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ORIGINAL ARTICLE

Antibacterial Potency of Indonesian Randu Honey Against *Staphylococcus* sp.

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ABSTRACT

Introduction: Honey is known to be used as an antimicrobial to treat infections caused by bacteria. Randu honey is produced by flower-sucking bees cultivated in the tropical forest and consumed nectar from the flower of randu tree (*Cheiba pentandra*). The purpose of this study was to determine the activity of randu honey on the growth of Gram-positive bacteria, *Staphylococcus* sp. **Methods:** This study used well-diffusion method with gradient concentration of (20 µl, 40 µl, 60 µl, 80 µl and 100 µl). The bacteria used in this study were *Staphylococcus aureus* and *Staphylococcus epidermidis* which inoculated on Muller-Hinton agar media. The inhibition zone was measured after incubation for 18-24 hours to determine the inhibition of honey randu against the growth of bacteria. **Results:** There was a difference in the minimum concentration of randu honey which can inhibit the growth of Staphylococcus aureus and Staphylococcus epidermidis. The inhibition zone was found at the concentration of 40 µl randu madu (0.67 mm ± 1.15), 60 µl (7.67 mm ± 7.50), 80 µl (10 mm ± 9.17), and 100 µl (22.67 mm ± 3.05) for *Staphylococcus epidermidis*, while 80 µl (2.67 mm ± 4.62) and 100 µl (13 mm ± 1.73) for *Staphylococcus aureus*. There was no significant effect ($p>0.05$) of the randu honey concentrations on the diameter of the inhibition zone of *Staphylococcus aureus* (0.09) and *Staphylococcus epidermidis* (2.97). **Conclusion:** It was concluded that madu randu has potential as an antibacterial against the growth of *Staphylococcus* bacteria.

Keywords: Randu honey, Staphylococcus, Antibacterial

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INTRODUCTION

Honey has been used as an antimicrobial against bacterial infections for a long time. The antibacterial properties contained in honey come from the presence of inhibin which acts as an antimicrobial and also several factors such as the osmotic nature of honey which is a highly saturated sugar solution. About 84% percent of honey is a mixture of fructose and glucose which can inhibit the the bacterial growth depending on the species (1). Several studies reported that honey can act as infection control against *Salmonella typhi* (2), *Streptococcus mutans* (3), *Pseudomonas aeruginosa*, *Enterococcus sp.*, and coagulase-negative *Staphylococcus* (4).

Staphylococcus sp. often causes resistance to several antibiotics; therefore, these bacteria often cause difficult therapeutic problems (5). *Staphylococcus aureus* is the main pathogen in humans, almost everyone will experience several types of *Staphylococcus aureus* infections during their life, ranging from food poisoning or mild skin infections to severe infections that may be life threatening (3). Severe *Staphylococcus aureus* infections cause pneumonia, meningitis, urinary tract infections, osteomyelitis, and endocarditis. *Staphylococcus aureus* can also lead to nosocomial infections and toxic shock syndrome (6). Meanwhile, *Staphylococcus epidermidis* has become increasingly common in recent decades. *Staphylococcus epidermidis* produces a kind of poisonous substance. These bacteria are able to produce biofilms that make it easier for them to stick to the surface of plastic or glass tools such as catheters. Biofilms owned by the bacteria *Staphylococcus epidermidis* are more resistant to phagocytosis and certain antibiotics (7,8).

In Indonesia, various plants that can produce nectar, such as calliandra, rubber, randu, rambutan, mango, and others, so that several kinds of honey can be found with different types and characteristics according to the origin of the plant nectar source. One type of honey produced in Indonesia is randu honey. This honey comes

from honeybees that are bred in the forest and consume nectar from flower randu (*Cheiba pentandra*). The physical characteristics of the honey are yellow brown in color, slightly thick, quite tasty and can be stored for a long time. About 75% of total honey production collected by beekeepers in East Java was from flower randu (9). Thus, it is interesting to find the potential of randu honey as antimicrobial against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

MATERIALS AND METHODS

Preparation of Randu Honey Concentration

This study used five concentrations of randu honey including 20 µl (20 µL of randu honey and 100 µL sterile distilled water), 40 µl (40 µL of randu honey and 100 µL of sterile distilled water), 60 µl (60 µL of randu honey and 100 µL of sterile distilled water), 80 µl (80 µL of honey randu and 100 µL of sterile distilled water) and 100 µl (10). Concentration was made by dissolving the honey with sterile distilled water. And using two control groups including chloramphenicol (30 µg) as a positive control and sterile distilled water as a negative control.

Preparation of Bacteria

Bacterial suspension was made by taking 1 - 2 ose of cultured Staphylococcus aureus and *Staphylococcus epidermidis* and mixed with 0.9 NaCl to obtain the standard of 0.5 McFarland or equivalent to 10^8 CFU / ml of bacteria (11). A standard solution of 0.5 McFarland was made by dissolving 99.5 mL of 1% sulfuric acid and 0.5 mL of 1.175% barium chloride (12). Each bacteria with confirmed 0.5 McFarland standard was cultured on Mueller Hinton agar (MH) using a cotton swab and incubated at 37°C for 18-24 hours then observed the growth of bacteria on media.

Antibacterial Sensitivity Test

Antibacterial sensitivity test was carried out by agar diffusion method using well diffusion. The well was made with a depth of ± 4 mm and a diameter of 5 mm on MH media using a loop. The well is made at a distance of 2 cm from the edge of the plate and 3 cm between the wells. Each well is labeled according to their respective treatments. Fifty µl of each treatment was placed in different wells and incubated at 1 x 24 hours at 37°C. Inhibition diameter was measured from the zone of inhibition observed in MH.

Data Analysis

The data of the diameter of the inhibition zone were analyzed using the one-way analysis of variance (ANOVA) at 5% significance level to determine the minimal concentration of randu honey which can inhibit the growth of *Staphylococcus sp.* The data were calculated with SPSS statistics 21 for windows. The classification of the level of inhibition zone for bacterial growth refers to Pelczar (13) categorized as no inhibition (0 mm), poor (<8 mm), moderate (8-10 mm), strong (11-20) and very strong (>20 mm).

RESULTS

The results showed that stretch honey was able to inhibit the growth of *Staphylococcus* bacteria at certain concentrations. The inhibition zone of *Staphylococcus aureus* culture media was formed at concentrations of 80 µl (2.67 mm ± 4.62) and 100 µl (13 mm ± 1.73) and there were no inhibition zones of 20 µl, 40 µl, and 60 µl. While the inhibition zone of *Staphylococcus epidermidis* was formed at concentrations of 40 µl (0.67 mm ± 1.15), 60 µl (7.67 mm ± 7.50), 80 µl (10 mm ± 9.17), and 100 µl (22, 67 mm ± 3.05) (Table I). The result of inhibition zone (Figure 1) can be seen by the presence of a clear zone around the well, which indicates no bacterial growth. This shows that honey contains compounds that can inhibit bacterial growth.

There was no statistical difference in the inhibition zone diameter between the concentrations of *Staphylococcus aureus* (0.09) and *Stapylococcus epidermidis* (2.97). However, based on the size of the inhibition zone formed, there are several differences in the levels of the inhibition zone at each concentration. In addition, there were 3 levels of inhibition zone from stretcher honey against *Staphylococcus aureus* bacteria, namely no inhibition (20 µl, 40 µl, and 60 µl), poor (80 µl), and moderate (100 µl). Whereas for *Stapylococcus epidermidis*, there are four levels of inhibition zones, namely no inhibition zone (20 µl), poor (40 µl and 60 µl), moderate (80 µl), and very strong (100 µl) (Table II).

Table I : The diameter of inhibition zone of randu honey againsts *Staphylococcus sp.*

Treatment	*Staphylococcus aureus*		*Staphylococcus epipdermidis*	
	Diameter in mm (Mean±SD)	CI (lower-upper)	Diameter in mm (Mean±SD)	CI (lower-upper)
20 µl	0	0-0	0	0-0
40 µl	0	0-0	0.67 ± 1.15^{d}	-2.20 – 3.54
60 µl	0	0-0	7.67 ± 0.57^{cd}	6.23 – 9.10
80 µl	2.67 ± 0.58^{c}	1.23 - 4.10	10 ± 3.78^{c}	-2.90 – 26.90
100 µl	14.33 ± 2.08^{b}	8.70 – 17.30	22.67 ± 3.05^{b}	15.08 – 30.26
Control (+)	30 ± 0.00^{a}	30-30	35 ± 1.72^{a}	25.39 – 42.61
Control (-)	0	0-0	0	0-0

Table II : The category of inhibition zone level of each concentration

Species of bacteria	Concentration	Category	p-value
Staphylococcus aureus	20 µl	no inhibition	0.09
	40 µl	no inhibition	
	60 µl	no inhibition	
	80 µl	poor inhibition	
	100 µl	moderate inhibition	
Staphylococcus epidermidis	20 µl	no inhibition	2.97
	40 µl	poor inhibition	
	60 µl	poor inhibition	
	80 µl	moderate inhibition	
	100 µl	very strong inhibition	

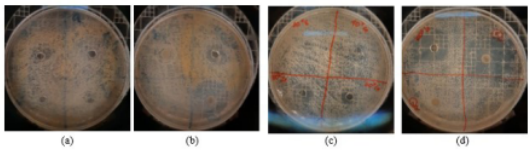

Figure 1 : The results of inhibition zone diameter in Staphylococcus aureus (a) treatments with 20 µl, 40 µl, 60 µl and 80 µl of honey (b) treatments with 100 µl of honey, Chloramphenicol 30 µg, and Disc Antibiotic Blank. Staphylococcus epidermidis (c) treatments with 20 µl, 40 µl, 60 µl and 80 µl of honey (d) treatments with 100 µl of honey, Chloramphenicol 30 µg, and Disc Antibiotic Blank.

DISCUSSION

The antimicrobial activity test of randu honey was conducted to determine the ability of randu honey to inhibit bacterial growth. The test was carried out by the well diffusion method against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. The media used for the antimicrobial test in this study was Mueller-Hinton Agar. Mueller-Hinton Agar is the standard agar medium for antibiotic susceptibility testing since it is contained the minimum requirement needed such as pH, cation concentration and thymidine content (14). Also, this medium contains sulfonamide inhibitors, trimethoprim, and tetracycline. This medium could also support the growth of non-fastidious pathogenic bacteria (15). Growth inhibition by an antibacterial substance can be observed in the presence of a clear zone around the well (16). The diameter of the inhibition zone is influenced by several factors, such as diffusibility of the antimicrobial agent, the concentration of antibiotics, the nature and composition of the medium, the presence of inhibition or stimulant substances, pH and incubation time (17).

The results also showed that a higher concentration of randu honey would increase the diameter of the inhibition zone. According to Roslizawati *et al.* (2013) (18), increasing the concentration of an antimicrobial substance can increase the content of active compounds that function as antibacterial, so that the ability of an antimicrobial substance to kill bacteria also increases. Another study using the well diffusion method conducted by Mursyida and Marwan (19) found that honey from Baserah can inhibit the growth of *Staphylococcus aureus* at concentrations of 25%, 50%, 75%, and 100% with an average diameter of the inhibition zone is 3.00 mm. 3.66mm, 5.00 mm, and 5.33 mm, respectively. Andriani et al. (20) conducted research on randu honey as an antibiotic against spoilage bacteria (*Pseudomonas fluorescence* FNCC 0071 and *Pseudomonas putida* FNCC 0070) with concentrations of 25%, 35%, 30% and 40%. In the media inoculated with *Pseudomonas fluorescens*, the diameter of the inhibition zone was 6.70mm (40% concentration), 5.96 mm (35% concentration), 5.53 mm (30% concentration), and 5.00 mm (20% concentration). While the media inoculated with *Pseudomonas putida* showed the diameter of the zone of inhibition with a mean of 7.30 mm (concentration 40%), 6.15 mm (concentration 35%), 5.20 mm (concentration 30%), and 5.00 mm (concentration 20 %).

The antibacterial properties of honey depend on the honeybee's metabolism, vegetable sources and environmental conditions that can affect the physical and chemical properties of honey. Therefore, several types of honey produce different bacterial inhibitory abilities. In addition, effectiveness is also influenced by the type of bacteria being tested; some honeys may exert a more significant effect on certain bacterial species. According to Machado *et al.* (21), several bacteria sensitive to honey include *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Paenibacillus larvae, Streptococcus spp., Salmonella spp., Shigella spp.*, and *Proteus* spp.

Fresh or unheated honey has been shown to have optimal bacteriostatic and bactericidal effects. This antibacterial effect mainly depends on the concentration of honey (22). In addition, Maddocks and Jenkins (23) proved that honey exhibits ant virulent effects on microorganisms by reducing the microorganisms' ability to obtain iron from the host and preventing the

development of infections. Honey also has Hydrogen Peroxide (H_2O_2) compounds which can inhibit the growth of *Staphylocccus aureus* (24). According to Aliyazicioglu and Boukraa (25), the antibacterial properties of honey include low pH, high sugar concentration and the presence of H_2O_2. At high sugar concentrations, bacteria will lose water due to osmotic pressure and will dry out, while low pH will inhibit bacterial growth.

Hydrogen peroxide is an important compound that is responsible for the antibacterial activity of honey peroxide. This compound is produced aerobically from glucose with glucose oxidase activity (21). The function of H_2O_2 in honey is to prevent the breakdown of raw honey, where the sugar concentration is not sufficient to prevent microbial growth (26). The study found that a mixture of hydrogen peroxide and ascorbic acid produced an antibacterial mechanism resulting in increased lysozyme lysis and bacterial death (27). In addition, several components can contribute to the antimicrobial activity of honey; this component is called non-peroxide. This substance is associated with antioxidant and protein compounds, such as lysozymes, flavonoids (flavones, flavonols, flavanones and dihydroflavonols) and other phenolic compounds (acids and cinnamic esters), methylglyoxal and bee peptides (28). These flavonoid compounds act as antibacterials by inhibiting nucleic acid synthesis and inhibiting the function of the cytoplasmic membrane of target bacteria (29).

CONCLUSION

Randu honey has an effect on the inhibition of the growth of *Staphylococcus* sp. The diameter of inhibition zone for *Stpahylococcus aureus* was formed at a concentration of 80 µl and 100 µl while for *Staphylococcus epidermidis* was formed at a concentration of 40 µl, 60 µl, 80 µl, and 100 µl. It's also concluded that increasing the amount of concentration will increase the diameter of the inhibition zone.

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