

Methanolic Extract From Cocoa Bean (*Theobroma Cacao L.*) As A Potential Active Ingredient In Mouthwash

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ABSTRACT

Dental plaque is a soft deposit firmly attached to the surface of teeth, formed from a mixture of saliva; such as mucin, remnants of oral tissue cells, leukocytes, lymphocytes with food debris, and bacteria resulting in the initial occurrence of tooth decay due to reduced pH. Unfortunately, currently available antiseptics-based mouthwash, used to prevent dental plaque formation, is thought to have a carcinogenic effect. Therefore, an alternative plaque was proposed using herbal antibacterial agents. Cocoa beans have anti-bacterial properties and are known to have active anti-bacterial agents, these substances are flavonoids, phenolics, alkaloids, terpenoids, saponins, and tannins. Herein, we aimed to determine the phytochemical content of methanol extract from cocoa bean (*Theobroma cacao L.*) and its antibacterial activities against *Streptococcus mutans* and subsequently studied its potential as a mouthwash active ingredient. Macerated methanolic extract of cocoa bean was investigated against clinical isolates of *S. mutans*. Qualitative phytochemical tests were further performed to observe the presence of flavonoids, phenolics, alkaloids, terpenoids, saponins, and tannins. The results showed that the growth of *S. mutans* colonies at a concentration of 1, 2, and 3% were 431×10^4 , 142×10^4 , and 59.5×10^4 CFU/mL, respectively. Addition of the extract at all concentrations (1–3%) did not affect the color, aroma, taste, and clarity of the mouthwash. An acute toxicity test on mice model confirmed that the cocoa bean-based mouthwash was safe.

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1. Introduction

Various mouth-related problems, such as bad breath and periodontal diseases, are caused by dental plaque. Dental plaque is a sticky glue that is formed on the surface of the teeth containing bacteria and its products. *Streptococcus mutans* play an important role in the formation of dental plaque by forming colonies that are tightly attached to the tooth surface and ferment sucrose into acid. Thus, lowering the pH of the tooth surface resulting in tooth mineralization (1). According to RIKESDAS 2018, 88.8% of Indonesian population experience dental health problems. The number reached 46.97% of Acehnese population, where 36,17% of them experienced dental caries (2). Children aged between 6 to 12 years old need intensive dental care, where 15 out of 30 students were reported experiencing dental caries with plaque caused by acid-producing bacteria (3). Based on an initial survey of students at Kayee Leu Elementary School, 80% of the 20 students had poor dental and oral hygiene and were prone to dental plaque. The formation of dental plaque can be overcome by using mouthwash containing antibacterial agents as a preventive treatment for throat infections (1). Plaque control can be done mechanically or chemically. Mouthwash is a chemical type of plaque control. Several chemical substances in mouthwash have antiseptic or antibacterial properties that function to inhibit plaque formation and gingivitis (4). The use of antiseptics in mouthwash is thought to have a carcinogenic effect on its users. The use of mouthwash with antiseptic content in

the form of alcohol can trigger oral cancer (5). There are many types of mouthwash available in convenience stores. One of the recommended substances in mouthwash is chlorhexidine, from the biguanide group. Chlorhexidine is the most effective of all therapeutic plaque control agents because it can ionically adhere to teeth and oral mucosal surfaces in high concentrations for long hours (4). Nonetheless, chlorhexidine has side effects if used repeatedly, such as staining of the teeth and altering taste (6). In this regard, research on herbal based mouthwash is proposed to offer a safe and effective plaque control (7). Polyphenols are commonly found active agents in cocoa beans (*Theobroma cacao* L.), dominated by catechins, epigallocatechin, anthocyanins, and procyanidins. Polyphenols have a preventive action against infectious and degenerative diseases including oral diseases, reducing the formation of biofilms and acid products from *S. mutans* that causes dental caries (8). Cocoa bean extract has been reported to possess a strong antibacterial effect against *Porphyromonas gingivalis* (9). Cocoa products contain inhibitors of the dextranase enzyme which plays a role in the formation of extracellular polysaccharide plaques from sucrose (9). The cocoa extract has the highest antioxidant activity with an IC50 value of 170 ppm (10). In another research, the extract from cocoa bean could inhibit bacterial growth from a concentration as low as 6.25% (7). Research conducted Purnamasari et al. (2010) reported that cocoa bean extract is effective against the growth of *S. mutans*. Furthermore, based on cell tests carried out on mice, cocoa bean extract could reduce cell damage (8). These antibacterial activities could be attributed to the content of phytochemicals in the cocoa bean extracts including flavonoids, alkaloids, tannins, terpenoids, and steroids. Flavonoids have an antibacterial mechanism that damages bacterial cell membrane, followed by the release of intracellular compounds. Tannins could act as antibacterial agent by inhibiting the reverse transcriptase and DNA topoisomerase enzymes which is antagonistic to the formation of bacterial cells. Moreover, phenolic compounds may disintegrate lipids in the plasma membrane of microorganisms (11). The methanol extract from cocoa beans acts as an antibacterial agent and has a high inhibiting rate on *S. mutans*. Based on the background, the researcher is interested in examining the effectiveness of cocoa bean mouthwash concentration on decreasing the plaque index in students of Kayee Leu Elementary School, Aceh Besar Regency, Indonesia.

2. Methods

The research design was a quasi-experimental study with a Post-Test Only control group. The model used in this study was female mice. The Research Location is Agricultural Laboratory and Veterinary Laboratory in Universitas Syiah Kuala Banda Aceh. The mouthwash formula was prepared at Pharmaceutical Laboratory, Health Polytechnic Aceh, in March-July 2022. The research sample of 1 kg dry cocoa beans was collected randomly in 2022 in the Geothermal Area of Sare Aceh Village, Lembah Selawah District, Aceh Besar. The mice sample consisted of 12 female mice. Data collection technique Research I

1. Preparation of Materials and Tools

All tools were sterilized; spray bottles, mortar and pestle, melamine, stirring spoon, and funnel. A dry sample of 1 kg cocoa beans was added in a blender and was macerated using methanol solvent for 3×24 hours, the filtrate was evaporated with Rotary.

2. Phytochemical Test

- a. Alkaloid Compound Test: 1 g dry sample was crushed, added 1 mL ammonia, then crushed, filtered, and added 10 mL of 0.5 N hydrochloric acid, shaken vigorously. After that, 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.
- b. Test for steroids, terpenoids, and saponins: dry sample of 10 g cocoa beans was mixed with distilled water, shaken vigorously for the presence of foam indicating saponin compounds, then hydrolyzed with HCl and tested with Liebermann-Burchard reagent. Green or blue color indicates steroidal saponins, and red color indicates triterpenoid saponins.
- c. Flavonoid test: dry sample of 10 g was mixed with 10 mL 80% ethanol, added 0.5 g magnesium metal, and 0.5 M HCl. A pink or purple color expresses the presence of flavonoids.

3. Procedure for making cocoa bean methanol extract mouthwash

The samples will be divided into different mouthwash concentrations of 1%, 2%, and 3%, considering that the National Agency of Drug and Food Control allows all herbal raw materials to be safely used below the concentration of 15%. Based on previous research, cocoa bean extract at small concentrations was able to inhibit the growth of *Streptococcus mutans*, using a positive control of 0.2% chlorhexidine and plain water as a negative control.

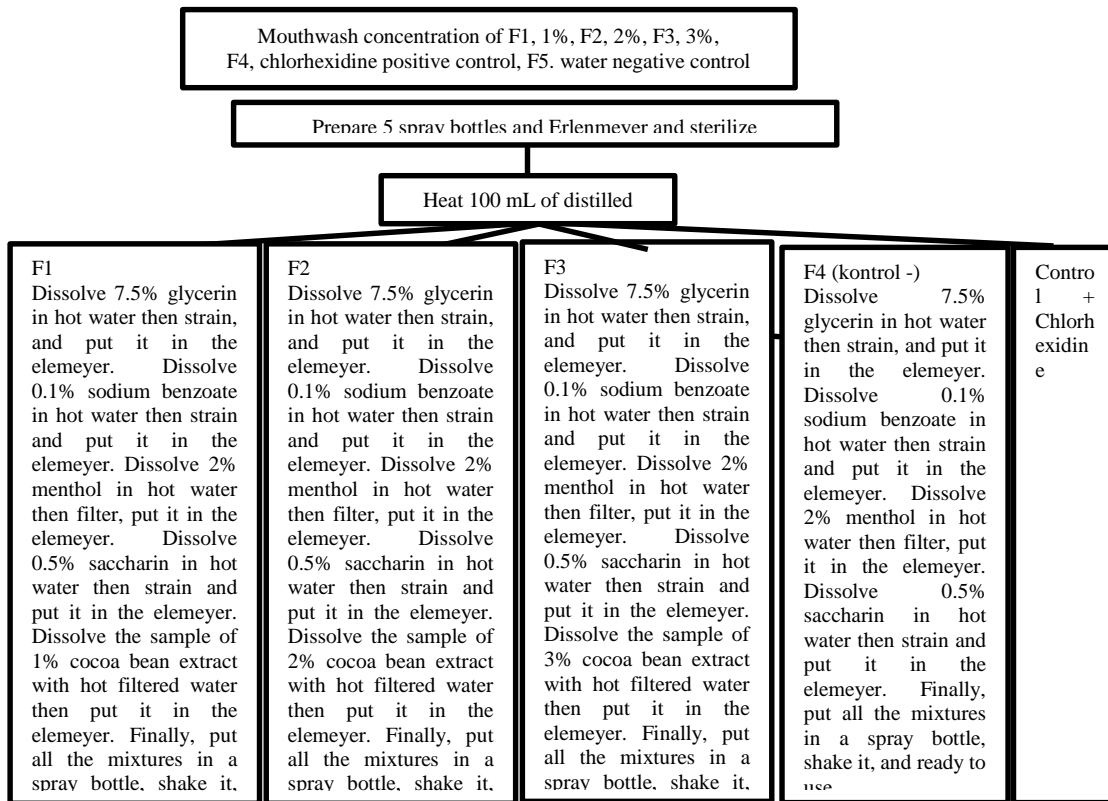


TABLE 1
MOUTHWASH FORMULATIONS WITH VARIOUS CONCENTRATIONS

Mixtures	Benefits	Composiiton %				
		F1%	F2%	F3%	Control +	Control -
Simplicia Cocoa beans	Active agent	1	2	3		
Glycerin		7.5	7.5	7.5		
Manthol	Aromatics	2	2	2		
Saccharin	Sweetener	0.5	0.5	0.5	0.2% chlorhexidin	Water
Sodium Benzoate	Preservative	0.1	0.1	0.1		
Distilled water	Diluent	100	100	100		

1. Evaluation of Mouthwash solution
Organoleptic visualization test (aroma, color, taste), clarity test, evaluation, mouthwash preparation starting with: visualization test, pH test, viscosity test, panelist test. The test was carried out for 3 weeks.
2. Organoleptic observation
Observations were made by inspecting the smell, color, and taste of the test preparations in week 1, week 2, week 3, and week 4.
3. Preference Test
The preference test was carried out by a group of 15 panelists. Each panelist was given a mouthwash preparation from the research. Panelists consisted of male and female dental health students. Testing is done by trying each formula individually. After gargling one formula, followed by another formula, gargling with plain water in between to neutralize the

sense of taste (12).

4. pH measurement

The pH value was measured using a pH meter that had been calibrated in advance using a standard buffer solution of pH 4 and 7. The pH of a good mouthwash is close to a neutral oral pH, between 6 and 7. The test was carried out for 30 days with observational data collection time in the 1st week, 2nd week, 3rd week, and 4th week.

5. Stability Test

The stability test was carried out using the centrifugation test method to see if there was a separation or not (13).

6. Concentration Test (MIC and MBC) of Methanol Extract from Cocoa Beans-based Mouthwash

Preparation of 3 test tubes with marked toothpaste concentrations of 1%, 2%, and 3%. The three tubes were filled with 3.5 mL of *Trypticase soy broth* (TSB), and 0.5 ml of *S. mutans* were added and then homogenized. Afterward, each tube was taken at an amount of 0.1 ml dripped on a petri dish according to the concentration and dropped onto TYS20B media, placed in an incubator for 48 hours. The observation is conducted after 48 hours.

7. Acute Toxicity Test of Methanol Extract from Cocoa Beans

Preparation of 12 rabbits; divided into 4 groups adapted for 5 days. Before being tested, fasted for 2 hours and was only given water. After adaptation, group 1 was given 1% extract, group 2 was given 2%, group 3 was given 3%, and group 4 was given distilled water 2 mL/kg body weight (BW). Clinical observations saw the development of body mass, stress, and death every day for 14 days.

3. Results And Discussion

1. Extraction Results

The process of maceration of cocoa beans with methanol for 3 × 24 hours was marked with a clear colored solvent, then filtered and concentrated using a rotary evaporator to separate the solvent used from the extract. The concentrated methanol extract obtained was 61.78 grams. Based on Table 1, the mass of the fresh sample is 2,500 grams, the dry mass is 1,300 grams, with 2.448% drying loss, the extract mass is 61.78 grams, and the percent yield 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 3
METHANOL EXTRACT RESULTS OF SAREE ACEH COCOA BEANS, LEMBAH SELAWAH DISTRICT, ACEH BESAR

NO	Sample Drying Method	Drying time (days)	Fresh sample mass before drying	Dry mass of simplicia	Drying shrinkage	Yield	Extract Mass %
1	Dry wind	14 days	2.500 grams	1.300 grams	2.448 %	4.75 %	61.78 grams

(% Extract yield = (Extract Mass / Sample Mass) x 100)

2. Phytochemical Test Results

Phytochemical tests determine the secondary metabolites contained in the cocoa bean plants. It was carried out on the methanol extract of Sare Aceh Aceh Besar cocoa beans. The tests included alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins. The results of secondary metabolites of cocoa bean methanol extract are as follows.

TABLE 4
PHYTOCHEMICAL TEST RESULTS

Secondary Metabolites	Cocoa bean methanol extract	Description
Phenolic	+	
Tannins	+	Cloudy white
Flavonoids	+	Pink/purple color
Steroids	-	Absent of green/bluish color
Terpenoids	+	Red
Saponins	+	Bubbles or foam
Alkaloids		
Dd	+	Brick Red Color
Mayer	+	White precipitate

Wagner

+

Brown precipitate

3. Result of Acute Toxicity of Cocoa Bean Methanol Extract

The acute toxicity test in this study aims to assess the drug safety in toxicity of the drug when used. The three levels of the methanol extract used were 1 mg/kg body weight (BW), 2 mg/kg BW, 3 mg/kg BW, and 2 mg/kg BW distilled water (14). The test preparations were administered once a day for 14 days and the research data were examined 24 hours after the administration. The toxicity of the methanolic extract was assessed by the mortality in test animals, symptoms of depression, hair standing, and symptoms of uncoordinated nerves. Based on Table 3, it can be seen that in the dose and mortality group of mice after the administration cocoa bean methanol extract mouthwash, there were no mice that died in 1, 2, and 3 mg/kg BW dose groups. All samples in control group (distilled water) survived.

TABLE 4
MICE MORTALITY AFTER INTERVENTION

Extract Dosage Group	Mice Death Count													
	Day-													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 mg/kg BW	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 mg/kg BW	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3 mg/kg BW	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2 mL distilled water	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABEL 5
MICE TOXICITY SYMPTOMS OBSERVATION RESULTS

Extract Dosage Group	Toxicity Symptoms	Number of toxicity symptoms on mice after extract administration													
		14 days of extract administration													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 mg/kg BW		-	-	-	-	-	-	-	-	-	-	-	-	-	
2 mg/kg BW	Standing hair, uncoordinated nerves, symptoms of depression	-	-	-	-	-	-	-	-	-	-	-	-	-	
3 mg/kg BW		-	-	-	-	-	-	-	-	-	-	-	-	-	
2 mL distilled water		-	-	-	-	-	-	-	-	-	-	-	-	-	

Based on Table 4, it can be seen that all test animals in each intervention dose group of cocoa bean methanol extract mouthwash, after 24 hours and the first day until the 14th day, did not show symptoms of standing hair, uncoordinated nerves, symptoms of depression. Thus, there were no signs of toxicity in mice.

TABLE 6

DOSE GROUPS BASED ON THE BODY MASS OF MICE								
Extract Dosage Group	Mass (Grams) After The Extract Administration							
	Day-							
	1	3	5	7	9	11	13	15
2 mL distilled water	23	23	23	24	26	27	28	28
	26	26	26	26	27	27	27	27
1 mg/kg BW	23	24	24	24	24	25	26	26
	25	24	25	25	25	26	26	27
2 mg/kg BW	25	25	25	25	26	26	26	27
	25	25	26	26	26	27	28	28
3 mg/kg BW	26	26	26	26	26	27	27	27
	25	25	25	26	26	27	28	28
	25	25	26	26	27	28	28	28
	25	25	26	26	27	27	27	27

Based on Table , it can be seen that all experimental animals in each group showed an increase in body mass. This means that the higher the dose given and the longer the dose given, it significantly affects body mass. A Normal body mass means no toxicity based on the mass of the mice and did not interfere with the health and appetite of the mice.

4. Mouthwash stability test

The mouthwash organoleptic test can be seen in Table 6, the duration of storage and the variation of concentration given to mouthwash did not affect the color, aroma, formation, and clarity. The pH of the cocoa bean methanol extract mouthwash during storage duration was within normal limits, pH was between 7 and 10. Testing the mouthwash stability, storage time and variations of mouthwash did not affect the quality of the mouthwash (13).

TABLE 7
COCOA BEAN EXTRACT MOUTHWASH ORGANOLEPTIC TEST

Formulation (%)	Observation	Week I	Week II	Week III
1	Color	Brown	Brown	Orange
	Aroma	-	-	Menthol
	Taste	Bitter	Bitter	Sweet
	Formaaation	Clear	Clear	Clear
2	Clarity	Clear	Clear	Clear
	Color	Brown	Brown	Orange
	Aroma	-	-	Menthol
	Taste	Bitter	Bitter	Bitter
3	Clarity	Clear	Clear	Clear
	Color	Dark brown	Dark brown	Orange
	Aroma	-	-	Menthol
	Taste	Bitter	Bitter	Sweet
0	Clarity	Clear	Clear	Clear
	Color	White	White	White
	Aroma	-	-	-
	Taste	Abit bitter	Abit bitter	Abit bitter
	Clarity	Clear	Clear	Clear

TABLE 8
COCOA BEAN METHANOL EXTRACT MOUTHWASH PH TEST RESULTS

Formulation %	Week 1	Week II	Week III
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 I	Week II	Week III
1	No separation phase	No separation phase	No separation phase
2	No separation phase	No separation phase	No separation phase
3	No separation phase	No separation phase	No separation phase
0	No separation phase	No separation phase	No separation phase

5. Antibacterial Activity of Cocoa Bean Methanol Extract Results

1) Bacterial Culture Results and Gram Stain

The *S. mutans* bacteria used in this study were 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. Before the inhibition test, gram staining was performed to ensure that the bacteria to be tested were *S. mutans*. Gram staining results under a microscope showed that the bacteria were round colonies, formed pairs of chains, and are purple in color, indicating *S. mutans* morphology.

TABLE 10
NUMBER OF *S. MUTANS* COLONIES RESULTED IN GRADED DILUTION

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

Table 10 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/dish.

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

The activity test of cocoa bean methanol extract on the growth of *S. mutans* was repeated twice. The average number of *S. mutans* colonies after the test showed the highest colony growth in the negative control (590 X10⁻⁴CFU/mL) and the lowest was in the positive control concentration (0 X10⁻⁴ CFU/mL). The number of bacterial colonies was also seen to decrease along with concentration increment as presented in Table 11.

TABLE 11
NUMBER OF COLONIES

Test substance concentration (%)	Number of <i>S. mutans</i> colonies		Average number of colonies (CFU/ml)
	1 st Repetition	2 nd Repetition	
1	261×10 ⁻⁴	278×10 ⁻⁴	269×10 ⁻⁴
2	138×10 ⁻⁴	118×10 ⁻⁴	128×10 ⁻⁴
3	66×10 ⁻⁴	53×10 ⁻⁴	59 ×10 ⁻⁴
Negative control -without extract	581×10 ⁻⁴	599×10 ⁻⁴	590×10 ⁻⁴
Positive control Ciprofloxacin, 10 µg/mL	0×10 ⁻⁴	0×10 ⁻⁴	0×10 ⁻⁴

The statistical test used in this study was One Way Anova which requires more than two groups, the distribution, and homogeneity of the data variance should be the same. This study had 5 groups, consisting of 3 treatment groups 1%, 2%, 3%, and 2 control groups, non-extract negative control group, and Ciprofloxacin 10 g/mL as the positive control. The results of the normality test showed that the distribution and homogeneity of the variance research data were normal with a value (P-value = 0.000) or normally distributed. 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, rejects 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 cocoa bean methanol extract mouthwash on the growth of *Streptococcus mutans* was aimed at a concentration of 1%, and minimum bactericidal concentration (MBC) was not found in this study.

6. Discussion

In this research, cocoa bean extract was obtained by using the maceration technique for the active substance extraction process using methanol as a solvent. This method was chosen because it is relatively simple, accessible, and uncomplicated (15). Methanol solvent was chosen because of its ability to attract polar active agents. After the maceration process, phytochemical tests were carried out to examine the active substances contained in cocoa bean extract; such as flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids. The composition of compounds contained in plants is influenced by various internal factors (varieties/genes) and external factors (sunlight, rainfall, soil structure, and climate). The bacteria used in the study were *S. mutans*, which are the main agent causing dental caries. *S. mutans* have various virulence factors; such as adhesion and colonization. *S. mutans* metabolism will increase in an acidic environment.

This study selected certain concentrations based on the provisions of toxicity according to the National Agency of Drug and Food Control which allows the use of test preparations below 15%. The concentration used in this study was 1%, 2%, and 3% (16). The LSD test results on the number of *Streptococcus mutans* colonies that had been given cocoa bean extract with concentrations of 100%, 50%, and 25%, show no bacterial growth. While the conditions for normal bacterial colonies are 30-300 CFU/dish (8).

A multilevel dilution method was performed in determining the antibacterial effect of cocoa bean extract mouthwash in this study. This diluted or sequential dilution method is a process of gradual dilution of a substance in a solution. The purpose of this method is to reduce the number of microbes that have been suspended in the solution. The study results of the stratified dilution were only read at a 10^{-4} dilution and obtained 57 bacterial colonies that grew. This amount meets the requirements for Colony Forming Units (CFU) per plate, each Petri dish had an amount of 30-3000 colonies (17).

The antibacterial effect test results of cocoa bean extract on the growth of *S. mutans* showed antibacterial effect at concentrations of 1%, 2%, and 3%, with a minimum inhibitory concentration of 1%. This result was proven that there was a reduction in the test results 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 can act as antibacterial and antifungal (18).

The statistical test used in this study was One Way ANOVA. From the Anova table in the Sig column, P-value = 0.000 is obtained. Thus, at the level of significance = 0.05, rejects H_0 . In conclusion, concentration affects the number of colonies that grow. The minimum inhibitory concentration (MIC) of cocoa bean methanol extract mouthwash on the growth of *Streptococcus mutans* was aimed at a concentration of 1%, and minimum bactericidal concentration (MBC) was not found in this study.

Flavonoids are phenolic compounds that can inhibit the synthesis of nucleic acids from bacteria (19). Alkaloids can inhibit the formation of peptidoglycan in bacterial cell walls and cause bacterial cell lysis. Saponins can inhibit bacterial growth due to these compounds can reduce the surface tension of the bacterial cell wall, when interacting the bacterial cell wall will break (lysis).

Saponins interfere with the surface tension of the bacterial cell wall so antibacterial substances can enter. Polyphenols inhibit bacteria by protein denaturation, reacting with the glucosyltransferase enzyme and damaging the cytoplasmic membrane of bacterial cells that have peptidoglycan. Saponins' mechanism of action against bacteria is by increasing the permeability of bacterial cells by reacting to the aglycone structure. Tannins are thought to be able to shrink cell walls or cell membranes so that they disturb the permeability of the cell. Due to the disruption of permeability, cells struggle to live. Polyphenols act as antibacterial by poisoning the protoplasm, damaging as well as penetrating cell walls, and precipitating bacterial cell proteins (19). The toxicity study results showed that the administration of three doses of 1, 2, and 3 mg/kg BW, and 2 mL distilled water, did not result in death in test animals, and did not show any signs of altered behavior (behavioral profile), neurological symptoms (neurological profile), and showed no signs of poisoning for 24 hours and after the 14th-day administration (8)(16).

Organoleptic tests, pH tests, stability tests, and antibacterial activity tests were carried out. These tests aim to know the feasibility of the mouthwash preparations made. In organoleptic testing, odor, color, and dosage form were observed. Mouthwash preparations that are made have a

general liquid form. 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 any odor. The pH test was carried out using a pH meter showing in formulation II: 6.46, and formulation III: 6.67. The pH of the preparation must be the same as an oral pH, between 6 and 7. An acidic oral environment can cause corrosive teeth and an alkaline pH can interfere with taste. The degree of saliva acidity (pH) is important to improve tooth integrity because it can increase the occurrence of remineralization, where a decrement can cause tooth demineralization. minerals back. The remineralization process can reduce the possibility of caries by retrieving its minerals back (20).

4. Conclusion

Cocoa bean methanol extract contains secondary metabolites: alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins. The MIC in cocoa bean methanol extract was at a concentration of 1% (269×10^{-4} CFU/ml), where the MBC could not be determined. Toxicity test revealed no signs of poisoning on mice model.

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