

Effectivity of Cacao Rind Ethanol Extract in Inhibiting *Streptococcus Pyogenes* Growth *In Vitro*

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ABSTRACT

Introduction: Infectious disease is still a common cause of illness and death in developing countries, such as Indonesia. One of the bacteria that causes infectious disease is *Streptococcus pyogenes*. Cacao fruit is a large commodity in Indonesia and has benefit for human. Cacao's rind is known to contain several active compounds such as flavonoid and alkaloid that have antibacterial effect that can inhibit *Streptococcus pyogenes* growth. This research aims to evaluate the Minimum Bactericidal Concentration (MBC) of cacao rind ethanol extract in inhibiting *Streptococcus pyogenes* growth *in vitro*.

Methods: This research was a laboratory experimental study, testing antibacterial activity of cacao's rind ethanol extract in inhibiting growth of bacteria *Streptococcus pyogenes* using dilution method *in vitro* to know the MIC and MBC result. Sample of bacteria *Streptococcus pyogenes* was obtained from Balai Besar Laboratorium, Surabaya. Sample of cacao's rind ethanol extract was extracted at Balai Materia Medica, Batu.

Results: At the beginning this experiment was done to find the MIC and MBC of cacao's rind ethanol extract against the growth of bacteria *Streptococcus pyogenes*, but the researcher can only find the MBC result, because the extract color is very dark, so the turbidity result of tubes P1 – P7 cannot be compared to control tube. From the results, the researcher draws a table showing how turbid and dark those tubes are. More (+) signs means more turbid or darker the tube is. From dilution test, the MBC of cacao's rind ethanol extract against the growth of bacteria *Streptococcus pyogenes* is 12.5%.

Conclusion: Cacao's rind (*Theobroma cacao* L.) was quite effective in increasing the growth of bacteria *Streptococcus pyogenes* *in vitro*, the Minimum Bactericidal Concentration (MBC) is 12.5%

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Introduction

Infectious disease is still a common cause of illness and death in developing countries, such as Indonesia. Infectious disease gives enormous impacts on the patients, such as physical suffering, lower working capability, and productivity that will lead to material loss.¹

One of the bacteria that causes infectious disease is *Streptococcus pyogenes*, an extracellular gram positive bacteria that colonize the throat or skin which are highly potential in causing suppurative and non suppurative infections. The most common infections caused by *Streptococcus pyogenes* are pharyngitis, scarlet fever, and impetigo. Streptococcus has been an epidemic for the last ten years with M1 stereotype as predominant.²

Group A Streptococcus has developed and become resistant to clindamycin and macrolides antibiotic, such as erythromycin, azithromycin, and clarithromycin. Group A Streptococcus also becomes resistant to tetracycline.³

Cacao crop (*Theobroma cacao* L.) is a large commodity in Indonesia. Cacao fruit consist of three parts, 74% rough rind, 24% seed, and 2% fruit placenta. The most widely used part from cacao fruit is its bean or seed, cacao bean is one of the largest export commodity so the other parts of cacao plants go to waste and become an environmental problem. From the data above, it is known that cacao's rind is the largest waste from cacao plant. In 2007, the number of cacao's rind waste was 508.84 tons and 545.88 tons in 2008.^{4,5}

Cacao's rind is known to contain several active compounds such as flavonoid and alkaloid that have antibacterial effect. Cacao's rind also contain 3 importants polyphenols that have antioxidant effect, such as proantocyanidine, catechin, and antocyanidine.^{6,7}

Previous experiment showed that the type of cacao's rind extract named Lindak may inhibit growth of *Staphylococcus aureus*, *Streptococcus mutan*, *Eschericia coli*, and *Salmonella thypii* bacteria.⁸

Methods

This study was a laboratory experimental study, testing antibacterial activity of cacao's rind ethanol extract in inhibiting growth of bacteria *Streptococcus pyogenes* using dilution method in vitro to know the MIC and MBC result. Sample of bacteria *Streptococcus pyogenes* was obtained from Balai Besar Laboratorium, Surabaya. Sample of cacao's rind ethanol extract was extracted at Balai Materia Medica, Batu.

Cacao's rind was first dried under the sun and then turned into powder using a blender and weighed up to 2 kilograms. Then, maceration technique was used, up to 10 liters of 96% ethanol solvent. This maceration was done for 2 days and then evaporated using rotatory evaporator of 50 rpm speed for 2 days. From those process, extract of 50 mL was produced. (UPT Materia Medica Batu 2016)

Suspension of bacteria *Streptococcus pyogenes* was first prepared in Muller-Hinton Broth until the turbidity is equal to Mc Farland 0.5 or 1.5×10^8 CFU/mL.

First, 9 sterile tubes were prepared, 2 for negative and positive controls (K1 & K2) and 7 for treatments (P1 – P7). K1 tube as negative control was given 1 mL ethanol extract 100%. K2 tube as positive control was given 1 mL broth solution and 1 mL of bacteria suspension. P1 tube was filled with 1 mL of ethanol extract 100% and 1 mL bacterial suspension. P2 – P7 tubes were filled with 1 mL of broth. P2 was added with 1 mL of ethanol extract 100% and then mixed and 1 mL of it was taken to be moved to P3 tube. This mixture-moving steps were repeated until P7 tube. When the last tube came, remove 1 mL out of P7. From these steps, it was obtained tube P1 with 100% concentration, P2 with 50% concentration, P3 with 25% concentration, P4 with 12.5% concentration, P5 with 6.25% concentration, P6 with 3.125% concentration, and P7 with 1.56% concentration. All tubes were incubated in 37°C temperature for 24 hours. This procedure was replicated for four times.

Results

At the beginning this experiment was done to find the MIC and MBC of cacao's rind ethanol extract against the growth of bacteria *Streptococcus pyogenes*, but the researcher can only find the MBC result, because the extract color was very dark, so the turbidity result of tubes P1 – P7 cannot be compared to control tube. From the results, the researcher draws a table showing how turbid and dark the tubes were. More signs (+) means more turbid or darker the tube was.

From the dilution test, samples were taken from each tube and were planted in blood agar plate, then were incubated for 24 hours in 37°C temperature. MBC s used to find out how much minimum concentration is needed to kill that bacteria. The MBC of cacao's rind ethanol extract against the growth of bacteria *Streptococcus pyogenes* is 12.5%.

Table 1. Results of dilution test.

Replication	Cacao's rind ethanol extract							Control	
	100 %	50 %	25 %	12.5 %	6.25 %	3.125 %	1.56 %	+	-
1	+++++	++++	+++	+++	++	+	+	-	+++++
2	+++++	++++	+++	+++	++	+	+	-	+++++
3	+++++	++++	+++	+++	++	+	+	-	+++++
4	+++++	++++	+++	+++	++	+	+	-	+++++

Source: Research Data, Processed

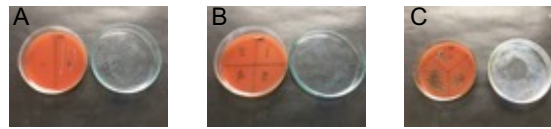


Figure 1. Planting the dilution results on blood agar plates. A. Control; B. Treatment 1-4; C. Treatment 5-7.

Table 2. Results of MBC test.

Replication	Cacao's rind ethanol extract							Control	
	100 %	50 %	25 %	12.5 %	6.25 %	3.125 %	1.56 %	+	-
1	-	-	-	-	+	+	+	+	-
2	-	-	-	-	+	+	+	+	-
3	-	-	-	-	+	+	+	+	-
4	-	-	-	-	+	+	+	+	-

Source: Research Data, Processed

Discussion

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of antimicrobial that can prevent growth of an organism after being subcultured on free antibiotic media. MBC of cacao's rind ethanol extract against the growth of bacteria *Streptococcus pyogenes* was found at 12.5%. The bactericidal effect of cacao's rind ethanol extract s because of its active compounds that work as antibacterial. Those active compounds are flavonoid and alkaloid. Alkaloid is an alkaline that consists of nitrogen atoms, thus it can cause protein coagulation which leads to disruption of the components of bacterial cell wall peptidoglycan. This condition causes bacterial cell wall cannot be fully formed and leads to cell death.⁶

Flavonoid works by inhibiting the function of bacteria's cytoplasm membrane, energy metabolism, and nucleic acid synthesis. In inhibiting nucleic acid synthesis, beta-flavonoid ring creates hydrogen bond with some nucleic acid bases and then these bonds can inhibit the synthesis of bacteria's DNA and RNA. Bacteria's cytoplasm membrane function is inhibited by lowering the membrane cell wall permeability, impairing the lipid bilayer membrane, and harm barrier function and thus can cause cell death. Inhibition of energy metabolism is related to RNA, DNA, protein, and cell wall synthesis activity.⁹

The extraction of cacao's rind was done using ethanol 96% solvent. This concentration means that water component is only 4%. Ethanol is a solvent with universal properties that can dissolve polar and non-polar compounds. With such capability, it is expected that all active compounds needed can be fully extracted. The higher the purity of ethanol, the less water content, thus facilitating separation of active components from its solvent. Just like other experiment, ethanol is the best solvent for extracting tannin from *Mimosa pudica* plant. Rosella flower (*Hibiscus sabdariffa* L) 96% ethanol extract was also proven to be more active than 30% ethanol extract with greater inhibition area and smaller Minimum Bactericidal Concentration.^{10, 11}

Conclusion

Cacao's rind (*Theobroma cacao* L.) is quite effective in increasing the growth of bacteria *Streptococcus pyogenes* in vitro, the Minimum Bactericidal Concentration (MBC) is 12.5%.

CONFLICT OF INTEREST

The author stated there is no conflict of interest in this study.

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