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Effect of catechin concentration on transparent soap as antibacterial activity

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Abstract

Gambir was an extract derived from the gambier plant (Uncaria gambir, Roxb). Traditionally gambier was used by the community in West Sumatera for a traditional event. In industry, gambier was used as a dye for textiles, pharmaceutical, cosmetics and as an antioxidant and antibacteria. Catechin was one of the main compounds in gambier which has several bioactivities, such as antioxidant, antifungal and disinfectant. In this study, the use of catechin compound will be carried out as a disinfectant of transparent soap. The study was carried out in three stages, namely extraction of catechin from gambier, transparent soap making and the microbial assay of the soap. Extraction of catechin was conducted by crystallization method in 12.5% ethanol solution at a temperature of 5-10 oC. The process of making soap uses six formulas of catechin. The microbial assay was used the agar diffusion method with two test bacteria namely Escherichia coli, and Staphylococcus aureus. The results showed that the five types of soap had inhibitory power on both test bacteria. The using of catechin concentration 0.2 g / 100 g - 0.8 g / 100 g was effective in inhibiting the growth of test bacteria. The difference in the concentration of catechin in soap had an effect on the inhibition power of both test bacteria.

Keywords: antibacterial, catechin, E. coli, gambier, S. aureus, soap

INTRODUCTION

Disinfectant soap is a cleaning agent that is used to maintain body health and hygiene. It have the antibacterial agents that can either kill or inhibit the bacterial cells [1]. Disinfectant soap is used to wash hands or take a bath so that the disinfectant content in the soap can be used against disease-causing bacteria (pathogens). Disinfecting soap can be used to clean the surface of the skin with surface tension and bind particles, oil, bacteria, and viruses. Previously, disinfectant soap was only used in hospitals, but now the use of disinfectant soap has become widespread as a daily necessity in homes.

The utilization of natural products from plants as disinfectants needs to be developed considering the synthetic disinfectants that used in soap products can increase the immunity system of pathogenic bacteria. Besides that it is also reported that chemicals as disinfectants in soap can cause skin irritation, affect and interfere with thyroid hormone function, and are carcinogenic [2]. In addition, synthetic antibacterial compounds used in soap can produce harmful substances such as dioxin and chloroform that can pollute the environment. The compound has been detected in sewage treatment plant effluents, surface, ground and drinking water [3]. Some medicinal plant are reported to have antibacterial activity, such as Glycyrrhiza glabra (Licorice) [4], C.papaya [5], Allium Kurrat [6]. Lately, there is a desire to get disinfectants from natural products in herbal soap [7,8].

Catechin, one of the the natural products is found in gambier plant [9,10], green tea leaf extract [11-15]. *Terminalia arjuna* bark [16], *Toona sureni bark* [17], *Murraya koenigii* [18], *Acasia catechu* [19] and *Acacia nilotica* [20]. Catechin is one of the the natural products obtained from leaves and twigs of gambier (*Uncaria gambir*, Roxb) which has antioxidants [21], disinfectants [22], anticariogenic [23], antifunga [24] and antibactery

activities [25,26]. It was used in treating and preventing infectious diseases[27].

MATERIAL AND METHODS

Materials anf chemicals

Gambiers were obtanined from Surantih district of West Sumatera. Castor oil was obtanined from Medan, Nort Sumatera. The chemicals and reagents used were grade analytical grade. Microorganisms tested in this study were *Escherichia coli* and *Staphylococcus aureus*.

Extraction of Catechin from Gambier:

Gambier were grinded to get a smaller sizes to form the powder up to 100 mesh. 100 grams of the powders were dissolved with 500 mL ethyl acetate in the erlenmeyer using a hot plate equipped with a magnetic sirrer. Heating was done for 1 hour at 60 °C until all the powder dissolves. The solution was filtered to separate the gambier solution from the impurities. The solvent was evaporated using a rotary evaporator to separate ethyl acetate to form a extract. The extract was dissolved in 125 mL ethanol 2.5% at 60 °C. Furthermore, the solution was cooled in ice-water at a temperature of 5-10 °C for 2-3 hours to form the crystal of catechin. The catechin was separated using a Büchner funnel equipped with a vacuum pump. This catechin separation process was carried out 7-10 times to yield the pure crystal. The catechin was analyzed by using the melting point method, thin layer chromatography (TLC), and FTIR.

Determination of The Saponification Value (SV)

A sample of 5 grams of castor oil was put in a flask, then added 50 mL alcoholic KOH and refluxed for 1 hour. After the reflux process was completed, 1 mL pp indicator was put into erlenmeyer. The solution was titrated with 0.5 N HCl until the pink color disappeared. Repeat the procedure for the blank. The saponification value was determined by the formula:

$$SV = \frac{(b-a)xNx56.1}{W(q)}$$

where:

SV = saponification value b = mL KOH needed for blank titration a = mL KOH needed for sample titration N = Normality of HCl W= weight of sample

Transparent Soap Making

The castor oil was heated in a stainless steel pan on a magnetic stirrer hotplate at a temperature of 65-70 °C for 5 minutes. Beside that NaOH 30% solution was heated a 250 mL beaker at 65 °C for 5 minutes. Put slowly the 30% NaOH solution into castor oil to form soap and keep the temperature at 65-70 °C while stirring using a magnetic stirrer until homogeneous. Heated stearic acid in a 100 mL beaker to become liquid. Dissolve sugar with aquadest in a 100 mL beaker while heated. Put into the mother soap slowly: liquid stearic acid, glycerin, sugar solution, comperland, 95% alcohol, coloring and catechins. Continued stirring and heating at 65 °C until the soap solution was homogeneous. Added deodorizer and other additives as needed, then pour into the mold. Labeled the soap, then the soap wrapped in plastic packaging.

Disinfecting Activity Testing

Nutrient Agar (NA) was heated in a sterile flask then let it cool at 40-50 °C. An amount of 15 mL NA was poured into a sterile petri dish (9 cm in diameter) and left at room temperature. Distribute homogeneously as much as 0.1 mL of bacterial culture into the medium in a petri dish. By aseptic the soap plates (20 mm diameter) from formula I, II, III, IV, V, and a comparison were placed on the surface of the agar by making a slight press. Place the petri dish in an incubator at room temperature for 18-24 hours. Inhibition area was measured in mm.

RESULTS AND DISCUSSION *Extraction and Purification of Catechin*

Extraction and purification of catechin from gambier produced white catechins as much as 11.03 g (11.03%) as shown in Fig.1.



Fig.1- Catechin from gambier

Analysis of catechin

Determination of the melting point

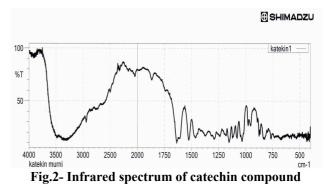
Determination of melting point for catechins using digital SMP apparatus melting point apparatus. The results of determining the melting point of gambier catechin showed that gambier catechin melt at a temperature of 175-177 °C.

Thin Layer Chromatography (TLC)

The sample was dissolved in ethyl acetate, then it was spotted on a TLC plate. It was inserted in the eluent (*n*-hexane eluent: Ethyl acetate (3: 7)) in the cumber. The results of catechin testing by TLC produced a single spot with Rf 0.375.

FTIR Spectrum Analysis

From the analysis of infrared spectrum (Fig.2), it showed that the sample contains a hydroxyl group. This was proven by the high intensity that appears at 3350 cm⁻¹ caused by OH bending vibrations. The appearance of two strong uptake at 1650 cm⁻¹ and 1525 cm⁻¹ strengthens the aromatic absorption system. Interpretation of combination analysis supports samples that have OH groups in the aromatic ring.



Disinfectant testing

Disinfectant testing was done by determining the inhibition zone of soap against two test bacteria, namely *E.coli* and *S. aureus*. The Zones of inhibition revealed to Fig.3. The results of the inhibition test can be seen in Table 1.



Fig.3- The zones of inhibition reveal lack of growth of the tested bacteria.

test, E.coli and S. aureus		
Cathecin (gram/100 g oil)	Inhibition zone in diameter (mm)	
	E. coli	S. aureus
0	20	20
0,2	26	30
0,4	35	33
0,6	44	32
0,8	36	36
Disinfectant soap	24	29

 Table 1: Inhibition zone of the soaps to two bacterial test, E.coli and S. aureus

CONCLUSION

The using of catechin concentration 0.2 g / 100 g - 0.8 g / 100 g was effective in inhibiting the growth of test bacteria on transparent soap. The difference in the concentration of catechin in the soap had an effect on the inhibition power of both test bacteria.

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