of Enterococcus faecalis, and as a control variable concentrations of extracts of gambier and the contact time with the bacteria Enterococcus faecalis.

Tools: Analytic balance (Sartorius, Germany), Petri dish (Pyrex, Germany), Round bottom flask (Pyrex, Germanny), Pipette instruments (Iwaki Pyrex, Germany), a tool of reflux (IKA, Germany), Beaker glass (Pyrex, Germany), ordinary filter paper, a Bunsen burner (Pyrex, Germany), Buchner Funnel (Schott Duran, Germany), measuring cup (Pyrex, Germany), Rotary evaporator Tool (Buchner, Germany), spoon stirrer (Pyrex, Grmny), porcelain glass, tube couplers (Pyrex, Germany), autoclavng (AU-American), tube racks, Incubator (Ecocell, Germany), paper labels, Biological safety cabinets, Snowman markers, Paper disc 0.5 mm, stainless steel

Tweezers, Cotton swab, Calipers 0.05 (Shinwa, Japan).

Ingredients: Gambier (from Padang, West Sumatra), NaOCl 5.25%, Ethanol 96%, Bacterial isolates of E.faecalis, Medium Brain Heart Infusion Broth, sterile distilled water, Medium Mueller-Hinton Agar, methylated, DMSO 10%. The study was conducted in three stages. The first, extract of Gambier made with reflux and rotary method. Gambier broken into small pieces and then weighed as much as 600 grams, then put into a sterile round-bottom flask which had been filled 800ml of ethanol. Round-bottom flask which is containing gambier and ethanol is heated to reflux tool for ± 2 hours at 70 ° C. Gambier and ethanol that has been filtered and then dirotavapor refluxing at 60 ° C for ± 1 hour to obtain a thick extract, and then allowed to stand for 2x24 hours on the bath until completely condensed. After that, it was made gambier extracts with varying concentrations. Gambir extract weighed using an analytical balance respectively of 0.07 g, 0.105 g, 0.14 g, 0.175 g, 0.21 g and 0.245 gr obtained from dilution formula. Concentration = mass / volume. Gambir extract which has been weighed is then diluted with 7 ml of 10% DMSO solution to obtain a concentration of 1%, 1.5%, 2%, 2.5%, 3% and 3.5%. After that, it put in a tube couplers and labeled in accordance concentration. The second stage is the determination of minimum inhibitory concentration (MIC) against the growth of bacteria Enterococcus faecalis. Prepared eight tube couplers, each of it is filled with medium BHIB much as 5 mL. In each tube then added bacteria isolate of E.Faecalis much as 0.2 mL. After that, the first six of each tube is added 5 mL Gambier extract 1%, 1.5%, 2%, 2.5%, 3% and 3.5%, tubes 7th and 8th respectively add 5 mL (NaOCl 5.25%) as a positive control and 5 mL of sterile distilled water as a negative control. All the tubes were incubated at 37°C for 24 hours and 48 hours, then it was observed the presence or absence of bacterial growth of E. faecalis which is characterized by the occurrence of turbidity in the tube. Minimal Inhibitory Concentration is determined by observing the concentration of what first looks clear. Solution in a tube that looks cloudy indicate the bacterial growth. When MIC is known, then the third stage is a test of resistibility or test the inhibition of antibacterial activity against E.Faecalis Gambir extract by contact time. The test material is the same as Gambier extract concentration on the determination of the MIC. Three petri dishes that are sterilized in an autoclave filled with medium Mueller-Hinton Agar. Cotton swab dipped in a bacterial culture tube and is pressed into the tube wall to prevent water leak. Cotton swab then described evenly across the surface of a petri dish containing medium. Eight pieces of paper dish dipped, in each sample is then placed and pressed probe in order to really stick to the surface of the medium. Six for each sample of extract gambier concentration, one for a negative control (sterile distilled water), and another for the positive control (5.25% NaOCl). , Petri dish and then incubated for 3x24 hours at a temperature of 370C. Measurement of the inhibition zones conducted with calipers after 24 hours, 48 hours and 72 hours. This procedure is performed three times (three times replication). Data were analyzed using SPSS version 16.0 statistical analysis for windows with One-way ANOVA test and LSD test on ($p < 0.05$).

3. Results

3.1. Minimal Inhibitory Concentration (MIC) of Gambier Extract against Bacteria Growth of Enterococcus faecalis

Minimal Inhibitory Concentration (MIC) of the extract Gambier on bacterial growth of E. faecalis is 1%, seen

on the tube couplers which is containing 1% Gambier extract seems more clearly than the tube couplers which contain the negative control, but slightly more turbid than the positive control (Table 1):

Table 1: The level of turbidity bacteria Enterococcus faecalis on BHIB medium after being given the extract of Gambier for 24 hours and 48 hours

The level of turbidity in	Sample							
	1%	1,5%	2%	2,5%	3%	3,5%	Control (+) (NaOCl 5.25%)	Control (-) (Steril distilled water)
24 Hours	2	2	2	2	2	2	1	3
48 Hours	1	1	1	1	1	1	1	3

Explanation :[25] : 1 = Clear; 2 = Slightly turbid ; 3 = Turbid

After the next 48 hours (Table 1), it seems that all Gambier extract concentration on the tube couplers become clear as tubes containing NaOCl 5.25% (positive control) while the tube is containing sterile distilled water (negative control) is remain turbid.

3. 2. Extract Gambier Antibacterial Activity Test Against Bacteria Growth Enterococcus faecalis

After the observation of the samples were incubated for 24 hours, 48 hours and 72 hours, the obtained test results gambier extracts for antibacterial activity against the growth of bacteria Enterococcus faecalis. The average and standard deviation of broad zones of inhibition of each extract Gambir, positive control and negative control after incubation can be seen in Table 2:

Table 2: The average diameter of the inhibition zones of each extract Gambir against bacteria Enterococcus faecalis after incubation for 24 hours, 48 hours and 72 hours

Sample	Replication	The average diameter of the inhibition zones after incubation (mm) ± standard of deviation		
		24 Hours	48 Hours	72 Hours
1%	3	21,39 ± 2,20	23,50 ± 0,76	25,49 ± 3,53
1,5%	3	21,06 ± 3,43	24,50 ± 3,29	22,04 ± 5,75
2%	3	17,83 ± 2,25	18,13 ± 1,81	18,29 ± 2,09
2,5%	3	13,94 ± 4,50	15,28 ± 0,78	17,22 ± 2,02
3%	3	12,94 ± 2,00	13,61 ± 1,00	14,87 ± 1,76
3,5%	3	16,89 ± 3,33	17,61 ± 1,78	20,09 ± 5,31
Positive Control	3	7,83 ± 1,74	14,72 ± 5,45	16,11 ± 5,48
Negative control	3	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00

At table 2, it appears that Gambier extract with a concentration of 1% is the best to effectively inhibit the growth of bacteria E. faecalis compared with a concentration of 1.5%, 2%, 2.5%, 3%, 3.5% and a positive control. Gambier extract at a concentration of 3% and the contact time of 24 hours, showing a zone of inhibition smallest of 12.94 mm (see the chart below).

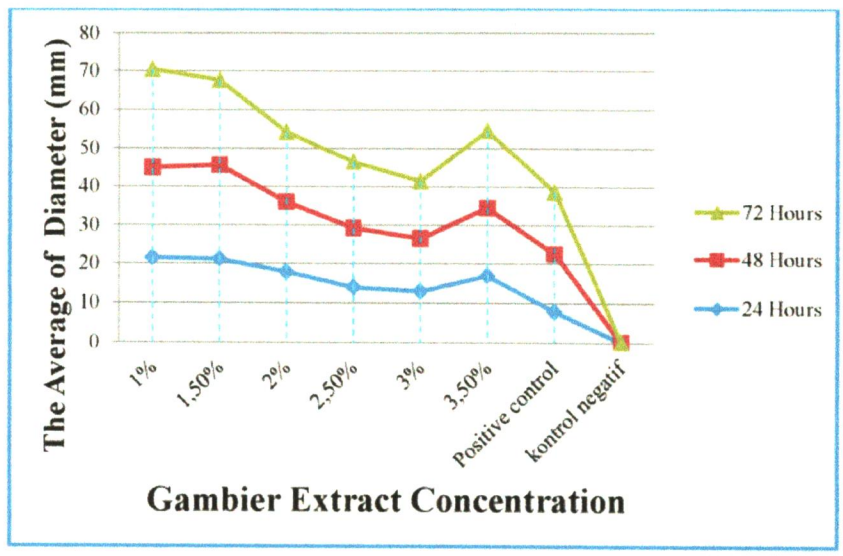

Figure 1: The average diameter of the inhibition zones of each extract Gambir against bacteria Enterococcus faecalis after 24 hours of incubation, 48 hours and 72 hours.

In the graph, it can be seen that the inhibition zones formed after the application of Gambier extract is greater than the inhibition zones formed by the positive control and a negative control.

To determine whether there is a significant difference between the concentration of extract of Gambier with negative control and a positive control after incubation of 24 hours, 48 hours and 72 hours, then do one way Anova. Statistical analysis by one-way ANOVA test showed that after incubation of 24 hours, 48 hours and 72 hours there is a significant difference between the concentration of the extract Gambir digunakam with negative control and a positive control in inhibiting the growth of bacteria E. faecalis. ANOVA test results showed a significant difference, because it followed primarily to test the Least Significant Difference (LSD) to know there are no significant differences either between Gambir extract concentrations or with a negative control and a positive control.

From the results of LSD test with a contact time of 24 hours, there is a significant difference ($p < 0.05$) between concentrations of extracts of gambier 1%, 1.5%, 2% and 3.5% as well as the negative control and a positive control, were at a concentration of 2.5% ($p = 0.416$) and 3% ($p = 1.00$) there is no significant difference. At 48 hours of contact time a significant difference ($p < 0.05$) only looks at a concentration of 1% ($p = 0.01$), 2.5% ($p = 0.025$) and 3% ($p = 0.049$). At the contact time of 72 hours there was no significant difference ($p < 0.05$) between all Gambir extract concentration, as well as the positive control and a negative control.

4. Discussion

The purpose of this study was to determine the inhibitory concentration and the minimal contact time of extracts of gambier (Uncaria gambier Roxb) on the growth of the bacteria E. faecalis. As was mentioned earlier, that E. faecalis has been the cause of the disease post endodontic treatment. Up to 90% of enterococcal infections in humans are caused by Enterococcus faecalis [8,9].

At the concentration of 1.5%, 2%, 2.5%, 3% and 3.5% inhibition zones that formed smaller than the concentration of 1%, which means a concentration of 1% was the most effective concentration inhibiting the growth of bacteria Enterococcus faecalis. In line with research on Gambier extract inhibitory to bacteria aureus Stapylococcus conducted by IW Merta et al found that there is effect of different concentrations of the extract Gambier against bacterial growth inhibition Stapylococcus aureus [10].

On the positive controls were also seen their zone of inhibition but not measuring inhibition zones on gambier extracts, it does showing that the extract Gambir more effectively inhibit the growth of bacteria Enterococcus faecalis compared to the positive control (NaOCl 5.25%), while the negative control (sterile distilled water) does not visible zone of inhibition as sterile distilled water is neutral, it does not contain material that is antibacterial (Table 1). In this study, the minimum inhibitory concentration Gambier extract that effectively inhibits the growth of bacteria E. faecalis in the concentration of 1% and a contact time of 24 hours. These results are consistent with the results of research conducted by Yulia Sari Risnawati, that increased contact time will increase the chemical reaction between the antiseptic to bacteria so that more bacteria are inhibited. Activities other than an antiseptic is influenced by environmental factors such as temperature, pH and the presence of materials - organic material, its ability to inhibit the growth of bacteria is also influenced by the concentration and time contact [11].

Previous studies that examined the antibacterial effects of extracts of gambier. In this study, the positive control used is a solution of sodium hypochlorite (NaOCl), that is known as a strong antibacterial activity, can kill bacteria very quickly even in low concentrations [12].

The test results of gambier extracts for antibacterial activity against the growth of bacteria Enterococcus faecalis performed using agar diffusion method, after an incubation period of 24 hours shows that at Gambier extract with a concentration of 1% seen their zones of inhibition of 21.39 mm. The same condition also occurs after an incubation period of 48 hours with a zone of inhibition of 23.50 mm, more increasing in the incubation period of 72 hours. It shows that the inhibition zones formed by the more comprehensive gambier extracts is 25.49 mm. These results indicate that the extract of Gambier very effective in inhibiting the growth of bacteria Enterococcus faecalis (Tabel2).

Gambier extract antioxidant known as antimicrobial, it is linked to the phenolic content in the form of catechins. These catechins are flavonoids that can be found in green tea, black tea, Gambier, wine and other food crops such as fruits and cocoa. As a medicinal plant, Gambier often used for medicinal mixtures, such as burns, headaches, diarrhea, dysentery, mouthwash, mouth sores, skin pain, facilitate the digestive process in the

stomach and intestines, and can be one of the formulas in the manufacture of lozenges according to Lutfi research Chabib and his colleagues [13,14].

Until now, there has been not found to report the results of research on the effectiveness gambier extracts as an alternative material root canal irrigation solution or medikame of root canal that could inhibit the growth of bacteria Enterococcus faecalis in the different of concentration and contact time. This is the proof of the novelty of this study.

5. Conclusions and Suggestions

5.1 Conclusion

Based on the formulation of the problem, hypothesis, and the results of this study, it concluded that:

1. The Extract of gambier (Uncaria gambier Roxb.) is effectively inhibits the growth of bacteria Enterococcus faecalis.

2. On a 1% concentration and contact time of 24 hours, extracts of gambier (Uncaria gambier Roxb) most effectively inhibit the growth of bacteria Enterococcus faecalis.

5.2 Suggestion

To progress of the further research, it is recommended:

1. The research Should carried out using bacteria Enterococcus faecalis were directly isolated from the root canal of the patient.

2. It should be done the further research on the other species that also persistent in root canals such as species of Candida albicans.

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