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Formulation and Antibacterial Activity of Natural Disinfectant Combination of *Psidium guajava* and *Piper betle* Leaf Infusion Against *Staphylococcus aureus*

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ABSTRACT
Spraying disinfectant all over the body in the disinfection room is being used to prevent the spread of the SARS-CoV-2 as the cause of the Covid-19 disease. Due to chemical disinfectants have high toxicity to the body, a relatively safe natural disinfectant is needed. The purposes of this study were to obtain a natural disinfectant solution formula combination of guava leaf infusion (*Psidium guajava* L.) and betle leaf infusion (*Piper betle L.*) and to determine the antibacterial activity of these formulas. In this research, a combination formula of guava leaf and betle leaf infusion with three formulas were formulated, contains 20% guava leaf infusion and 25% betle leaf with ratio of 1: 3, 1: 1, and 3:1 respectively, namely F2, F3 and F4. F0 was chemical disinfectant as positive control and F1 as negative control. Antibacterial activity test against *Staphylococcus aureus* conducted on a petri dish using disc diffusion method. The highest inhibition zone was formed by F4 with average diameter of 10.2 mm, categorized as strong level of antibacterial activity against *Staphylococcus aureus*. The results confirmed that natural disinfectant solution formula combination of guava leaf infusion and betle leaf infusion had potential agents as natural disinfectant.

Keywords: *Natural disinfectant, Psidium guajava, Piper betle, leaf infusion, antibacterial activity, Staphylococcus aureus*

1. INTRODUCTION

The spread of the SARS-CoV-2 virus as the cause of the Covid-19 disease is increasingly becoming massive throughout the world. Various efforts to prevent the transmission of this virus have been carried out, ranging from the use of antiseptic hand sanitizers, the use of masks, social distancing, self-isolation at home and also including maintainingthe cleanliness of the house and clothing using disinfectants [1].

The mechanism of action of disinfectants in inactivating coronavirus has similarity in their mechanism of killing *Staphylococcus aureus* bacteria, namely by damaging the viral bilayer membrane and damaging the permeability of the bacterial cell membrane [2].

Spraying of disinfectants all over the body in disinfection booths is currently being used. The Environmental Protection Agency (EPA) does not recommend the use of disinfectant products that have not been tested for efficacy when used with fogging, electrostatic spray or spray application methods [3].

Inhalation of chlorine gas and chlorine dioxide can cause severe irritation of the respiratory tract [4]. The use of hypochlorite solutions at low concentrations continuously for a long period of

time can cause skin irritation and damage to the skin. Use at high concentrations may result in severe skin burns. Although data are still limited, inhalation of hypochlorite can cause irritation to the respiratory tract [4].

The World Health Organization (WHO) does not recommend the use of alcohol and chlorine on all body surfaces because they will harm mucous membranes of the body such as the eyes and mouth [5]. Phenol was initially used as a disinfectant in household cleaners (Dettol, Phenol) [6]. In the concentration of 0.01 – 1%, phenol is bacteriostatic while in 1.6% solution is a bactericidal which can cause protein coagulation. Phenol bonds with protein are easily separated, so that phenol can penetrate into intact skin [7]. Phenol solution is fungicidal used for sterilization of excreta and medical equipment. In toxicology this compound is important, because it is often used in suicide attempts. Against the mucosa of the gastrointestinal tract and mouth, this material is caustic and corrosive. The central nervous system (CNS) causes excitation followed by depression [8].

Guava leaves (*Psidium guajava* L.) contain quite a lot of phenolic compounds including tannins and flavonoids [9]. The tannins contained in guava leaves are 90,000-150,000 ppm or about 9%. Tannins as antimicrobials are caused by the presence of pyrogalol groups and galloil groups which are phenol groups that can inhibit bacterial growth or killthem by reacting with bacterial protein cells so that protein denaturation occurs [10]. The presence of denaturation of the bacterial cell wall causes disruption of bacterial metabolism resulting in damage to the cell wall which eventually causes cell lysis, so that guava leaves are antimicrobial [10].

Betle leaf can be used as an antibacterial because it contains 4.2% essential oil, which mostly consists of betephenol, caryophyllen (sisquiterpene), chavicol, cavibetol, estragol, and terpenes [11]. The main components of essential oils consist of phenols and their derivative compounds. One of the derivative compounds is chavicol which has a bactericidal power five times stronger than phenol [11]. The antibacterial power of betle leaf (*Piper betle* L.) essential oil is due to the presence of chavicol compounds which can denature bacterial cell proteins [12]. In addition to functioning as an antibacterial, flavonoids contain chavicol and cavibetol which are derivatives of phenol which have antibacterial power five times that of ordinary phenol against *Staphylococcus aureus.*

By looking at the increasing conditions of the Covid-19 outbreak, the need for natural and safe disinfectants for the body, as well as looking at the natural resources of guava leaves and betle leaves which contain chemicals that have antiseptic power, this research was carried out to formulate natural disinfectant preparations from the infusion of guava leaves (*Psidium guajava* L.) and betle leaves (*Piper betle* L.) and obtained the level of their antibacterial activity against *Staphylococcus aureus*.

2. MATERIAL AND METHODS

2.1. Materials

Guava leaves (*Psidium guajava* L.), betle leaves (*Piper betle* L.), X brand disinfectant, *Staphylococcus aureus* (ATCC 25923) , Nutrient Broth media (*Himedia)*, spiritus, sterile cotton, 0.9% NaCl, distilled water, Tool Technical and analytical balance (*Ohaus*), pH meter (*Eutech Instrument pH 510, Singapore*), stove,infusion pan, autoclave (*Techmech*), incubator (*IN55*), hot plate (*Cimarec SP88857105),* glassware (*pyrex*), test tube, petri dish (*Corning*), micro pipette (*AMT-Y09 200),* bunsen, thermometer .

2.2. Methods

2.2.1. Determination of Guava and Betle Plants

The guava and betle plants to be used were determined at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu.

2.2.2. Infusion Preparation

Guava leaf infusion (Psidium guajava L.) has a concentration of 20% as an antiseptic [11]. Fresh guava leaves were cleaned and then finely chopped. A total of 200 grams of fresh guava leaves and 1000 ml of distilled water were boiled to obtain an infusion concentration of 20%. Boiled

for 15 minutes after the water temperature was 90°C while stirring occasionally, then filtered to got only the infusion solution.

Betle leaf infusion (*Piper betle* L.) has a concentration of 25% as an antiseptic [10]. Fresh betle leaves were cleaned and then finely chopped. A total of 250 grams of fresh betle leaves and 1000 ml of distilled water were boiled to obtain an infusion concentration of 25%. Boiled for 15 minutes after the water temperature was 90°C while stirring occasionally, then filtered to got only the infusion solution [13].

2.2.3. Preparation of Disinfectant Solution

The formula for the disinfectant solution combination of guava leaf infusion (*Psidium guajava* L.) and betle leaf infusion (*Piper betle* L.) made as many as four formulas which can be seen in Table 1.

Table 1 Desinfectanct solution formulas

Ingredients	F0 (%)	F2 (%)	F3 (%)	F4 (%)
X brand disinfectant solution	100	-	-	-
Guava leaf infusion (20%)	-	25	50	75
Betle leaf infusion (25%)	-	75	50	25

2.2.4. Evaluation of Disinfectant Solution

2.2.4.1. Organoleptic Observations

Organoleptic observations were made on the color, aroma and taste of each formula.

2.2.4.2. pH Test

Measurement of the pH of the disinfectant solution using a pH meter that has been calibrated with a buffer solution of pH 4 and pH 7.

2.2.5. Sterilization of Tools and Materials

Tools and materials to be used were sterilized to avoid contamination in the test. All tools made of glass were sterilized using an autoclave, while other tools could be sterilized by glowing in a bunsen lamp or dipped in alcohol and passed over a bunsen flame.

2.2.6. Preparation of Media

Nutrient Broth as much as 20 grams was dissolved in 500 mL of distilled water and then heated and homogenized using a heating device and stirred evenly. The agar media must be completely homogeneous and prior to use, the media were sterilized using an autoclave at a temperature of 121ºC with a pressure of 1 atm for 15 minutes [13].

2.2.7. Preparation of Staphylococcus aureus

Staphylococcus aureus used in this study was cultured in a test tube. Pure cultures of *Staphylococcus aureus* bacteria were aseptically using a zigzag inscribed needle and incubated for 24 hours. Besides being recultured on agar slanted, bacteria were also recultured on petri dishes. Before being used in conducting the test,

Staphylococcus aureus was diluted. Dilution of *Staphylococcus aureus* was carried out by taking 2-3 bacterial colonies in a petri dish and putting it into a test tube containing 10 mL of physiological NaCl solution, homogenized using a vortex [13].

2.2.8. Antibacterial activity test with the Disc Diffusion Method

A filter paper plate containing a dilution of the disinfectant solution was placed on an agar medium that has been planted with *Staphylococcus aureus* which would be diffused into the agar medium. The observations were made for 24 hours [14].

This study used the infusion method as an extraction method, which if compared to the maceration method as in previous studies, this method is more simple and easier to carry out and applied by the community. The solvent used by the infusion method, namely distilled water, is also a solvent that is easy to obtain, cheap, stable, non-flammable, and non-toxic. The formula for the disinfectant solution combination of guava leaf infusion (*Psidium guajava* L.) and betle leaf infusion (*Piper betle* L.) made as many as four formulas, F0 as the standard, namely brand X disinfectant on the market, F1 as negative control, F2, F3 and F4 as the test solution. Organoleptic observations were made on the color, aroma and taste of each formula. The results of organoleptic observations can be seen in Table 2.

3. RESULTS AND DISCUSSION

Table 2 Results of organoleptic observations of guava leaf infusion, betle leaf infusion and guava leaf andbetle leaf disinfectant

Formula	Colour	Odor	Flavor
F0	Transparant	Phenol scent	Bitter
F 1	Transparant	No Odor	No Flavor
F 2	Dark Brown	Dominant aroma of betle leaf	Bitter
F 3	Dark Brown	Dominant aroma of betle leaf	Bitter
F 4	Dark Brown	Strong betle leaf aroma, a littleguava leaf aroma	Bitter

Measurement of pH was carried out to determine whether the disinfectant preparation was in accordance with the pH of the skin and was safe to use disinfectant on the skin. The results of pH observations can be seen in Table 3.

Table 3 The results of pH measurements on F0, F1, F2, F3 and F4

Formula	F0	F1	F2	F3	F4
pH	6.82 ± 0.03	7.01 ± 0.01	5.40 ± 0.02	5.33 ± 0.05	5.11 ± 0.01

Guava leaves (*Psidium guajava* L.) contain quite a lot of phenolic compounds including tannins and flavonoids, so that guava leaves are antimicrobial.

The presence of tannins and flavonoids is what causes guava leaf infusion to be acidic [15]. Betle leaf contains 4.2% essential oil, which mostly consists of betephenol, caryophyllen (sisquiterpene), chavicol, cavibetol, estragol, and terpenes. The main components of essential oils consist of phenols and their derivative compounds. One of the derivative compounds is chavicol which has a bactericidal power five times stronger than phenol. The presence of these substances causes the pH of the betle leaf infusion to be acidic [16]. Disinfectant solution infusion of guava leaves and betle leaf has an acidic pH because it contains most of the flavonoids and tannins. In the pH range obtained, the disinfectant solution infusion of guava leaf and betle leaf is still safe for the skin [17].

Microbiological tests to determine the effectiveness of natural disinfectants were carried out using the disc diffusion method. The filter paper plate was dripped with 20µl of disinfectant solution (F2,F3,F4) and a positive control with the infusion composition of betle leaf and guava leaf, respectively, 1:3, 1:1, 3:1. then placed on agar media that has been planted with Staphylococcus aureus. The clear area indicates the inhibition of the growth of microorganisms by the disinfectant solution on the surface of the agar medium [18]. The presence or absence of antibacterial activity is known by looking at the ability of the disinfectant solution tested by the formation of the diameter of the inhibition zone which is visible in the presence of a clear area around the filter paper disk [19]. The results of observations made within 24 hours, can be seen in Figure 1.

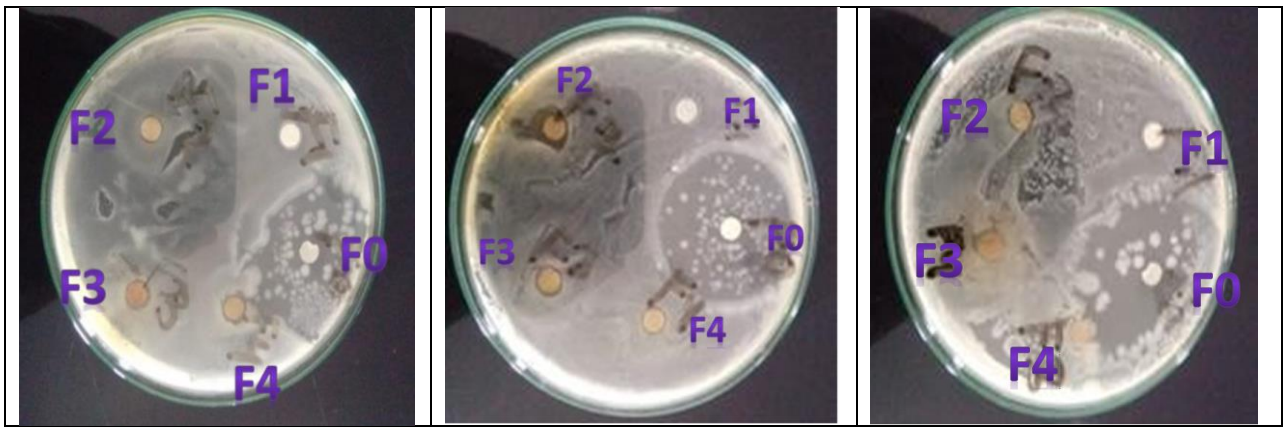

Figure 1 Observation results of the inhibition zone of the disinfectant solution against *Staphylococcus aureus* after being incubated for 24 hours for 3 times.

Figure 1 shows that an inhibition zone was formed around the disc in the positive control group as well as in F2, F3 and F4 with the composition of betle leaf and guava leaf infusions of 1:3, 1:1, 3:1, respectively. The largest inhibition zone was formed by infusion with a concentration of 75% guava leaves and 25% betle leaf with the smallest diameter of 10.1 mm, the 1argest diameter of 10.3 mm, and the average diameter of 10.2 mm. Disinfectant solution brand X as a positive control in this study showed an average inhibition zone of 40.2 mm. The results in Table 4 show that the greater the concentration of guava leaf infusion, the greater the inhibition zone formed. According to Davis and Strout (1971), the criteria for antibacterial strength were as follows: an inhibition zone diameter of 5 mm or less was categorized as weak, an inhibition zone of 5-10 mm was categorized as moderate, 10-20 mm was categorized as strong and an inhibition zone diameter of more than 20 mm was categorized as very strong. Based on these categories, the inhibitory agents produced by the infusion of guava leaf and betle leaf disinfectant solutions at concentrations of 25% and 75% were categorized as the strongest among the others. Infusion of guava leaves and betle leaf has antibacterial properties because it contains quite a

lot of phenolic compounds including flavonoids and tannins [20]. Thetannin content in guava leaves is 9%, which is more than other compounds contained in the leaves, namely 6% fat, 3% resin, and 0.4% essential oil (eugenol). The antibacterial power of tannins is due to the presence of pyrogalol groups and galloil groups which are phenol groups that can inhibit bacterial growth or kill them by reacting with protein cells from bacteria so that protein denaturation occurs [21]. Denaturation of proteins in the bacterial cell wall causes disruption of bacterial metabolism resulting in damage to the cell wall which eventually causes lysis [10]. Flavonoids have an antibacterial effect through their ability to form complex bonds with extracellular proteins of the bacterial cell wall, this will damage the integrity of the cell wall and eventually the cell wall is damaged and causes lysis [11].

Table 4 The results of antibacterial activity test of natural disinfectant solution betle leaf infusioncombination of guava leaf infusion against *Staphyloccocus aureus*

Formula	Inhibition Zone Diameter (mm)				
	Dish 1	Dish 2	Dish 3	Average	Activity Level
F0	46,7	30,4	43,6	40,2	Very Strong
F1	-	-		-	-
F2	6,5	5,8	6,7	6,3	Moderate
F3	9,9	9,7	9,2	9,6	Strong
F4	10,2	10,1	10,3	10,2	Strong

4. CONCLUSION

In this study, the best formula for natural disinfectant solution combined with guava leaf infusion (Psidium guajava L.) and betle leaf infusion (Piper betle L.) which can inhibit the growth of Staphylococcus aureus bacteria is formula F4 combination of 75% guava leaf infusion + 75% guava leaf infusion Betle Leaf 25% which has the highest inhibition against *Staphylococcus aureus* bacteria with an average inhibitory diameter of 10.2 mm. The results confirmed that natural disinfectant solution formula for a combination of guava leaf infusion (*Psidium guajava* L.) and betle leaf infusion(*Piper betle L.*) had pottential agents as natural disinfectant.

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