

Original Research Article

Effectiveness of Cocoa Seed Garmins to the Decrease of Dental Plaque Index

Ainun Mardiah^{1*}, Cut Aja Nuraskin¹, Teuku Salfiyadi¹, Nora Ushrina¹, Reca¹¹Poltekkes Ministry of Health Aceh Jl. Soekarno Hatta, Tingkeum, Darul Imarah, Lheu Blang, Banda Aceh, Aceh Besar District, Aceh, 23231, Indonesia

Article History

Received: 19.08.2022

Accepted: 21.09.2022

Published: 27.09.2022

Journal homepage:

<https://www.easpublisher.com>

Quick Response Code



Abstract: Dental and oral disease is a high prevalence in Indonesia 88.8% of the population has dental caries and periodontitis, 93% of children under 12 years of age have dental caries, 67.2% have experienced caries and 43.4% have not been treated. Dental plaque is a soft deposit that is firmly attached to the tooth surface, consisting of microorganisms that cause dental caries. Caries prevention and plaque control can be done by using mouth wash; it will be more effective if you rinse your mouth using herbal mouthwash that contains antioxidants and anti-bacterial. Cocoa Bean Extract (Theobroma Cacao L.) contains secondary metabolites, alkaloids, flavonoids, tannins, steroids and has anti-bacterial, anti-inflammatory properties and has a strong inhibitory effect on *S. mutans* bacteria. The purpose of the study: to determine the phytochemical content of cocoa beans, determine the effectiveness of the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (KBM) of mouthwash of cocoa bean methanol extract, determine the toxicity of mouthwash of cocoa bean methanol extract, determine the decrease in plaque index before and after gargling with methanol extract of cocoa beans (Theobroma Cacao L.), determine the effectiveness of the pH of the oral cavity before and after gargling methanol extract of cocoa beans (Theobroma Cacao L.). The research method is a laboratory experiment with a post-test only control group design. The research was conducted in the laboratory of the USK Faculty of Veterinary Medicine, and the USK FMIPA laboratory in 2022. Dry samples of 1 kg cocoa beans were macerated with 80% methanol for 3x24 hours; the filtrate was evaporated using a rotary evaporator to obtain a thick methanolic extract of cocoa beans. Phytochemical test results of methanol extract of cocoa beans contain compounds, phenolics, tannins, flavonoids, steroids, terpenoids, saponins, alkaloids. Minimum Inhibitory Concentration Test The average number of colonies of *S. mutans* after the test showed the highest colony growth in the negative control group (590 X10⁻⁴CFU/mL) and the least was in the positive control concentration (0 X10⁻⁴ CFU/mL). The results of the normality test showed that the distribution and homogeneity of the variance of the research data were normal with a value (P-value = 0.000) or spread normally, so it was continued with the ANOVA test. From the Anova table in the Sig column, P value = 0.000 is obtained. Thus at the level of significance = 0.05 we reject H₀. In conclusion, concentration affects the number of colonies that grow. Or there is a significant difference in the average number of colonies that grow based on each concentration. The minimum inhibitory concentration (MIC) of mouthwash of cocoa bean methanol extract against the growth of *Streptococcus mutans* was aimed at a concentration of 1% and no minimum killing concentration (MBC) was found in this study. Organoleptic test of mouthwash, duration of storage and variation of mouthwash concentration did not affect the quality of mouthwash. Acute toxicity test of methanol extract of cocoa beans in mice was not toxic. Conclusion Cocoa bean methanol extract contains secondary metabolic compounds and has MIC of cocoa bean extract against mutant streptococcus bacteria at a concentration of 1% and has no MBC and is not toxic. Suggestion, it is necessary to do further in vitro research by increasing the concentration of cocoa bean extract to obtain the Minimum Kill concentration (KBM) on the growth of *Streptococcus mutans*.

Keywords: Extract, seeds, cocoa, drug, gargle.

Copyright © 2022 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution **4.0 International License (CC BY-NC 4.0)** which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

INTRODUCTION

Dental and oral health is a very important thing that must be considered. The main cause of dental

disease is dental plaque which causes dental caries and inflammation of the periodontium. Dental plaque is a soft deposit firmly attached to the tooth surface,

consisting of microorganisms. Dental plaque is a major factor in the occurrence of dental caries. The result of this disease is not only tooth loss, but can spread to other organs [1].

Dental caries and periodontal disease are dental diseases with a high prevalence in Indonesia, with 88.8% of the population experiencing dental caries. According to the Indonesian Ministry of Health, 93% of children under 12 years have dental caries, 67.2% have experienced caries and 43.4% have not been treated [2].

Plaque control can be done mechanically or chemically. Chemically by using mouthwash some mouthwashes have antiseptic or antibacterial properties that work to inhibit plaque formation and gingivitis. The use of antiseptics in mouthwash is thought to have a carcinogenic effect on its users. Therefore, it will be more effective if added plaque control by gargling using herbal mouthwash containing antioxidants [3, 4].

Cocoa beans play an important role in health, one of which is as an antibacterial. Cocoa beans contain flavonoid compounds, tannins, and alkaloids that have anti-microbial effects. Based on research that has been done cocoa bean extract is effective in inhibiting streptococcus bacteria [5, 6]. Based on previous research, gargling with cocoa beans has an effect on dental plaque index, the higher the concentration given, the higher the decrease in dental plaque index [7]. Based on previous research, cocoa bean extract is not toxic, it can even reduce cell histology damage in mice [8].

Based on the description above, this researcher deserves to be a superior basic research Karna College produced a new theory and formulation of a new mouthwash with herbal ingredients from the cocoa plant that had never existed before and could be used for scientific development.

Research Problems

Based on the above background, the problem is "what is the concentration of the effectiveness of mouthwash (Mouthwash) of cocoa bean extract on reducing plaque index in the oral cavity in students of SDN Kayee Leu Aceh Besar.

Research Purposes

1. Special Purpose

1. Testing the phytochemical content of cocoa bean methanol extract.
2. Measuring the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MBC) of mouthwash of cocoa bean extract against the inhibition of *S. mutans*.
3. Quality test of cocoa bean methanol extract mouthwash.

4. Toxicity test of methanol extract of cocoa beans on mice.
5. Testing the plaque index before and after rinsing the mouthwash of cocoa bean extract methanol.

2. The Urgency of Research Excellence

This research is important to do considering the high number of dental caries in the community. Various promotional and preventive efforts have been made to reduce dental plaque as the cause of dental caries, but the dental caries rate is still high. Many people still do not know the benefits of cocoa beans for health, especially dental and oral health. Cocoa beans have several active components, one of which is polyphenols which can reduce the plaque index of the main bacterial biofilm formation that causes dental caries. Aceh is known as the third largest cocoa producer in the world. This researcher deserves to be a superior basic research university because it produces a new theory and a new mouthwash formulation.

METHODOLOGY

This type of research is experimental laboratory with post-test only control group. The research was carried out at the USK FMIPA Laboratory for phytochemical processes, extraction by maceration and rotary evaporator at the USK Agricultural Laboratory, Toxicity testing and MIC and KBM tests in the USK FKH laboratory. Plaque index test on students of SDN Kayee Leu Aceh Besar. Research time March to November 2022. Research sample Cocoa beans from the village of Sare Aceh Besar. Streptococcus mutas bacteria from the USK FKH Microbiology laboratory. Great for students aged 8-10 years who have index teeth with good dental conditions. Research tools and materials, autoclave, fairy cup, ose needle, measuring cup, rotary evaporator, spirit lamp, tube rack, filter paper, analytical balance, vortex, tweezers, cotton, elemeyer tube. Ingredients used, cocoa bean extract,

RESEARCH PROCEDURE

1. Sample collection of dry cocoa beans 1 kg

Extraction by maceration of cocoa beans 3x24 hours with 80% methanol solvent, the filtrate in the rotary evaporator obtained thick extract of cocoa beans methanol.

2. Phytochemical Test

Alkaloid Compound Test, 1g dry sample was crushed, added 1ml ammonia, crushed, filtered, added 10ml 0.5n hydrochloric acid, shaken vigorously then divided into three tubes. The addition of Mayer's reagent causes a white precipitate, Reagedragendorff causes a reddish precipitate, and Wagner's reagent causes a brown precipitate, indicating a positive presence of alkaloid compounds. Test for steroids, terpenoids and saponins, dry sample of 10g cocoa beans added with distilled water, shaken vigorously, the presence of foam indicated saponin compounds, then

hydrolyzed HCl and tested with Liebermann-Burchard reagent. Green or blue color indicates steroidal saponins, red color indicates triterpenoid saponins. Flavonoid test dry sample 10g enter 10ml 80% ethanol, plus 0.5g magnesium metal plus 0.5m hcl pink or purple color for the presence of flavonoids.

1. Making Mouthwash of Cocoa Bean Extract Methanol Concentration 1%, 2%, 3%

The stock concentration that will be varied is starting from a concentration of 1%, 2%, 3%, with the consideration that according to the POM all herbal raw materials are safe to use below 15% [9]. And based on preliminary research, cocoa bean extract at the smallest

concentration of 1% was able to inhibit mutant *Streptococcus* bacteria.

- a) Formula 1, heat 100 ml of distilled water at 90°C for 15 minutes then remove and let stand a few minutes. heat 20 ml of distilled water, remove, add 0.2 grams of saccharin, 1 gram of menthol, 7 grams of glycerin, 0.02 grams of sodium benzoate, add 1% cocoa bean extract, strain and add to formula 1, stir until homogeneous, then strain into a bottle. Repeat for formula 2.2% and formula 3.3% cocoa bean methanol extract. Formula 4 as a negative control (-) without using cocoa bean extract.

Table 1: Composition of mouthwash variations

BAHAN	KEGUNAAN	KOMPOSISI %			
		F1	F2	F3	F4 Kontrol
Ekstrak	Bahan aktif	1	2	3	
Manthol	Aroma	1	1	1	
Sakarín	Pemanis	0,2	0,2	0,2	
Natrium Benzoat	Pengawet	0,01	0,01	0,01	
Aquades	Pengencer	100	100	100	

- b) Organoleptic visualization test (aroma, color, taste), clarity test Observation for 3 weeks.
- c) Mouthwash pH test
- d) The stability test was carried out using the centrifugation test method to see if there was a separation or not [10].

2. Acute Toxicity Test Mouthwash Cocoa Seed Methanol Extract

Prepare 12 female mice; divide into 4 groups, each group consists of 3 mice. Adapt 5 days. Before being tested, fasted for 2 hours and was only given water. After adaptation, group 1 was given 1% extract, group 2, 2%, group 3, 3% and group 4 was given aquades 2 ml/kg body weight. Clinical observations every day for 14 days.

3. Concentration Test (MIC) and (KBM) Mouthwash Methanol Extract Cocoa Beans

Preparation of 3 test tubes marked concentrations of 1%, 2%, 3%. The three tubes were filled with 3.5 ml of Trypticase soy broth (TSB),

added 0.5 ml of *S. mutans* bacteria and then homogenized. Furthermore, 0.1 ml is taken from each tube, dropped into a petri dish according to the concentration and dripped onto TYS20B media, put in an incubator for 48 hours.

RESULTS AND DISCUSSION

1. Cocoa Bean Sample Extraction Results

The process of maceration of cocoa beans with methanol for 3 x 24 hours marked with a clear colored solvent, then filtered and concentrated using a rotary evaporator in order to separate the solvent used from the extract. The concentrated methanol extract obtained was 61.78 grams. Based on Table 2, the weight of the fresh sample is 2,500 grams, the dry weight is 1,300 grams, the drying loss is 2,448%, the extract weight is 61, 78 grams, and the yield percentage is 4.75%. The compounds contained in the ethanol extract are usually dominated by polar compounds such as flavonoids, glycosides, tannins, and some alkaloids.

Table 2: Methanol Extract Results of Sare Aceh Cocoa Beans, Lembah Selawah District, Aceh Besar

NO	Sample Drying Way	Drying time (days)	Fresh sample weight before drying gram	Dry weight of simplicia gram	Drying shrinkage %	yield	Extract weight %
1	Wind dry	14 days	2,500 grams	1,300 grams	2.448	4.75%	61.78 grams

$$(\% \text{ Extract yield} = (\text{Extract Weight} / \text{Sample Weight}) \times 100)$$

2. Phytochemical Test Results

Phytochemical test to determine secondary metabolites contained in plants. Phytochemical tests were carried out on methanol extract of Sare Aceh Aceh Besar cocoa beans. Phytochemical tests include tests for

alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics and tannins. The test results of secondary metabolites of cocoa bean methanol extract are as follows;

Table 3: Phytochemical Test Results

Secondary metabolites	Cocoa bean methanol extract	Information
Phenolic	+	
Tannins	+	Formed cloudy white
Flavonoids	+	Formed in pink/purple
Steroids	-	No green/bluish color is formed
Terpenoids	+	Formed in red
Saponins	+	Bubbles or foam is formed
Alkaloids		
dd	+	Brick red color
Mayer	+	A white precipitate is formed
Wakner	+	Formation of a brown precipitate

3. Result of Acute Toxicity of Cocoa Bean Extract Methanol

The acute toxicity test in this study aims to assess the safety of the drug in terms of the toxicity of the drug when used. The three levels of laban leaf methanol extract used were 1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW, and 2 mg/kg BW aquadest [11]. The test preparations were given once a day for 14 days and the research data were measured 24 hours after the administration of the test preparations. The toxicity of the laban leaf methanol extract was measured from the

number of dead test animals, symptoms of depression, hair standing, and symptoms of nervous incoordination.

Based on Table-4, it can be seen that the dose and mortality groups of mice after administration of mouthwash. In the methanol extract of cocoa beans, no dead mice were found in the 1g/bb kg dose group, the 2g/bb kg dose group and the 3 g/bb kg dose group, and the aquadest water control group. All 12 mice were alive.

Table 4: Mice Mortality Group after Intervention

Extract Dosage Group	Treatment	Mice Death Count													
		DAY													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 g	1g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 g	2g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 g	3g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 ml aquadest	2 ml water	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2 ml water	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2 ml water	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5: Results of Observation of Mice Toxicity Symptoms

Extract Dosage Group	Symptoms of Toxicity	treatment	AmountSymptoms of the toxicity of mice after administration of Extract													
			14 days of administration of extract													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 g	Standing feathers, incoordination nerves, Symptoms of depression	1g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
		1g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
		1g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
2 g		2g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
3 g		3g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
		3g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
		3g/g bb	-	-	-	-	-	-	-	-	-	-	-	-	-	
2 ml aquadest		2 ml water	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2 ml water	-	-	-	-	-	-	-	-	-	-	-	-	-	
		2 ml water	-	-	-	-	-	-	-	-	-	-	-	-	-	

Based on Table-5, it can be seen that, all the test animals in each treatment dose group of cocoa bean extract methanolic mouthwash after 24 hours and the first day until the 14th day did not show symptoms of

standing hair, nerves incoordination, symptoms of depression, which means There were no signs of toxicity in mice.

Table 6: Dose groups based on body weight of mice

Group Extract Dosage	Treatment	WEIGHT (GRAM) AFTER THE EXTRACT DAY							
		1	3	5	7	9	11	13	15
Aquades	2 ml water	23	23	23	24	26	27	28	28
	2 ml water	26	26	26	26	27	27	27	27
	2 ml water	23	24	24	24	24	25	26	26
Extract 1%	1g/g bb	25	24	25	25	25	26	26	27
	1g/g bb	25	25	25	25	26	26	26	27
	1g/g bb	24	25	25	25	26	26	26	26
Extract 2%	2g/g bb	25	25	25	26	26	26	27	27
	2g/g bb	25	25	26	26	26	27	28	28
	2g/g bb	26	26	26	26	26	27	27	27
Extract 3%	3g/g bb	25	25	25	26	26	27	28	28
	3g/g bb	25	25	26	26	27	28	28	28
	3g/g bb	25	25	26	26	27	27	27	27

Based on Table-6, it can be seen that all experimental animals in each group showed increased body weight. Which means that the higher the dose given and the longer the dose given, it significantly affects body weight? Normal body weight which means no toxicity based on the weight of the mice. Giving mouthwash of cocoa bean extract methanol did not interfere with the health and appetite of mice. This can be seen from the weight growth of mice for 14 days. All mice in each treatment group experienced a significant increase in body weight.

4. Mouthwash stability test

The organoleptic test of mouthwash can be seen in Table 7, the duration of storage and the variation of concentration given to mouthwash did not affect the color, aroma, shape and clarity. The pH of the mouthwash of cocoa bean extract methanol duration of storage was within normal limits, pH was 7-10. Testing the stability of mouthwash, storage time and variations of mouthwash did not affect the quality of the mouthwash [10].

Table 7: Organoleptic Test of Cocoa Bean Extract Mouthwash

% Formulation	Observation	first week	2nd week	3rd week
1%	Color	Chocolate	Chocolate	orange
	Scent	-	-	menthol
	Flavor	Bitter	Bitter	Sweet
	Form	Clear	Clear	Clear
	Clarity	Clear	Clear	Clear
2%	Color	Chocolate	Chocolate	orange
	Scent	-	-	menthol
	Flavor	Bitter	Bitter	Bitter
	Clarity	Clear	Clear	Clear
3%	Color	Dark brown	Dark brown	orange
	Scent	-	-	menthol
	Flavor	Bitter	Bitter	Sweet
	Clarity	Clear	Clear	Clear
0%	Color	White	White	White
	Scent	-	-	-
	Flavor	Slightly bitter	Slightly bitter	Slightly bitter
	Clarity	Clear	Clear	Clear

Table 8: Results of testing the pH of the mouthwash of cocoa bean extract methanol

% Formulation	week 1	second week	3rd week
1%	8.9	8.4	8.4
2%	8.6	7.8	7.8
3%	8.1	7.5	7.9
0%	8.9	7.7	8.3

Table 9: Stability test results of methanol extract of cocoa bean mouthwash

Formulation%	Consistency		
	week 1	second week	3rd week
1%	There is no phase separation	There is no phase separation	There is no phase separation
2%	There is no phase separation	There is no phase separation	There is no phase separation
3%	There is no phase separation	There is no phase separation	There is no phase separation
0%	There is no phase separation	There is no phase separation	There is no phase separation

5. Result of Antibacterial Activity of Cocoa Bean Extract methanol

1) Bacterial Culture Results and Gram Stain

The *S. mutans* bacteria used in this study were taken from pure cultures from the Microbiology Laboratory of the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh which were then cultured on TYS20B media and then incubated for 48

hours at 37°C. The results of bacterial culture showed spherical colonies that were growing on the surface of the medium. Prior to the inhibition test, gram staining was performed to ensure that the bacteria to be tested were *S. mutans* bacteria. The results of Gram staining under a microscope show that the bacteria are round, colonize, form pairs of chains and are purple in color. *S. mutans* morphology on TYS20B media.

Table 10: Number of *S. mutans* Colonies Resulted in Graded Dilution

	Dilution Rate	Colony Growth Average colony / petri dish
1	10-1	420
2	10-2	211
3	10-3	160
4	10-4	54
5	10-5	5

Based on the results in Table-10, it shows that the 4th dilution (10-4) has 54 colonies. The dilution level is feasible to be chosen for sample testing because it meets the colony requirements of 30-300 CFU/cup.

2) Results of Antibacterial Activity Test of Cocoa Bean Methanol Extract Mouthwash on the growth of *S. mutans*

In this study, testing the activity of cocoa bean methanol extract on the growth of *S. mutans* was

repeated 2 times. The average number of *S. mutans* colonies after the test showed the highest colony growth was in the negative control (590 X10-4CFU/mL) and the least was in the positive control concentration (0 X10-4 CFU/mL). The number of bacterial colonies was also seen to decrease with increasing concentration as presented in Table-11.

Table 11: Number of Colonies

Test material concentration	Number of colonies of <i>S. mutans</i>		Average number of colonies (CFU/ml)
	Repetition I	Repetition II	
1.0%	261 X10-4	278X10-4	269 X10-4
2%	138 X10-4	118 X10-4	128 X10-4
3%	66 X10-4	53 X10-4	59 X10-4
negative control –without extract	581 X10-4	599 X10-4	590X10-4
positive control Ciprofloxacin 10 g/ml	0X10-4	0X10-4	0X10-4

The statistical test used in this study is One Way Anova which has more than two groups; the distribution and homogeneity of the data variance are the same. This study had 5 groups; consisting of 3 treatment groups 1%, 2%, 3% and 2 control groups, extract negative control group and Ciprofloxacin 10 g/ml positive control. The results of the normality test

showed that the distribution and homogeneity of the variance of the research data were normal with a value (P-value = 0.000) or spread normally, so it was continued with the ANOVA test. From the Anova table in the Sig column, P value = 0.000 is obtained. Thus at the level of significance = 0.05 we reject H0. In conclusion, concentration affects the number of

colonies that grow. Or there is a significant difference in the average number of colonies that grow based on each concentration. The minimum inhibitory concentration (MIC) of mouthwash of cocoa bean methanol extract against the growth of *Streptococcus mutans* was aimed at a concentration of 1% and no minimum killing concentration (MBC) was found in this study.

DISCUSSION

In this study, cocoa bean extract was obtained by using maceration technique for the extraction process of the active ingredient components. This method was chosen because it is relatively simple, cheap and easy [12]. The maceration process was carried out using methanol as a solvent. The reason for using methanol as a solvent is because it has the ability to attract folar active ingredients.

After the maceration process, phytochemical tests were carried out to prove that the cocoa bean extract contained active substances such as flavonoids, phenolics, tannins, terpenoids, saponins, alkaloids. The composition of compounds contained in plants is influenced by various factors both internal factors and external factors. Internal factors such as the influence on varieties/genes and which include external factors, namely, the influence of sunlight, rainfall, soil structure, and climate in the area so that there are differences in the content of the active substance of cocoa beans.

This study used the bacterium *Streptococcus mutans* which is the main agent causing dental caries. *Streptococcus mutans* has various virulence factors such as adhesion, colonization. *Streptococcus mutans* metabolism at low pH will increase in an acidic environment.

In this study, the selection of concentrations based on the provisions of toxicity according to Balaipom the use of test preparations below 15 grams is a dose that is not harmful. So the dose used in this study was a concentration dose of 1%, 2%, 3% [9]. The results of the LSD test were the number of *Streptococcus mutans* colonies that had been given cocoa bean extract with concentrations of 100%, 50%, 25%, no bacterial growth, all bacteria died. While the conditions for normal bacterial colonies are 30-300 CFU/cup. Then the concentration of 1%, 2%, 3% is a dose that is not harmful for mouthwash [13].

The method of determining the antibacterial effect of mouthwash cocoa bean extract in this study used the multilevel dilution method. This diluted or sequential dilution method is a process of gradual dilution of a substance in solution. The purpose of this method is to reduce the number of microbes that have been suspended in solution. In this study, the results of the stratified dilution were only read at the 10⁻⁴ dilution and obtained as many as 57 bacterial colonies that grew.

This amount meets the requirements for Colony Forming Units (CFU) per mile, where the requirements of this method are on petri dishes grown in the amount of 30-3000 colonies/cup [14].

The test results of the antibacterial effect of cocoa bean extract on the growth of *Streptococcus mutans* showed that cocoa bean extract had an antibacterial effect at concentrations of 1%, 2%, 3%, with a minimum inhibitory concentration of 1%. This result was proven that there was a reduction in the colonies growing in the medium after being incubated for 24 hours. This is because cocoa bean extract contains secondary metabolic compounds such as flavonoids, phenolics, tannins, terpenoids, saponins, alkaloids, and mayer, which have various precise properties such as antibacterial, antifungal. Each of these secondary metabolic compounds has a mechanism to inhibit the growth of bacteria.

The statistical test used in this study is One Way Anova which has more than two groups; the distribution and homogeneity of the data variance are the same. This study had 5 groups; consisting of 3 treatment groups 1%, 2%, 3% and 2 control groups, extract negative control group and Ciprofloxacin 10 g/ml positive control. The results of the normality test showed that the distribution and homogeneity of the variance of the research data were normal with a value (P-value = 0.000) or spread normally, so it was continued with the ANOVA test. From the Anova table in the Sig column, P value = 0.000 is obtained. Thus at the level of significance = 0.05 we reject H₀. In conclusion, concentration affects the number of colonies that grow. Or there is a significant difference in the average number of colonies that grow based on each concentration. The minimum inhibitory concentration (MIC) of mouthwash of cocoa bean methanol extract against the growth of *Streptococcus mutans* was aimed at a concentration of 1% and no minimum killing concentration (MBC) was found in this study.

This is because the chemical compounds contained in the cocoa bean extract only inhibit the growth of *S. mutans* bacteria (bacteriostatic) so that the bacteria can grow back after the antibacterial activity is reduced. The formation of inhibition produced by the methanolic extract of laban leaves on the growth of *S. mutans* in this study was presumably because the extract contained active compounds such as flavonoids, saponins, and alkaloids that have antibacterial properties [15].

Flavonoids are phenolic compounds by inhibiting the synthesis of nucleic acids from bacteria and inhibiting bacterial mortality [16]. Alkaloids can inhibit the formation of peptidoglycan in bacterial cell walls and cause bacterial cells to lyse. Saponins can inhibit bacterial growth because these compounds can

reduce the surface tension of the bacterial cell wall, when interacting the bacterial cell wall will break or lysis.

When saponins interfere with the surface tension of the bacterial cell wall, antibacterial substances will enter the cell and interfere with metabolism so that the bacteria will die. Polyphenols inhibit bacteria by denaturing proteins by reacting with the glucosyltransferase enzyme and damaging the cytoplasmic membrane of bacterial cells that have peptidoglycan. The mechanism of action of saponins against bacteria is by increasing the permeability of bacterial cells due to the reaction of the aglycone structure of the saponins into the lipid layer so that pores are formed on the cell membrane. Tannins are thought to be able to shrink cell walls or cell membranes so that they interfere with the permeability of the cell itself. Due to the disruption of permeability, cells cannot carry out living activities so that their growth will be hampered or even die. Saponins by lowering surface tension resulting in increased permeability or cell leakage and resulting intracellular compounds will come out, causing cells to die. Polyphenols have potential as antibacterial by poisoning protoplasm, damaging and penetrating cell walls and precipitating bacterial cell proteins [17].

The results of the toxicity study showed that the administration of three doses of 1 mg/kg bb, 2 mg/kg bb, 3 mg/kg bb, 2 ml of distilled water, did not cause death in the test animals, and did not show any signs of behavior (behavioral profile), did not show neurological symptoms (neurological profile), and did not show signs of poisoning for 24 hours and was given the test preparation on the 14th day. This is in accordance with the statement from Balaipom for use test preparations below 15 grams are a dose that is not dangerous. So the dose used in this study was a concentration dose of 1%, 2%, 3% [1, 3, 9].

The tests carried out were organoleptic tests, pH tests, stability tests and antibacterial activity tests. This test was carried out with the aim of knowing the feasibility of the mouthwash preparations made. In organoleptic testing, the odor, color and dosage form were observed. Mouthwash preparations that are made have a liquid form which is a characteristic of mouthwash in general. In terms of color, the preparation produces a brown color that matches the color of the cocoa fruit plant, in terms of smell; the preparation does not have an odor. The pH test was carried out using a pH meter; pH testing is carried out to measure the degree of acidity of the preparation. The pH obtained is in the formulation II, 6.46 and formulation III 6.67. The pH of the preparation must be with an oral pH of 6-7. This is so that the mouthwash is not acidic because it can be corrosive to the teeth or if it is alkaline it can interfere with taste. The degree of acidity (pH) of saliva is an important part in improving tooth integrity

because it can increase the occurrence of remineralization, where a decrease in salivary pH can cause tooth demineralization minerals back [19].

CONCLUSION

Methanol extract of cocoa beans contains secondary metabolic compounds of alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics and tannins. Minimum Inhibitory Concentration (MIC) in cocoa bean extract in methanol extract was at a concentration of 1% (269 X10⁻⁴ CFU/ml), and did not indicate the Minimum Kill Concentration (KBM). Toxicity test to three levels of dose 1 mg/kg bw, 2 mg/kg bw, 3 mg/kg bw, 2ml distilled water, showed no signs of poisoning.

Suggestion

It is necessary to do further in vitro research by increasing the concentration of cocoa bean extract to obtain the Minimum Kill concentration (KBM) on the growth of *Streptococcus mutans*.

BIBLIOGRAPHY

1. The surprise. Dental Disease Prevention, Jakarta: Egc.P.104. Published Online 2011.
2. Riskesdas, K. (2018). Main Results of the Da Sar Health Research (Riskesdas). *Journal of Physics A: Mathematical and Theoretical*, 44(8), 1-200. <https://doi.org/10.1088/1751-8113/44/8/085201>.
3. Baitariza, A., Ghazali, A., & Rosmiati. (2020). Formulation of Mouthwash Solution for Preventing Dental Plaque Pineapple Peel Extract (*Ananas Comosus* L. Merr). *J Sabdariffarma*, 6(1), 33-42.
4. Ristianti, N., Jk, W. (2015). Differences in the Effectiveness of Herbal and Non Herbal Mouthwash against Plaque Accumulation in the Oral Cavity. 2, 31-36.
5. Dame, J. P., & Angela, P. K. E. (2020). Effectiveness Test Of Cocoa Seed (*Theobroma Cacao* L.) Extract As An Antibacterial Ii Various Concentrations On *Streptococcus Pyogenes*. *Maj Kedok Uki 2020*, 36(1), 8-13.
6. Sepriyani, R. (2020). Effectiveness of Cocoa Seed Extract (*Theobroma Cacao* L.) As Antimicrobial Against *Streptococcus Mutans*.
7. Fazillah. (2015). Effect of Gargling With Cocoa Bean Extract (*Theobroma Cacao* L.) On Dental Plaque Index Study Against Al Itqon-Banat Islamic Boarding School, *Unissula Institutional Respotory*.
8. Yessi. (2010). Effect of Cocoa Bean Extract on Paracetamol Hepatotoxicity in Mice.
9. Amir, S. (2014). Food and Drug Supervisory Agency of the Republic of Indonesia.
10. Kono, S., Yamlean, P., & Sudewi, S. (2018). Formulation of Patikan Kebo (*Euphorbia Hirta*) Herbal Mouthwash Preparations and Antibacterial Test of *Prophyromonas Gingivalis*. 7(1), 37-46.
11. Saryanto. (2015). Acute and Sub-Chronic Toxicity Test of Herbal Medicine for Fibro Adenoma Mammariae (Fam) Saryanto. *Proceedings*, (2008),

- 42-50.
12. Dent, M., Uzelac, V., & Penic, M, B. (2013). The Effect Of Extraction Solvent, Temperature, And Time On The Composition And Mass Fraction Of Polyphenols In Dalmation Wild Sage (*Savia Offcinalis* L) Ectrac. *Biotechnol*, 51(1), 84-91.
 13. Purnamasari, D., Munadzirroh, E., & Yogiartono, R. (2010). Concentration of Cocoa Bean Extract as a Natural Material In Inhibiting the Growth of *Streptococcus Mutans*. *Pdgi*, 59(1), 14-18.
 14. Mubarak, Z., & Chismirina, S. H. (2016). Antibacterial Activity of Beehive Natural Propolis Extract Against the Growth of *Enterococcus Faecalis*. *Jounal Syiah Kuala Dent Soc*, 175-185.
 15. Wuri, H. S. (2011). Inhibitory Test of Saga Leaf Methanol Extract Against *Streptococcus Mutans* Growth.
 16. Chismirina, S., & Poppy, A. (2011). Jamblang Fruit Extract Against *Streptococcus Mutans* Growth As The Main Cause Of Caries. *Dentika*, 16(2), 144-148.
 17. Afni, N., Said, N., & Yuliet. (2015). Antibacterial Activity Test of Areca Nut Extract Toothpaste (*Areca Catechu* L.) Against *Streptococcus Mutans* and *Staphylococcus Aureus*. *Galen J Pharm* 48-58. Issn 2442-8744.
 18. Sinensis, C., Kunze, L., Mice, P., Sundari, D., Nuratmi, B., & Winarno, M. (O2009). Acute Toxicity (Ld 50) And Test Of Green Tea Leaf Extract (*Camellia Sinensis* (Linn.) Kunze) In *Mice*, 19, 198-203.
 19. Lely, M. (2017). Effect (Ph) of Saliva on the Occurrence of Dental Caries in Preschool Age Children. *Health Researcher Bull*, 45(4), 241-248. Doi:10.22435/Bpk.V45i4.6247241-248

Cite This Article: Ainun Mardiah, Cut Aja Nuraskin, Teuku Salfiyadi, Nora Usrina, Reca (2022). Effectiveness of Cocoa Seed Garmins to the Decrease of Dental Plaque Index. *EAS J Dent Oral Med*, 4(5), 123-131.