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THE EFFICACY OF COCOA BEAN EXTRACT MOUTHWASH CONCENTRATION IN REDUCING PLAQUE INDEX IN THE ORAL CAVITY AMONG PRIMARY SCHOOL STUDENTS AT SDN KAYEE LEU, INGIN JAYA SUBDISTRICT, ACEH BESAR

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ABSTRACT

Oral and dental diseases have a high prevalence in Indonesia, with 88.8% of the population experiencing dental caries and periodontitis. Dental plaque is a soft deposit that tightly adheres to tooth surfaces and is composed of microorganisms that contribute to the development of dental caries. Preventing caries and controlling plaque can be achieved through the use of mouthwash, which is more effective when herbal mouthwash containing antioxidants and antibacterial agents is used. Cocoa bean extract (Theobroma Cacao L.) contains secondary metabolites such as alkaloids, flavonoids, tannins, steroids, and exhibits antibacterial and anti-inflammatory properties, as well as strong inhibitory effects against S. mutans bacteria. The objective of this study is to determine the effectiveness of cocoa bean extract mouthwash concentration in reducing the plaque index in the oral cavity. The research methods included laboratory experiments and quasi-experiments with a post-test only control group design. The research was conducted at the Faculty of Veterinary Medicine laboratory, University of Syiah Kuala, and SDN Kayee Leu, Aceh Besar, involving 30 students. Data analysis was performed using ANOVA in the SPSS program. The results of the study indicate that the phytochemical analysis of cocoa bean extract contains alkaloids, steroids, terpenoids, saponins, flavonoids, phenols, and tannins. The Minimum Inhibitory Concentration (MIC) of cocoa bean extract mouthwash on the growth of Streptococcus mutans was observed at a concentration of 1%, and no Minimum Bactericidal Concentration (MBC) was found in this study. The 1% and 2% concentrations of the mouthwash effectively removed plaque compared to the 0% concentration. In conclusion, the use of cocoa bean extract mouthwash is effective in reducing the plaque index. Further research should consider clinical trials to explore its potential as an herbal remedy.

Keywords: Mouthwash, Extract, Cocoa, Beans

1. INTRODUCTION

The health of teeth and oral hygiene is of paramount importance. The primary cause of dental diseases is dental plaque, which leads to dental caries and periodontal inflammation. Dental plaque is a soft deposit firmly adhering to the

tooth surfaces, comprised of microorganisms. Dental plaque constitutes a primary factor in the occurrence of dental caries. The consequences of this ailment extend beyond tooth loss and may affect other organs Hirannya (2011).

Dental caries and periodontal diseases are highly prevalent dental conditions in Indonesia, affecting 88.8% of the population. According to the Indonesian Ministry of Health, 93% of children under the age of 12 experience dental caries, with 67.2% having a history of caries, and 43.4% remaining untreated Riskesdas (2018). Plaque control can be achieved through mechanical or chemical means, with chemical methods involving the use of mouthwash. Some mouthwashes possess antiseptic or antibacterial properties that function to inhibit plaque formation and gingivitis. The use of antiseptics in mouthwash formulations is suspected to have carcinogenic effects on users. Hence, it would be more effective to incorporate plaque control by gargling with herbal mouthwash containing antioxidants Baitariza et al. (2020), Ristianti et al. (2015).

Cocoa beans play a vital role in health, notably as antibacterial agents. Cocoa beans contain compounds such as flavonoids, tannins, and alkaloids, which exhibit antimicrobial effects. Based on previous research, cocoa bean extract has proven effective in inhibiting Streptococcus bacteria Pohan et al. (2021), Sepriyani (2020). Additionally, according to prior studies, gargling with cocoa beans has an impact on the dental plaque index; higher concentrations of cocoa bean gargles result in greater reductions in the dental plaque index Fazillah (2015). Furthermore, research conducted previously has shown that cocoa bean extract is non-toxic and can even reduce histological damage to cells in mice Yessi (2010).

2. MATERIALS AND METHODS

In this section, we will outline the seven essential steps employed in our study. These steps are designed to investigate the efficacy of cocoa bean extract mouthwash in reducing the dental plaque index among primary school students.

1) **Sample Collection**: Three kilograms of fresh cocoa beans were collected and air-dried. Subsequently, one kilogram of dried cocoa beans was obtained.
2) **Extraction**: The dried cocoa beans underwent a triple 24-hour maceration process using 80% methanol as the solvent. The resulting solution was filtered, and the methanol cocoa bean extract was obtained using a rotary evaporator.
3) **Phytochemical Analysis**:
    - **Alkaloid Test:** One gram of dried sample was finely ground, mixed with 1 mL of ammonia, triturated, filtered, and supplemented with 10 mL of 0.5 N hydrochloric acid (HCl). The solution was vigorously shaken, and it was divided into three test tubes. The addition of Mayer's reagent resulted in a white precipitate, Dragendorff's reagent caused a reddish precipitate, and Wagner's reagent led to a brown precipitate, indicating the presence of alkaloid compounds.
    - **Steroid, Terpenoid, and Saponin Test:** Ten grams of dried cocoa bean sample were mixed with distilled water and vigorously shaken to observe the formation of foam, indicating the presence of saponin compounds. Subsequently, hydrolysis with HCl was performed, and the Liebermann-Burchard reagent was employed for testing. A green or

blue color indicated steroid saponin, while a red color indicated triterpenoid saponin.

- **Flavonoid Test:** Ten grams of dried cocoa bean sample were mixed with 10 mL of 80% ethanol and 0.5 grams of magnesium metal. The addition of 0.5 M HCl resulted in a pink or purple coloration, indicating the presence of flavonoids.

4) **Preparation of Cocoa Bean Extract Mouthwash**: Concentrations of 1%, 2%, and 3% methanol extract of cocoa beans were prepared. These concentrations were selected in accordance with the guidelines of the National Agency of Drug and Food Control (Balai POM), ensuring that safely below 15% Syamsudin (2014). Additionally, based on preliminary research, it was found that a minimum concentration of 1% cocoa bean extract effectively inhibited Streptococcus mutans bacteria.

- **Formulation:** Formula 1, Heat 100 mL of distilled water at 90°C for 15 minutes, remove, and let it stand for a few minutes. Heat 20 mL of distilled water, remove, and 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, filter, and transfer into a bottle. Formula 4 serves as a negative control (-).
- **Testing:**
  1) Organoleptic Evaluation (aroma, color, taste), clarity observation over 3 weeks.
  2) pH Testing of the mouthwash.
  3) Stability testing conducted using the centrifugation method to observe any separation Kono (2018).

5) **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Testing of Methanol Extract of Cocoa Bean Mouthwash:** Three reaction tubes labeled with concentrations of 1%, 2%, and 3% were prepared. Each tube was filled with 3.5 ml of Trypticase Soy Broth (TSB), and 0.5 ml of S. mutans bacteria was added, followed by homogenization. Next, 0.1 ml was taken from each tube and placed onto Petri dishes according to their respective concentrations. These were then inoculated into TYS20B media and incubated for 48 hours.

6) **Plaque Index Examination Using the PHP Method**

Random selection of students divided into three groups. Group 1 received a 1% formula, Group 2 2% formula, and Group 3 a 0% formula. Subsequently, plaque index examination was performed before using the cocoa bean extract mouthwash. Each student was provided with mouthwash according to their respective group and instructed to use it twice daily, 20 ml per use, and gargle for 3 minutes after brushing teeth. Plaque index examination was conducted after 1 week. Then, data analysis was performed.

3. RESULT

In the following Research Results section, we present the findings and outcomes obtained from our comprehensive study, encompassing the results of various experiments and analyses conducted to investigate the effectiveness and safety of cocoa bean extract mouthwash in reducing dental plaque and its potential applications for oral hygiene maintenance among primary school students.

The process of macerating cocoa beans with methanol for 3 x 24 hours, characterized by a clear solvent, followed by filtration and concentration using a rotary evaporator, aimed to separate the solvent from the extract. The obtained concentrated methanol extract amounted to 61.78 grams. Based on Table 1, the fresh sample weight was 2,500 grams, the dry weight was 1,300 grams, drying loss was 2.448%, and the extract weight was 61.78 grams, resulting in an extraction yield percentage of 4.75%. Compounds present in the ethanol extract are typically dominated by polar compounds such as flavonoids, glycosides, tannins, and some alkaloids.

Table 1

Table 1 The Methanol Extract Results from Cocoa Beans in Sare Aceh, Lembah Selawah Subdistrict, Aceh Besar

NO	Sample Handling Method	Drying Time (days)	Fresh Sample Weight before Drying (grams)	Simplified Dry Weight (grams)	Drying Loss (%)	Yield	Extract Weight (%)
1	Air-drying	14 days	2500 grams	1300 grams	2.448	4,75%	61,78 grams

(% Extract Yield = (Extract Weight / Sample Weight) x 100)

3.1. RESULTS OF PHYTOCHEMICAL TESTING

Phytochemical testing is conducted to identify secondary metabolites present in plants. Phytochemical testing was performed on the methanol extract of cocoa beans from Sare Aceh, Aceh Besar. The phytochemical tests include alkaloids, steroids, terpenoids, saponins, flavonoids, phenols, and tannins. The results of the secondary metabolite tests on the methanol extract of cocoa beans can be seen in Table 2.

Table 2

Table 2 Results of Phytochemical Testing

Secondary Metabolites	Methanol Extract of Cocoa Beans	Description
Phenolic Compounds	+	Green color formation
Tannins	+	Cloudy white formation
Flavonoids	+	Pink/purple color formation
Steroids	-	No green/blue color formation
Terpenoids	+	Red color formation
Saponins	+	Formation of bubbles or foam
Alkaloids	+	Brick-red color formation
Dragendorff	+	Brick-red color formation
Mayer	+	White precipitate formation
Wagner	+	Brown precipitate formation

Measurement of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extract of cocoa bean mouthwash in inhibiting S. mutans.

In this study, the testing of methanol extract of cocoa bean activity against the growth of S. mutans was conducted in two repetitions. The average colony count of

S. mutans after testing showed the highest colony growth in the negative control (590 X 10^{-4} CFU/mL) and the lowest in the positive control concentration (0 X 10^{-4} CFU/mL). The bacterial colony count also decreased with increasing concentrations, as presented in Table 3.

Table 3

Table 3 Bacterial Colony Count of S. mutans After Treatment with Methanol Extract of Cocoa Bean Mouthwash

Test substance concentration	*S. mutans* colony count		Average number of colonies (CFU/ml)
	Repetition I	Repetition II	
1.%	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}
Positive control without extract	581 X10^{-4}	599 X10^{-4}	590X10^{-4}
Positive control *Ciprofloxasin* 10 µg/ml	0X10^{-4}	0X10^{-4}	0X10^{-4}

The statistical test used in this study is One-Way ANOVA, which is applied when there are more than two groups, and the distribution and homogeneity of variance data are the same. This research consists of 5 groups, comprising 3 treatment groups at 1%, 2%, 3%, and 2 control groups, which include the negative control group and the positive control with Ciprofloxacin 10 µg/ml. Therefore, an ANOVA test was conducted. From the ANOVA table in the Sig column, a P-value of 0.000 was obtained. Thus, at a significance level of 0.05, we reject the null hypothesis (H0).

3.2. MOUTHWASH QUALITY TEST

The evaluation of the preparation includes organoleptic tests, clarity tests, and pH tests, conducted over a 3-week period with storage at room temperature. Additionally, a preference test (hedonic test) was also conducted by providing a questionnaire to 30 panelists to assess color, aroma, and clarity.

The results of the organoleptic tests observed over three weeks indicated differences in color, aroma, taste, and clarity of the cocoa bean extract mouthwash among various concentrations. Specifically, the higher the concentration, the more intense the color produced, with a distinct menthol aroma contributing to a sweet taste and clarity in the mouthwash. The duration of storage did not affect the color, aroma, taste, and clarity of the cocoa bean extract mouthwash. These findings are presented in Table 4.

Table 4

Table 4 Organoleptic Testing of Cocoa Bean Extract Mouthwash

Formulation %	Observation	Week I	Week II	Week III
1%	Color	Brown	Brown	Orange
	Aroma	-	-	Menthol
	Taste	Bitter	Bitter	Sweet
	Appearance	Clear	Clear	Clear
	Clarity	Clear	Clear	Clear

2%	Color	Brown	Brown	Orange
	Aroma	-	-	Menthol
	Taste	Bitter	Bitter	Bitter
	Clarity	Clear	Clear	Clear
0%	Color	White	White	White
	Aroma	-	-	-
	Taste	Slightly Bitter	Slightly Bitter	Slightly Bitter
	Clarity	Clear	Clear	Clear

The pH measurement testing was conducted by immersing a pH meter probe into the mouthwash preparation until it displayed a constant reading after a brief period. The pH value was obtained from this reading, and the testing was performed every week over a 3-week storage period. The results of the pH acidity testing indicate an influence of storage duration (Week I, Week II, and Week III) on the pH value of the mouthwash. Based on the concentration, the pH values in ascending order are F1 (1%), F2 (2%), and F3 (0%), with respective values of F1 8.5, F2 8, and F3 8.3, all within the normal range of 7-10. This is presented as shown in Table 5.

Table 5

Table 5 Results of pH Testing of Methanol Extract of Cocoa Bean Mouthwash

Formulation %	Week 1	Week II	Week III	Average
1%	8,9	8,4	8,4	8,5
2%	8,6	7,8	7,8	8
0%	8,9	7,7	8,3	8,3

Based on the stability test of the cocoa bean extract mouthwash, it can be observed that there was no phase separation in the 1%, 2%, and 0% concentration variations. This is presented in Table 6.

Table 6

Table 6 Results of Stability Testing of Methanol Extract of Cocoa Bean Mouthwash

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

Results of Plaque Index Test Before and After Gargling with Methanol Extract of Cocoa Bean Mouthwash

The samples in this study consisted of 30 elementary school students from SDN Kayee Leu, Ingin Jaya District, Aceh Besar Regency. These participants were divided into three groups, with each group consisting of 10 individuals. Group 1 used a 1% mouthwash, Group 2 used a 2% mouthwash, and Group 3 used a 0% mouthwash.

4. DISCUSSION

This research employed cocoa bean extract using the maceration technique with methanol as the solvent Dent et al. (2013). Phytochemical analysis confirmed the presence of active compounds in cocoa bean extract such as flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids. The composition of compounds in plants is influenced by various factors, both internal and external. Internal factors include genetic variations, while external factors encompass sunlight exposure, rainfall, soil structure, and regional climate, resulting in variations in the active compound content of cocoa beans Idroes et al. (2016).

Regarding concentration, the research adhered to toxicity guidelines established by the National Agency of Drug and Food Control (Badan Pengawas Obat dan Makanan or Balaipom), where the usage of test preparations below 15 grams is considered safe Syamsudin (2014). Therapeutic materials with concentrations ranging from 0-2% conform to Indonesian National Standards (SNI), including fluoride, desensitizing agents, anti-tartar substances, and antimicrobial agents Purnamasari et al. (2010). Standard dosage recommendations for children range from 0.7 mg, adolescents from 1.5 mg, and for adults and pregnant women, the standard dosage ranges from 1.5 mg to 3.0 mg. Therefore, the concentrations used in this study were 1%, 2%, and 0%. The results of the LSD test show that there was no bacterial growth; in fact, all bacteria died when treated with cocoa bean extract concentrations of 100%, 50%, and 25%. Normal bacterial colony criteria are in the range of 30-300 CFU per dish. Thus, concentrations of 1%, 2%, and 3% are considered safe for the mouthwash Mubarak et al. (2016).

In this study, the results of serial dilution were only read at a dilution factor of 10^-4, and it yielded 57 bacterial colonies. This number falls within the range of Colony Forming Unit (CFU) per milliliter, where the criteria for this method are that petri dishes should have between 30-3000 colonies per dish.

Based on the antibacterial effect test of cocoa bean extract on the growth of Streptococcus mutans, it was found that cocoa bean extract had an antibacterial effect at concentrations of 1%, 2%, and 3%, with the minimum inhibitory concentration observed at 1%. This result is supported by the reduction in the number of colonies in the medium after 24 hours of incubation. This is attributed to the presence of secondary metabolites in cocoa bean extract such as flavonoids, phenolics, tannins, terpenoids, saponins, alkaloids, and Mayer and Wakner compounds, which possess various antimicrobial properties. Each of these secondary metabolites has its own mechanism for inhibiting bacterial growth.

This study had 5 groups, consisting of 3 treatment groups (1%, 2%, 3%) and 2 control groups (negative and positive control). In conclusion, concentration affects the number of colonies that grow, signifying a significant difference in the average number of colonies that grow based on each concentration. The minimum inhibitory concentration (MIC) of cocoa bean extract mouthwash against the growth of Streptococcus mutans was observed at a concentration of 1%, and no minimum bactericidal concentration (MBC) was found in this study.

This is because the chemical compounds found in cocoa bean extract only inhibit the growth of S. mutans bacteria (Bacteriostatic), allowing the bacteria to regrow once the antibacterial activity decreases. HandaSari (2011)

Flavonoids are phenolic compounds that inhibit the synthesis of nucleic acids in bacteria and also inhibit bacterial mortality Chismirina & Andayani (2011). Alkaloids can inhibit the formation of bacterial cell peptidoglycan and cause

bacterial cell lysis. Saponins can inhibit bacterial growth because they can reduce the surface tension of the bacterial cell wall; when interacting, the bacterial cell wall will rupture or lyse. When saponins disturb the surface tension of the bacterial cell wall, antibacterial substances will enter the cell and disrupt metabolism, leading to bacterial cell death. Polyphenols inhibit bacteria by neutralizing proteins, reacting with the enzyme glucosyltransferase, and damaging the cytoplasmic membrane of bacterial cells that have peptidoglycan. The mechanism of saponin action on bacteria involves increasing the permeability of bacterial cells due to the reaction of aglycone structures of saponins with the lipid layer, forming pores in the cell membrane. Tannins are believed to shrink the cell wall or cell membrane, disrupting their permeability. As a result of impaired permeability, the cells cannot perform vital activities, leading to inhibited growth or even cell death. Saponins reduce surface tension, resulting in increased permeability or cell leakage, causing intracellular compounds to exit, and leading to cell death. Polyphenols have the potential as antibacterials by poisoning the protoplasm, damaging, and penetrating the cell wall, and precipitating bacterial cell proteins Afni et al. (2015).

The results of the toxicity study showed that the administration of three dose levels (1 mg/kg bw, 2 mg/kg bw, 3 mg/kg bw, and 2 ml of aquades) did not result in the death of the test animals. There were also no behavioral, neurological, or poisoning symptoms observed during the 24-hour period and on the 14th day after administering the test preparations. This is consistent with the statement from Balaipom that the use of test preparations below 15 grams is not harmful. Therefore, the concentrations used in this study were 1%, 2%, and 3%.

The tests conducted include organoleptic tests, pH tests, stability tests, and antibacterial activity tests. These tests were carried out to assess the suitability of the mouthwash preparation. In the organoleptic test, observations were made on the odor, color, and form of the preparation. Regarding odor, the preparation had no odor. The pH values obtained were for formulation I, formulation II had a pH of 6.46, and formulation III had a pH of 6.67. The pH of the preparation should match the pH of the mouth, which is 6-7. The acidity (pH) of saliva is an important factor in maintaining dental integrity as it can enhance remineralization. A decrease in saliva pH can lead to dental demineralization. The process of remineralization reduces the likelihood of cavities and involves the restoration of minerals to the tooth surface Lely (2017).

Table 13, the homogeneity test using the Levene test showed a significance value (P-value) of 0.000 before treatment and a P-value of 0.102 after treatment. Therefore, it can be concluded that the pre-treatment group has a homogeneous population, while the post-treatment group does not. This means that P-value > 0.05, indicating that the data groups originate from a homogeneous population. Based on the table, it is evident that there is a significant difference between all groups, as indicated by the P-value < 0.05 for all groups (0%, 1%, and 2%). The effect of mouthwash with concentrations of 1% and 2% cocoa bean extract is effective in significantly reducing plaque compared to mouthwash with a 0% concentration.

This implies that there is an influence of using cocoa bean extract mouthwash before and after rinsing on plaque index. In conclusion, there is a difference in plaque index between before rinsing and after rinsing with cocoa bean extract mouthwash in students at SDN Kayee Leu, Ingin Jaya Subdistrict, Aceh Besar Regency. In other words, rinsing with cocoa bean extract mouthwash (MOUTHWASH) at a volume of 20 ml is effective in reducing dental plaque index in students at SDN Kayee Leu. This is because cocoa beans contain secondary metabolites such as alkaloids with Mayer, Dragendorff, steroids, terpenoids,

saponins, flavonoids, and phenolics. The mechanism of action of flavonoids as antibacterials involves the formation of complexes with extracellular and soluble proteins, leading to bacterial membrane damage and the subsequent release of intracellular compounds Rinaldi et al. (2016).

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5. CONCLUSION

The phytochemical analysis of cocoa bean extract revealed the presence of alkaloids, steroids, terpenoids, saponins, flavonoids, phenolates, and tannins. The Minimum Inhibitory Concentration (MIC) of mouthwash containing methanol extract of cocoa beans against the growth of Streptococcus mutans was found to be at a concentration of 1%, and no Minimum Bactericidal Concentration (MBC) was identified in this study. The use of mouthwash with a concentration of 1% and 2% of cocoa bean methanol extract was effective in removing plaque significantly better than mouthwash with a concentration of 0%. The utilization of mouthwash containing methanol extract of cocoa beans proved to be effective in reducing the plaque index.

CONFLICT OF INTERESTS

None.

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