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RESEARCH ARTICLE

Antibacterial Effect of Semendo Coffee Beans (Coffea Canephora) Extract Against Streptococcus Sanguinis In Vitro Growth

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ABSTRACT

Background: *Streptococcus sanguinis is one of the most dominant bacteria in early colonization of plaque formation. Robusta coffee beans (Coffea canephora) was reported to have antibacterial properties because it contained compounds such as alkaloids, flavonoids, saponins, tannins, and steroids.* ***Purpose:*** *This study aimed to determine the antibacterial effect of Semendo coffee beans extract to inhibit the bacterial growth of S. sanguinis.* ***Material and method:*** *This study was an in vitro experimental laboratory. This study used Robusta coffee beans origins from South Sumatera called Semendo coffee beans. The treatment group used Semendo coffee beans extract with concentrations of 2.5%, 5%, 10%, 20%, and 40%. The positive control used 0,2% chlorhexidine gluconate and the negative control used sterile distilled water. The antibacterial potency test was used dilution method to MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) test, and disk diffusion method to inhibitory zone test.* ***Result:*** *The result showed that the MIC of Semendo coffee beans against S. sanguinis couldn't be determined because it was blocked by the color of the extract while MBC was 5%. The average diameter of the inhibition zone formed on 2.5% Semendo coffee beans extract was 12,0±0,4082 mm and bigger along with higher concentration. These results suggested that the extract of Semendo coffee had an antibacterial effect against the growth of S. sanguinis on 2,5%-40%.*

Keywords: *Semendo coffee beans, Streptococcus sanguinis.*

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INTRODUCTION

Dental plaque is a complex biofilm formed on the tooth surface covered with saliva.[1] Plaque is a local cause of disease in the oral cavity due to the activity of microorganisms contained in plaque.[2] This plaque buildup can cause various diseases, such as caries and periodontal disease.[3] Based on the 2018 Basic Health Research (*RISKESDAS*), the prevalence of dental and oral problems related to caries and periodontal disease in Indonesia reached 57.6%.[4] Plaque formation can depend on the adhesion of bacteria to the salivary components that are absorbed on tooth surface.[1] The process is dominated by *Streptococci* bacteria, and among these bacteria, one of the bacteria that can initiate plaque formation is *Streptococcus sanguinis*.[5]

Streptococcus sanguinis bacteria play a role in early colonization of dental plaque formation. These bacteria have virulence factors in the form of PilA, PilB, PilC, and SsaB (saliva-binding protein) which mediate the attachment of *S. sanguinis* to tooth pellicle by forming protein bonding with the α-amylase saliva.[6] In addition, *S. sanguinis* provides a space for the attachment of other bacteria such as *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*, resulting in the maturation of dental plaque.[6,7]

Plaque can be removed by performing periodontal treatments, such as mechanical therapy, followed by antimicrobial therapy, such as antibiotics prescription.[8] However, antibiotics are often used not in relevance with the rules of their usage, causing bacteria becoming resistant to antibiotics.[9] In addition, mouthwash also contains chemical compounds which often bring side effects. Therefore, to overcome the side effects of these chemicals, compounds derived from natural ingredients such as plants are developed which can be used as medicine. Natural ingredients are generally considered safer compared to chemical ones because they rarely have side effects.[10]

One of the natural ingredients that can be used as an alternative to periodontal disease treatment is coffee.[11] Robusta coffee (*Coffea canephora*), a kind of coffee, can be used for this treatment because this coffee contains several compounds including alkaloids, flavonoids, saponins, tannins, and steroids which are thought playing a role in inhibiting the growth of plaque bacterial colonies.[12] Regarding the role of inhibiting bacteria, one research was conducted comparing the antibacterial properties between Robusta coffee and Arabica coffee against *Lactobacillus acidophilus*. The research showed that Robusta coffee has better bacteriostatic activity in 25% concentration, presumably because there is a big difference in the concentration of active compounds contained in Robusta coffee compared to Arabica coffee.[13] In this study, no testing was carried out at a concentration below 2.5%, because the concentration followed the previous research result.

Indonesia, being based on geographic location, has various types of coffee, one of which is Robusta Semendo originating from South Sumatra. The data for 2014 in South Sumatra, especially Muara Enim Regency, showed that Semendo coffee production reached 136,000 tons per year with a plantation area of 256,000 hectares throughout South Sumatra.[14,15] Semendo coffee is a typical coffee product that is widely consumed by the community and easy to find.[16] However, based on the results of literature reviews, there has never been any research regarding the antibacterial activity of this coffee against plaque bacteria such as *Streptococcus sanguinis.*

Based on the above explanation, the researcher is interested in researching to determine the effectiveness of Semendo coffee bean extract, which is part of the Robusta coffee, against the growth of *Streptococcus sanguinis* bacteria.

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MATERIALS AND METHODS

This research is a laboratory experimental study with a posttest control group design. The research was conducted at the Pharmacy Laboratory of Mathematics and Natural Sciences Faculty, Sriwijaya University; the Microbiology Laboratory of Medicine Faculty and the Pharmacy Biology Laboratory of Pharmacy Faculty, North Sumatra University (*USU*). The research sample was *Streptococcus sanguinis* obtained from Microbiology Laboratory of Medicine Faculty of USU with seven treatment groups; extract concentrations of 2.5%, 5%, 10%, 20%, 40%, chlorhexidine gluconate 0.2% as positive control, and sterile distilled water as negative control with 4 replications of the test.

The inclusion criteria in this research were coffee beans taken from the *Semendo Darat Laut*, Muara Enim, beans from the Robusta type with red fruit and green bean (green coffee). The exclusion criteria were beans from rotten fruit and roasted coffee beans.

The test of Semendo coffee bean extract against *Streptococcus sanguinis* was carried out using the dilution method to obtain the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, as well as the disc diffusion method to obtain the inhibition zone value. The coffee bean extract was obtained through maceration method using 96% solvent Etanol for 24 hours. The preparation of Semendo coffee bean extract was carried out at the Pharmacy Laboratory of Mathematics and Natural Sciences Faculty, Sriwijaya University by immersing the simplicia of Semendo coffee beans that have been made for 24 hours, occasionally stirred, filtered and evaporated with a rotary evaporator until a thick 100% extract is obtained. Then, the 100% Semendo coffee bean extract was tested with phytochemical test at the Pharmacy Biology Laboratory of Pharmacy Faculty at USU to determine the antibacterial compounds contained in the extract.

The 100% coffee bean extract was first diluted to several concentrations; 2.5%, 5%, 10%, 20%, and 40% using sterile distilled water. After the dilution was carried out, it was continued to the test method to obtain the MIC value by filling the test tube with nutrient broth media and adding a *Streptococcus sanguinis* suspension. Then, all the treatment groups were dropped on each test tube that had been marked and covered with aluminum foil. The test tube was incubated for 24 hours and the turbidity was observed. The MIC value was determined based on the tube with the lowest concentration and no turbidity or clearness was formed when compared to the positive control which showed a bacteriostatic effect. After the MIC test, the MBC test was carried using all treatment groups in the MIC test tube for the subculture test.

The subcultures were carried out by taking bacterial cultures in the MIC test tube using a sterile loop needle which then implanted on the MHA media using the streak technique. Then, the petri dishes were incubated in an incubator (Thermo Scientific IGS 180, USA) at 37ºC for 24 hours. The value of MBC is determined based on the calculation of bacterial colonies growth on petri dishes using conventional method.

The inhibition test was carried out by the disc diffusion method. It was conducted on a petri dish that had been swabbed with *S. sanguinis* bacterial suspension using a sterile cotton swab. Then, the paper discs were placed on each marked petri dish and each was dripped with the seven treatment groups. The inhibition zone can be seen from the presence or absence of the clear zone formed around the disc paper which then measured using a caliper.

The results obtained in the inhibitory test were then analyzed using SPSS (IBM SPSS Statistic version 22, USA). The data analysis begins with a normality test using the Shapiro-Wilk test and homogeneity test using Lavene's test. If the two tests show normal and homogeneous data ($p < 0.05$), the analysis continues to the parametric statistical test with One-Way ANOVA to determine the differences

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between groups, proceeded with the Tukey HSD test to determine the magnitude of the differences between groups.

RESULTS

The phytochemical tests on 100% Semendo coffee bean extract showed that the extract contained alkaloids, flavonoids, saponins, tannins, and steroids. Meanwhile, the results of MIC and MBC tests on the extract against *Streptococcus sanguinis* can be seen in Table 1 and Table 2.

Table 1. The turbidity of *Streptococcus sanguinis* on MIC test.

No	Treatment	Result			
		Repetition I	Repetition II	Repetition III	Repetition IV
1.	2,5%	Turbid	Turbid	Turbid	Turbid
2.	5%	Turbid	Turbid	Turbid	Turbid
3.	10%	Turbid	Turbid	Turbid	Turbid
4.	20%	Turbid	Turbid	Turbid	Turbid
5.	40%	Turbid	Turbid	Turbid	Turbid
6.	Positive control	Clear	Clear	Clear	Clear
7.	Negative control	Turbid	Turbid	Turbid	Turbid

Table 1 shows that the test tubes that experienced turbidity were found in all concentration groups. The clarity and turbidity in this MIC test could not be distinguished due to the influence of coffee bean extract color. Therefore, the MIC value could not be determined. On the other hand, the results of the MBC test are shown in Table 2.

The MBC value was determined based on the calculation of bacterial colonies growth on petri dishes using conventional method (Table 2). It shows that the petri dishes with the lowest concentration and no bacterial growth was found at a concentration of 5%.

Tabel 2. Growth of *Streptococcus sanguinis* on MBC test.

No	Treatment	Total of bacterial colonies (CFU/ml)				Average
		RepetitionI	Repetition II	Repetition III	Repetition IV	
1.	2,5%	97	102	83	116	99,5
2.	5%	0	0	0	0	0
3.	10%	0	0	0	0	0
4.	20%	0	0	0	0	0
5.	40%	0	0	0	0	0
6.	Positive control	0	0	0	0	0
7.	Negative control	>300	>300	>300	>300	>300

The antibacterial test procedure was continued to the inhibition zone test using the disc diffusion method. The results of the inhibition test are shown in Table 3. Table 3 shows that all Semendo coffee bean extracts tested had the ability to inhibit the growth of *Streptococcus sanguinis* bacteria with the largest inhibition zone diameter was found at a concentration of 40% with an average diameter of 26.9 mm.

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Table 3. Inhibition zone diameter of Semendo coffee extract against *S. sanguinis*

Repetition	Inhibition Zone Diameter (mm)						
	Negative control (-) sterile distilled water	Positive control (+) CHX 0,2%	Semendo coffee bean extract 2,5%	Semendo coffee bean extract 5%	Semendo coffee bean extract 10%	Semendo coffee bean extract 20%	Semendo coffee bean extract 40%
1	0	36,0	12,0	15,5	19,5	24,0	27,0
2	0	35,5	11,5	14,5	19,0	23,0	26,5
3	0	36,0	12,5	14,0	19,6	23,5	27,0
4	0	35,0	12,0	14,5	19,5	24,0	27,1
Total	0	142,5	48,0	58,5	77,6	94,5	107,6

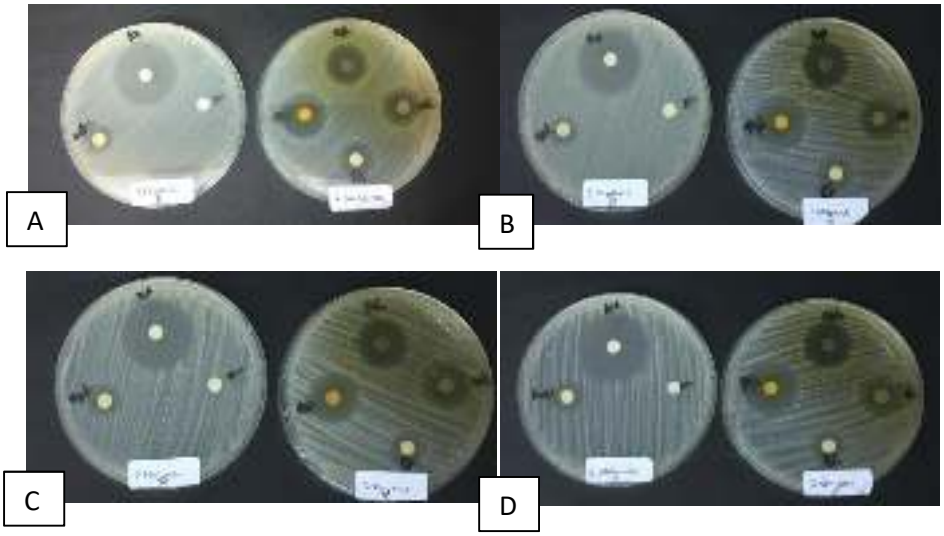
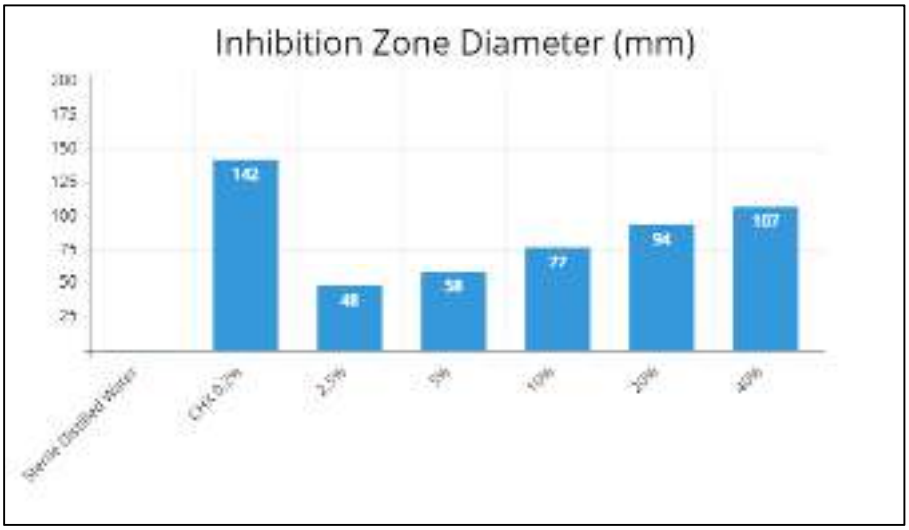


Figure 1. Antibacterial inhibition of the sample group against *S. sanguinis* bacteria
A. 1 repetition, B. 2 repetition, C. 3 repetition, D. 4 repetition
B.



Graphic 1. Inhibiton zone diameter (mm)

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Table 4. Tukey's Post-Hoc analysis test results

Group	Semendo coffee been extract 2,5%	Semendo coffee been extract 5%	Semendo coffe been extract 10%	Semendo coffee been extract 20%	Semendo coffee been extract 40%	CHX 0,2%	Sterile distilled water
Semendo coffee been extract 2,5%		0,000	0,000	0,000	0,000	0,000	0,000
Semendo coffee been extract 5%			0,000	0,000	0,000	0,000	0,000
Semendo coffee been extract 10%				0,000	0,000	0,000	0,000
Semendo coffee been extract 20%					0,000	0,000	0,000
Semendo coffee been extract 40%						0,000	0,000
CHX 0,2%							0,000
Steril distilled water							

The inhibition zone test data analysis was then performed using SPSS. The normality and homogeneity test showed that the data were normally distributed and homogeneous with a significance value of more than 0.05, respectively. Then, the data analysis was continued to the One-Way ANOVA test which showed the results among the treatment groups of Semendo coffee bean extract had a value of p = 0.000. Because the p value <0.05, it means that the values between the treatment groups of Semendo coffee bean extract had significant difference. The analysis was continued to Tukey HSD Post-hoc analysis and it was shown that the results of all treatment groups had a value of p = 0.000. Because the p value <0.05, it can be concluded that the overall test group had significant difference (Table 4).

DISCUSSION

The MIC test results showed that the turbidity and clarity of the test tube could not be determined because the color of the extracts was concentrated, making it difficult to determined the MIC value. This is also in line with a research revealing that the MIC value could not be determined.[17] From the results of the MBC test, it can be concluded that Semendo coffee bean extract (*Coffea canephora*) has the activity of killing *Streptococcus sanguinis* bacteria. It is as shown in Table 2 where the petri dishes in the MBC test had no bacterial colony growth starting from a concentration of 5%. A research showed that Robusta coffee bean extract (*Coffea canephora*) can kill bacterial colony growth starting from a concentration of 3.125%.[8]

The diffusion test showed that the inhibitory response began to appear at a concentration of 2.5% which was indicated by the formation of the inhibition zone diameter on the media. Thus, the diameter had an average of 12.0 mm and it got bigger as the extract concentration value increased. A research concluded that the greater the concentration of the coffee bean extract, the greater the inhibition zone formed.[18] The difference appearing in the inhibition zone is due to differences in the amount of active substances contained in each concentration. The greater a concentration, the greater the components of the active substance

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contained in it, so the inhibition zone formed is also different for each concentration.[19]

The antimicrobial activity against *Streptococcus sanguinis* bacteria can be explained due to the presence of antibacterial compounds as evidenced by the results of phytochemical tests. Based on the results of phytochemical tests, Semendo coffee bean extract *(Coffea canephora)* contains alkaloids, flavonoids, saponins, tannins, and steroids.[12] In addition, it was shown that these compounds were proven to be able to inhibit the growth of the *Streptococcus sanguinis* bacteria.[20]

Some of alkaloid compounds found in coffee are caffeine and trigonelin.[18] Caffeine is an alkaloid compound whose antibacterial ability is due to the presence of alkaline groups containing nitrogen. The presence of this group, when in contact with bacteria, will trigger reaction with amino acid compounds to change their structure and arrangement. This change will affect the balance of genetic and bacterial DNA, causing damage and lysis.[18,21] Meanwhile, trigonelin, which is a pyridine alkaloid, works as an antibacterial by disrupting the stability of the bacterial cytoplasmic membrane which results in an imbalance in the metabolic function of these bacteria, subsequently resulting in inhibition of bacterial growth.[8,22]

The antibacterial activity of flavonoids is carried out by damaging the bacterial cell membrane through differences in polarity between the lipids that make up DNA and the alcohol groups in flavonoid compounds, resulting on damages of which these compounds can enter the nucleus of bacterial cells.[18] Tannins have an antibacterial mechanism by working on polypeptides of the bacterial cell membrane, triggering a chain of process that end up on lysis.[23] Another phenol compound that may also be contained in Semendo coffee beans (*Coffea canephora*) is chlorogenic acid, as mentioned in a research which identified the presence of chlorogenic acid in Robusta coffee beans. Chlorogenic acid acts as an antimicrobial by increasing the permeability of the outer membrane and plasma membrane significantly which causes decreased defense function and leakage from the cell nucleus.[8] However, in this research, the content of chlorogenic acid in Semendo coffee beans was not tested and analyzed.

Another content identified in coffee is saponins. Saponins act as antibacterial by hydrolyzing the cell walls. Damage to the cell wall can cause loss of its semi-permeability property so that it cannot select the entering and exiting substances, such as water and enzymes.[20] While steroids work to inhibit bacterial growth by damaging the plasma membrane of bacterial cells, causing the cytoplasm to leak and leave the cell, which in turn causes cell death.[23]

The average diameter of the inhibition zone in the chlorhexidine gluconate control group was 0.2% higher than the Semendo coffee bean extract group, which was 35.6 mm with a statistically significant difference. Chlorhexidine gluconate 0.2% is a gold standard mouthwash that has antibacterial properties that can precipitate cytoplasmic acid proteins, resulting in changes in cell wall permeability and leakage of bacterial cells.[24] Therefore, from the results of this research, it can be seen that although Semendo's coffee bean extract (*Coffea canephora*) has antibacterial properties, its ability has not yet match the 0.2% chlorhexidine gluconate antibacterial power. The limitation of this study is that it cannot determine the MIC value because it is blocked by the dark color of the extract. For further research, MIC testing can be carried out using a lower concentration.

It can be concluded that the Semendo coffee bean extract (*Coffea canephora*) has antibacterial effect to ~~kill~~ *Streptococcus sanguinis* with a MBC value of 5% and an average diameter of the inhibition zone at 12 mm, starting from a concentration of 2.5%.

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