Testing The Antibacterial Efficacy of Cocoa Bean Extractagainst The Growth of *Staphylococcus Aureus*

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Abstract
Background: Staphylococcus aureus is a gram-positive bacterium that is the causative agent of a wide variety of clinical diseases. Infections caused by this pathogen are prevalent in both community and hospital. Treatment of these infections is challenging due to the antibiotic resistance produced by these bacteria. On the other hand, cocoa beans, which contain polyphenols, flavonoids, alkaloids, saponins, and tannins, have potential as antibacterials. This study aims to assess the effectiveness of cocoa bean extract as an antibacterial alternative against Staphylococcus aureus. Methodology: The study used true experimental design with post-test only control group design. This study tested the effect of cocoa bean extract with concentrations of 25%, 50%, 75%, and 100% on the growth of Staphylococcus aureus bacteria as well as 2 control groups (positive control using vancomycin antibiotic and negative control using aquadest). The process of making cocoa bean extract involved maceration with 97% ethanol, followed by evaporation to obtain a thick extract. Antibacterial tests were performed using antibiotic disks dipped in cocoa bean extract and measurement of inhibition zone diameter. Result: This study shows that cocoa bean extract (Theobroma cacao) has antibacterial activity that varies depending on the concentration used. The higher the concentration of cocoa bean extract, the larger the diameter of the inhibition zone formed. Conclusion: There is an inhibitory effect of cocoa bean extract on the growth of Staphylococcus aureus in vitro.
Keywords: *Staphylococcus aureus and Cocoa beans (Theobroma cacao).*

I. INTRODUCTION

Bacteremia is a condition in which living bacteria are present in the blood. Bacteria are divided into two groups that are the most common causes of bacteremia. The first is gram-positive bacteria, namely *Staphylococcus aureus*, and the second is gram-negative bacteria, namely *Escherichia coli* [1]. Bacteria are prokaryotic organisms that constitute a large part of the normal flora of the body without causing infection [2]. The presence of normal flora in the body does not always provide benefits, because under certain circumstances, this normal flora can cause disease. One example of normal flora in the body is the bacterium *Staphylococcus aureus*. The bacterium *Staphylococcus aureus* can be found on the surface of the skin in approximately 20% of healthy adults and in the nasal mucosa of approximately 30% of healthy adults [3], [4]. However, when *Staphylococcus aureus* enters the bloodstream, it can cause infectious diseases, one of which is bacteremia resulting from treatment in an unsterile hospital environment. Common sources in hospitalized patients include the respiratory tract and indwelling catheters, particularly central venous catheters. Untreated urinary tract infections are also among the most common causes of community-acquired bacteremia [5]. The treatment of *Staphylococcus aureus* remains a challenge due to antibiotic resistance caused by the bacterium *Staphylococcus aureus*, which produces Penicillin-Binding Protein (PBP2α) encoded by the *mecA* gene and has low susceptibility to methicillin.

Cocoa beans have the scientific name *Theobroma cacao*. Cocoa beans are widely used because they are a raw material for chocolate production. In addition to being used in chocolate production, cocoa beans contain flavonoids and can also act as anti-inflammatory agents because they contain polyphenolic compounds. Cocoa beans contain polyphenols, flavonoids, alkaloids, saponins, and tannins, which provide many benefits to the body. Polyphenols are biologically active compounds that are widely distributed in plants and are among the most common phytochemicals with beneficial health effects, consisting of flavonoids, saponins, and tannins [6]. Polyphenols have antibacterial activity by acting as toxins in the protoplasm, damaging and penetrating the cell wall [7]. Flavonoids have also been identified as polyphenolic compounds that can provide antibacterial activity through various mechanisms of action. In several studies, flavonoids have been shown to inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [8]. Alkaloids are also present as heterocyclic compounds containing nitrogen. Furthermore, within the chemical core structure, alkaloids play a role in disrupting the components that make up peptidoglycan in bacterial cells, causing the cell wall layer to not form properly and resulting in cell death [9], [10]. In addition, saponins work by increasing the permeability of bacterial cell membranes, resulting in hemolysis or causing bacterial cells to rupture or lyse. Tannins, meanwhile, act by targeting the polypeptide components of the bacterial cell

wall, causing the formation of the cell wall to become incomplete and ultimately resulting in bacterial cell death [11]. Based on this background, long-term antibiotic use and irrational antibiotic use can lead to resistance, while cocoa beans (*Theobroma cacao*) contain antibacterial compounds and may potentially serve as an alternative to antibiotics. Therefore, I am interested in investigating the effectiveness of cocoa bean extract (*Theobroma cacao*) as an antibacterial agent against *Staphylococcus aureus*.

II. METHODS

The research method used was a *true experimental design* using a *post-test-only control group design*, in which the researcher only observed the results after the treatment or intervention had been administered. The experimental research was conducted by providing treatment in the form of cocoa bean extract at concentrations of 25%, 50%, 75%, and 100%, and then observing its effect on the growth of *Staphylococcus aureus*. Subsequently, observations and measurements were carried out in the treatment groups after receiving the intervention, consisting of six groups, namely four treatment groups and two control groups. The positive control used antibiotic vancomycin disk, while the negative control used distilled water. The treatment groups were P1, P2, P3, and P4, representing cocoa bean extracts at concentrations of 25%, 50%, 75%, and 100%, respectively. This research was conducted from August to October 2024. The preparation of the cocoa bean extract was carried out at the Medical Laboratory of Universitas Muhammadiyah Sumatera Utara. Identification of the *Staphylococcus aureus* bacteria and antibacterial testing of the cocoa beans were conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, and the FMIPA Laboratory, Universitas Sumatera Utara.

The process of preparing the cocoa bean extract initially involved preparing 1.5 kg of cocoa beans. The cocoa beans were washed until clean and dry, the pulp was removed, and the beans were dried in an oven for 2 days at a temperature of 60°C. The dried beans were then ground using a grinder or blender until finely powdered and subsequently sieved to obtain the powder (*simplisia*). The cocoa bean powder was then macerated by soaking it in 1.3 L of 97% ethanol as a solvent for 3 days, with stirring performed each day. Afterward, the macerated cocoa beans were filtered to obtain the liquid cocoa bean extract. The liquid extract was then evaporated using a *rotary evaporator* until a concentrated extract of 20 mL was obtained. The antibacterial testing procedure involved 16 samples and 4 additional petri dishes as reserves, all containing *Staphylococcus aureus*. The petri dishes had previously been prepared with *Mueller-Hinton Agar (MHA)*, which functioned as the medium for bacterial cultivation. Using an inoculating loop, a small amount of *Staphylococcus aureus* colony was taken and mixed with 1 mL of NaCl, with the turbidity compared with the *McFarland* standard. The bacterial suspension was then inoculated onto the petri dishes containing *Mueller-Hinton Agar (MHA)*, and one paper disk was placed in each dish. The paper disks had previously been immersed in the extracts mixed with DMSO (*dimethyl sulfoxide*) at concentrations of 25%, 50%, 75%, and 100%. Vancomycin was administered using antibiotic disk. Subsequently, all media were incubated in an incubator at 37°C for 24 hours. At the final stage, the diameter of the inhibition zone was measured using an automatic vernier caliper in millimeters (mm).

Number Of Replications

The total number of samples in this study was 16 samples, consisting of four treatment groups, with four replications for each group. The treatment groups consisted of four groups with concentrations of 25%, 50%, 75%, and 100%. In addition, a positive control group using vancomycin antibiotic disks and a negative control group using distilled water were also included.

Data Analysis

This study used a parametric test after confirming that the data followed a normal distribution. A Shapiro-Wilk normality test was conducted to determine whether the research data were normally distributed. If the data were confirmed to be normally distributed, a *One-Way ANOVA* test was subsequently performed to determine whether there were significant differences among the groups being tested. This was followed by a *Post-Hoc LSD* test to determine which groups had significant differences. If the data were not normally distributed, a parametric test could not be performed, and the non-parametric Kruskal-Wallis test was used to determine significant differences among groups, followed by the Mann-Whitney test to identify which groups showed significant differences at each concentration of cocoa bean extract.

III. RESULTS AND DISCUSSION

The results of this study were obtained from the clear zones (mm) produced by the cocoa bean extract and measured using an automatic vernier caliper. The diameters of the clear zones produced by the cocoa bean extract and the control groups against the growth of *Staphylococcus aureus* at several concentrations are presented in millimeters (mm).

Inhibitory Activity of Cocoa Bean Extract (*Theobroma cacao*) Against *Staphylococcus aureus* and the Effectiveness of Cocoa Beans Against *Staphylococcus aureus*

Kelompok	Pengulangan				Rata-rata
	1	2	3	4	
25%	8,35	9,05	6,40	5,35	7,29
50%	8,3	9,85	10,4	11,5	10,01
75%	8,95	12,6	10,95	10,6	10,88
100%	12,45	13,65	11,35	15,80	13,31
Kontrol (+)	22,35	24,15	23,21	22,40	23,02
Kontrol (-)	0	0	0	0	0

The results of administering several concentrations of cocoa bean extract showed differences in the clear zones obtained. At a concentration of 25%, the cocoa bean extract in the second replication produced the highest clear zone, with a value of 9.05 mm. At a concentration of 50%, the highest clear zone was obtained in the fourth replication, with a value of 11.5 mm. At a concentration of 75%, the highest clear zone was obtained in the second replication, with a value of 12.6 mm. Meanwhile, at a concentration of 100%, the highest clear zone was obtained in the second replication, with a value of 13.65 mm. In the positive control group using vancomycin, the highest clear zone was obtained in the second replication, with a value of 24.15 mm, while the negative control group using distilled water did not produce a clear zone.

IV. DISCUSSION

A previous study conducted by Dame J Pohan entitled "The Effectiveness Test of Cocoa Bean Extract (*Theobroma cacao*) as an Antibacterial Agent Against *Streptococcus pyogenes*" found that the most effective antibacterial effect was produced at a concentration of 100% [12]. In addition, a previous study conducted by Dafista Diyantika concerning morphological changes in *Staphylococcus aureus* following exposure to cocoa bean extract reported that cocoa bean ethanol extract at a concentration of 15.6 mg/mL could cause morphological changes in *Staphylococcus aureus* [12]. Based on another study conducted by Helda Dwiya Lestari entitled "Antibacterial Activity of Cocoa Bean Extract Against *Staphylococcus epidermidis*," the most effective concentration was 100%, and the higher the concentration, the greater the antibacterial effect produced [13]. Compared with the present study, the strongest inhibitory effect was also observed at a concentration of 100%.

V. CONCLUSION

Based on the results of the research and discussion, it can be concluded that cocoa bean extract (*Theobroma cacao*) has antibacterial activity against the growth of *Staphylococcus aureus* at concentrations of 25%, 50%, 75%, and 100%. A concentration of 100% cocoa bean extract (*Theobroma cacao*) produced the most effective inhibition zone in inhibiting the growth of *Staphylococcus aureus*.

Following the study on the effectiveness of cocoa bean extract (*Theobroma cacao*) against the growth of *Staphylococcus aureus in vitro*, further research is recommended to investigate the antibacterial effects of cocoa bean extract using other methods, such as *in vivo* methods. Future studies may also examine cocoa bean extract against *Staphylococcus aureus* obtained from different sources of infection and expand the testing to other microorganisms, such as fungi, viruses, and parasites.

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