

Influence of *Theobroma cacao* L on the adhesion of *Streptococcus mutans*

Rahmi Syaflida, Gostry Aldica Dohude, Isnandar, Risa Sepriyani*

Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia

*Corresponding Author: rahmi.syaflida@usu.ac.id

ARTICLE INFO : Received, April 15, 2023; Revised, June 12, 2023; Accepted, August 10, 2023; Published, December 20, 2023
ORCID : Syaflida R (0000-0002-3501-2939); Dohude G A (0000-0002-2972-9749); Isnandar (0000-0001-8364-6760); Sepriyani R (0000-0002-9342-9142)
DOI : [10.24815/jds.v8i2.29878](https://doi.org/10.24815/jds.v8i2.29878)

ABSTRACT

Background: Indonesia is a tropical country with various plants ranging from forest products to agriculture and plantations. One of the plants with a lot of beneficial potential as a traditional medicine is the cacao plant (*Theobroma cacao* L.). Cocoa beans have a high content of polyphenol compounds, which significantly contribute as antioxidants, anti-cancer, anti-diabetes, anti-hypertension, and anti-inflammation. Ketekin, flavonoids, and tannins are compounds in cocoa beans known to have antimicrobial properties. **Objective:** This study aimed to determine the effectiveness of the antimicrobial power of cocoa bean extract in inhibiting the growth of *Streptococcus mutans* bacteria. **Methods:** This research is an experimental laboratory research. The study began with the manufacture of cocoa bean extract made into five concentrations, namely 50%, 25%, 12.5%, 6.25%, and 3.125%. This research was conducted by measuring the diameter of the inhibition zone through the Kirby-Bauer disk diffusion method using chlorhexidine 0.2% positive control and DMSO negative control. **Results:** The results showed at the largest concentration of 50%, the average value of the inhibitory zone obtained was 19.10 ± 0.25 mm, and the smallest concentration of 3.12% was 7.25 ± 0.29 mm. **Conclusion:** The study concludes that Cocoa bean extract effectively inhibits the growth of *Streptococcus mutans*. **Keywords:** Cocoa beans extract, *Streptococcus mutans*, Antimicrobial

ABSTRAK

Latar belakang: Indonesia merupakan negara tropis yang memiliki keanekaragaman tumbuhan mulai dari hasil hutan, pertanian, dan perkebunan. Salah satu tumbuhan yang memiliki banyak potensi manfaat sebagai obat tradisional adalah tanaman kakao (*Theobroma cacao* L.). Biji kakao memiliki kandungan senyawa polifenol yang tinggi yang berperan besar sebagai antioksidan, antikanker, antidiabetes, antihipertensi, dan antiinflamasi. Ketekin, flavonoid, dan tanin merupakan senyawa yang terkandung dalam biji kakao yang diketahui memiliki sifat antimikroba. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas daya antimikroba ekstrak biji kakao dalam menghambat pertumbuhan bakteri *Streptococcus mutans*. **Bahan dan Metode:** Penelitian ini merupakan penelitian eksperimental laboratorium. Penelitian diawali dengan pembuatan ekstrak biji kakao yang dibuat menjadi 5 konsentrasi yaitu 50%, 25%, 12,5%, 6,25%, dan 3,125%. Penelitian ini dilakukan dengan mengukur diameter zona hambat melalui metode difusi cakram Kirby-Bauer menggunakan kontrol positif klorheksidin 0,2% dan kontrol negatif DMSO. **Hasil:** Hasil penelitian menunjukkan pada konsentrasi terbesar 50% diperoleh nilai rata-rata zona hambat sebesar $19,10 \pm 0,25$ mm dan konsentrasi terkecil sebesar 3,12% sebesar $7,25 \pm 0,29$ mm. **Kesimpulan:** Berdasarkan hasil penelitian disimpulkan bahwa ekstrak biji Kakao efektif dalam menghambat pertumbuhan *Streptococcus mutans*. **Kata Kunci:** Ekstrak Biji kakao, *Streptococcus mutans*, Antimikroba

1. Introduction

Tooth and mouth disease is the sixth highest disease complained by Indonesian society and ranks as the fourth most expensive disease in its treatment. Based on the Household Health Survey, the prevalence of caries in Indonesia reached 90.5%. Tooth decay due to dental hard tissue, if left for too long without treatment, will eventually cause bacteria to invade the pulp tissue, resulting in pulp death (necrosis). The spread of infection can continue into the periapical tissue, resulting in a periapical abscess. The leading bacterial cause of dental caries is *Streptococcus mutans*.^{1,2}

One of the plants with many beneficial potentials, both food technology and to be developed as traditional medicine, is the cocoa plant (*Theobroma cacao* L.). The community widely uses part of the cocoa bean as an antioxidant that can reduce the formation of cancer-causing free

radicals. Cocoa also contains bioactive compounds which are useful in preventing the accumulation of cholesterol in blood vessel walls. The results showed that processed cocoa bean products such as chocolate and chocolate drinks are a rich source of specific/specific antioxidants in the form of higher catechins, epicatechins, procyanidins, and polyphenols compared to green tea, red wine, and blueberries. Cocoa beans contain quite large polyphenol compounds. The polyphenol content in cocoa beans includes 33- 42% catechins, 23-25% leukocyanidins, 5% anthocyanins, and tannins. While the fat-free powdered cocoa beans contain 5-18% polyphenol compounds.^{3,4}

The existence of a higher polyphenol composition than cocoa products or their derivative products greatly contributes to the health of the body because it has a role as an antioxidant, anti-cancer, anti-diabetes, antihypertension, anti-inflammatory, can relieve stress, prevent dental caries, improve ability, improve endurance against hemolysis, and makes the heart-healthy. ³ Research on cocoa beans as antimicrobials began to be carried out because of the high levels of polyphenolic compounds in cocoa beans. In addition, microbial resistance to several antibiotic products is a current problem and phenomenon, and various types of synthetic antimicrobial products, such as Chlorhexidine 0.2% mouthwash, also have negative effects on oral health, such as the appearance of stains on the teeth and cheek mucosa and irritate. On the oral mucosa, several microbiologists are concerned about finding alternative antimicrobials that can cure diseases caused by bacteria. New antimicrobial alternatives can be sourced from nature, one of which is by utilizing secondary metabolites derived from cocoa bean plants.^{5,6}

2. Material and Methods

This study was approved by the Health Research Ethics Commission (KEPK) at the Faculty of Medicine, University of North Sumatera. The sample was the *Streptococcus mutans* bacteria cultured in the Microbiology Laboratory of the General Hospital of North Sumatera University. This study is an experimental laboratory with five different concentrations, namely 50%, 25%, 12,5%, 6,25%, and 3,125%, Chlorhexidine 0,2% as a positive control and DMSO as a negative control, which is repeated as many as four times. This study used the diffusion test with the Kirby-Bauer method.

2.1. Extract Preparation of Cocoa Bean

Production and dilution of cocoa bean extract were conducted at the Laboratory of Traditional Medicine, Faculty of Pharmacy, University of North Sumatra. The maceration technique makes cocoa bean extract. One kg of cocoa beans is cleaned from the pulp of seeds, then dried by aerating for $\pm$ 24 hours. In the next step, the cocoa beans are cut roughly and aerated again until they dry for $\pm$ 48 hours, then mashed with a blender until they become powdery as much as 0.2 kg. The cocoa bean powder is put into the macerator, and then ten parts of the solvent, 70% ethanol, are added. Soak for the first 6 hours while stirring occasionally, then let stand for 18 hours. The filtrate was then concentrated with a rotary evaporator machine for 2 hours to separate the solvent from the cocoa bean extract until a concentrated extract of 50 mL was obtained. After getting the cocoa bean extract was diluted using DMSO to obtain each extract with a concentration of 50%, 25%, 12.5%, 6.25%, and 3.125%.

2.2. Antibacterial Test of Cocoa Bean Extract

Identification, breeding, and sample testing were conducted at the Microbiology Laboratory of the General Hospital of North Sumatra University. Cultured bacterial colonies are taken with sterile oase three times, then put into a test tube containing 0.45% Sodium Chloride, and then homogenized with a vortex mixer. After the vortex process, the suspension was obtained using a spectrophotometer at a wavelength of 600 nm to obtain an absorbance of 0.55 McFarland. Then, the bacterial suspension is taken with a sterile cotton swab and then applied completely to the surface of Muller Hilton Agar media by making a full scratch repeated on the petri dish. Then, place the blanc disc in each marked concentration area and drop the cocoa bean extract, Chlorhexidine 0.2%, and DMSO on the blanc disc using a micropipette, as much as 20 μ . Petri dishes are incubated in an incubator for 24 hours at 37°C. After 24 hours, the diameter of the clear zone formed around the blanc discs was measured using a caliper.

3. Result and Discussion

Testing the effectiveness of cocoa bean extract (*Theobroma cacao* L.) on the growth of *Streptococcus mutans* was carried out on cocoa bean extract (*Theobroma cacao* L.) concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, positive control, and negative control to determine the diameter of inhibition for each treatment group. In this study, the inhibition diameter was determined using the diffusion method. The results showed that cocoa bean extract (*Theobroma cacao* L.) concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, and the positive control found a clear zone around the blank disc, indicating growth inhibition. *Streptococcus mutans* bacteria with different inhibitory diameters. Meanwhile, the negative control did not find a clear zone, so it had no inhibition diameter (Table 1).

The mean diameter of inhibition of cocoa bean extract (*Theobroma cacao* L.) concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and positive control in inhibiting the growth of *Streptococcus mutans* was 7.25 ± 0.29 mm; 8.10 ± 0.25 mm; 10.10 ± 0.25 mm; 13.90 ± 0.25 mm; 19.10 ± 0.25 mm; and 33.3 ± 0.29 mm. These results show that the minor inhibition diameter was at a concentration of 3.125%, 7.25 ± 0.29 mm, and the largest was at a concentration of 50%, 19.10 ± 0.25 mm.

Table 1. Diameter Value of Inhibition of Cocoa Bean Extract (*Theobroma cacao* L.)

Groups	Concentration	Repetition (mm)				Average diameter (mm)	SD
		1	2	3	4		
Cocoa Bean Extract (<i>Theobroma cacao</i> L.)	50%	19,5	19	19	19	19,10	0,25
	25%	13,5	14	14	14	13,90	0,25
	12,5%	10	10,5	10	10	10,10	0,25
	6,25%	8	8	8	8,5	8,10	0,25
	3,125%	7,5	7	7	7,5	7,25	0,29
Positive Control (Chlorhexidine 0.2%)		33,5	33	33,5	33	33,3	0,29
Negative Control (Aquadest)		0	0	0	0	0	0,0000

Additionally, the Shapiro-Wilk test was used to determine the normality of the data from the value of the antimicrobial efficiency test findings of cocoa bean extract (*Theobroma cacao* L.). The research data in this study obtained p 0.05, indicating from the run tests' outcomes that they were not regularly distributed. If the data are not normally distributed, non-parametric statistical tests, such as the Kruskal-Wallis test, might be used to continue the analysis (Table 2).

Table 2. Results of statistical tests of Kruskal Wallis antibacterial effect of cocoa bean extract (*Theobroma cacao* L.)

No	Group	N	Average $\pm$ SD	p-value
1	I	4	$19,10 \pm 0,25$	0,000*
2	II	4	$13,90 \pm 0,25$	
3	III	4	$10,10 \pm 0,25$	
4	IV	4	$8,10 \pm 0,25$	
5	V	4	$7,25 \pm 0,29$	
6	VI	4	$33,3 \pm 0,29$	

Based on the results of the Kruskal Wallis test, the value of $p = 0.000$ ($p < 0.05$) means that there is a significant difference in the diameter of inhibition between cocoa bean extract (*Theobroma cacao* L.) concentrations of 3.125%, 6.25%, 12.5%, 25%, 50% with the positive control (Chlorhexidine 0.2%) in inhibiting the growth of *Streptococcus mutans* bacteria. These results show that cocoa bean extract (*Theobroma cacao* L.) has antimicrobial properties to inhibit the growth of *Streptococcus mutans* bacteria. Based on the results of research that has been carried out, cocoa bean extract can inhibit the growth of *Streptococcus mutans* bacteria. This is evidenced in the extract of cocoa beans with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, and Chlorhexidine 0.2% as a positive control seen a clear zone around the blank disc, while DMSO as a negative control no visible clear zone on the blank disc.

The clear zone indicates the inhibition of microorganism growth, namely *Streptococcus mutans*, by antimicrobial agents on the surface of the agar medium. According to David and Stout in 1971, the classification of bacterial growth inhibition responses based on clear zones formed can

be grouped into four groups: extreme when the inhibition zone is > 20 mm, strong 10-20 mm, moderate 5-10 mm, and weak. The inhibitory power generated by cocoa bean extract is caused by the ability of cocoa beans to inhibit bacterial growth, which is influenced by the content of polyphenols that can damage the cell walls of bacteria that contain peptidoglycan. The presence of several antibacterial compounds, such as catechins, flavonoids, tannins, and anthocyanins, also causes the antibacterial properties of cocoa bean extract.⁷

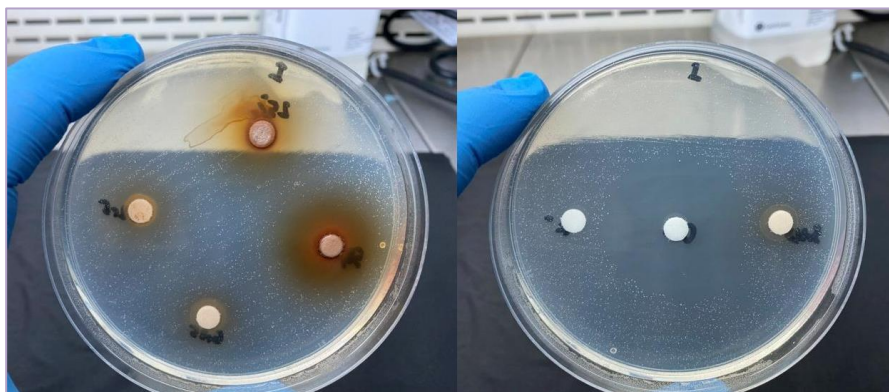


Figure 1. The inhibition zone formed for cocoa bean extract, 0.2% chlorhexidine, and DMSO

Flavonoids are polyphenol compounds that work by denaturing and coagulating bacterial cell proteins. These mechanisms cause damage to the composition and changes in the permeability mechanism of cell walls. Catechins are secondary metabolites naturally produced by plants and belong to the flavonoid group. This compound has the antimicrobial activity of its phenol group. Catechins can damage the cytoplasmic membrane, causing leakage of important metabolites that activate the bacterial enzyme system.^{8,9}

Tannins will inactivate the adhesion of microbial cells found on the cell surface, enzymes bound to cell membranes, and cell wall polypeptides that will cause damage to cell walls. The mechanism of action of tannins causes the loss of permeability properties of cell membranes, so the entry of substances such as water, nutrients, and enzymes is not selected. When the enzyme comes out of the cell, cell metabolism will be inhibited, resulting in the formation of ATP (Adenosine Three Phosphate) needed for cell growth and propagation.^{10,11} Anthocyanins are a class of flavonoids that provide color pigments in cocoa bean plants and can also function as antioxidants and antimicrobials. The mechanisms underlying antimicrobial activity in anthocyanins include cell membranes and intracellular interactions of these compounds.^{12,13} Bacteria exposed to anthocyanins will experience irregularity in the outer membrane and leak cytoplasm, which causes the release of cell components. This will cause damage to bacterial cells and will cause lysis, resulting in bacterial cell death.^{14,15}

4. Conclusion

From the results of the study on the effectiveness of cocoa bean extract (*Theobroma cacao* L.) as an antimicrobial against *Streptococcus mutans*, it can be concluded that cocoa bean extract (*Theobroma cacao* L.) has an antimicrobial effect against *Streptococcus mutans* with a significance value of $p=0.000$ ($p<0.05$) with a minor inhibition diameter at a concentration of 3.125% of 7.25 ± 0.29 mm and the largest inhibition diameter at a concentration of 50% namely 19.10 ± 0.25 mm.

5. References

1. Sondang P, Hamada T. Menuju gigi dan mulut sehat pencegahan dan pemeliharaan. Edisi revisi. Medan: USU Press, 2015; 5-10; 2-4
2. Tarigan R. Perawatan pulpa gigi. Jakarta: Widya Medika, 1994 :181-94.
3. Ackar, D., K.V. Landic, M. Valek, D. Subaric, B. Milicevic, J. Babic and H. Nedic. Cocoa polyphenols : Can we consider cocoa and chocolate as potential functional food. J of Chemistry 2013;13 : 289-96.
4. Hii, C.L., C.L. Law, Suzannah S, Misnawi. Polyphenols in cocoa (*Theobroma cacao* L.). Asian Jurnal of food and agro-industry 2009; 2(4) : 702-22.
5. Ranneh Y, Ali F, Esa NM. The protective effect of cocoa (*Theobroma cacao* L.) in colon cancer. Jurnal of nutrition and food science. 2013; 3(2) : 190-3.

6. Sudibyo A. Peranc coklat sebagai produk pangan derivat kakao yang menyehatkan. Jurnal Riset Industry 2012; 6(1) : 23-40.
7. Dewi NR, Sasongko H. Perbandingan aktivitas antimikroba ekstrak infusa daun dari sembilan jenis suku piperace terhadap staphylococcus aureus dan candida albicans. Jurnal Ilmu Alam dan Tek Terapan 2019; 1(1): 1-5.
8. Sari R, Alana lulu, Apridamayanti P. Penentuan FICI kombinasi ekstrak etanol kulit daun lidah buaya (Aloe vera (L) burm.f) dan gentamisin sulfat terhadap bakteri Escherichia coli. Pharm Sci Res 2017; 4(3) : 132-42.
9. Hafidhah N, Hakim RF, Fakhurrazi. Pengaruh ekstrak biji kakao (*Theobroma cacao* L.) terhadap pertumbuhan enterokokus faecalis pada berbagai konsentrasi. Jurnal Canius Dent 2017; 2(2) : 92-6.
10. Habiburrahman B, Hasyim M, Rahim B. Formulasi dan uji stabilitas fisik ekstrak kulit buah kakao (*Theobroma cacao* L.) pada sediaan pasta gigi sebagaiantibakteri terhadap streptococcus mutans. Jurnal Pharma and Med Sci 2016; 1(2) : 5-10.
11. Wulandari, Suswati E, Misnawati. Efek antibakteri ekstrak etanol biji kakao (*Theobroma cocoa* L.) terhadap pertumbuhan shigella dysentria secara in vitro. Jurnal medika planta 2012; 1(5) : 69-73.
12. Mulyatni AS, Budiani A, Taniwiryono D. Aktivitas antibakteri ekstrak kulit buah kakao (*Theobroma cacao* L.) terhadap escherichia coli, bacillus subtilis, dan staphylococcus aureus. Jurnal Menara Perkebunan 2012; 80(2) : 77-84.
13. Kumalasari DV, Suswati E, Misnawati. The effect of ethanolic extract of cocoabeans (*Theobroma cacao* L.) as an antibacterial against pseudomonas aeruginosa in vitro. E-j Pustaka Kesehatan 2015; 3(1) : 29-33.
14. Yumas M. Pemanfaatan limbah kulit ari biji kakao (*Theobroma cacao* L.) sebagai sumber antibakteri streptococcus mutans. Jurnal Industri Hasil Perkebunan 2017;12(2): 7-20.
15. Atikah AR, Hendrik SB Tuti K. Antibacterial effect of 70% ethanol and waterextract of cacao beans (*Theobroma cacao* L.) on aggregatibacteractino-mycetemcomitans. J Dent 2016; 49(2) : 104-9.

Authors Contribution

Contribution	Syaflida R	Dohude GA	Isnandar	Sepriyani R
Concepts or ideas	√	√	√	√
Design	√	√	√	√
Definition of intellectual content	√			
Literature search	√	√	√	√
Experimental studies	√			
Data acquisition	√	√		√
Data analysis	√	√		√
Statistical analysis	√		√	√
Manuscript preparation	√		√	√
Manuscript editing	√	√		√
Manuscript review	√	√		√



JDS (Journal of Syiah Kuala Dentistry Society) is an Open Access Journal licensed under a Creative Commons Attribution-ShareAlike 4.0 International License. This license authorizes the utilization, replication, modification, distribution, and reproduction of the article in any medium or format, provided that due credit is given to the original Author (s) and the source, a link to the Creative Commons license is provided, and any alterations made to the article are duly indicated.

Citation Format: Syaflida R, Dohude GA, Isnandar, Sepriyani R. Influence of *Theobroma cacao* L on the adhesion of *Streptococcus mutans*. J Syiah Kuala Dent Soc. 2023; 8(2): 162–166.

Publisher's Note: The authors of this article assert that all claims made herein are exclusively their own and may not necessarily reflect the views of their respective affiliated institutions or those of the publisher, editors, and reviewers. The publisher does not provide any guarantee or endorsement for any product subject to evaluation in this article or any claim made by its manufacturer.