

# Inhibition of Propolis and Trigona spp's honey towards Methicilin-Resistant Staphylococcus aureus and Vancomycin-Resistant Staphylococcus aureus

Leka Lutpiatina<sup>1</sup>, Ratih Dewi Dwiyanti<sup>1</sup>, Anny Thuraidah<sup>1</sup>

<sup>1</sup>Medical Laboratory Technology Poltekkes Kemenkes Banjarmasin, Mistar Cokrokusumo  
Street 4a Banjarbaru, Indonesia

## ABSTRACT

Propolis and Trigona spp honey have functioned as anticancer, antiviral, antifungal and antibiotic. Isolates of Staphylococcus aureus resistant to Methicillin and Vancomycin found in the surgical treatment room and ICU of Ratu Zalecha Hospital Martapura. The purpose of this research was to determine the inhibitory zone of MRSA, VRSA to the propolis extract and honey of Trigona spp at the concentration of 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml and 1000 mg/ml. Also to know the concentration of the propolis extract and honey of Trigona spp in inhibiting the growth of MRSA, VRSA. The type of this research was true experimental with the design of Pretest-Posttest With Control Group Design. The study was conducted from April 2015 to June 2015 in Banjarbaru, South Kalimantan Indonesia. The objects of research are propolis and honey of Trigona spp hives in the Barabai area. The dependent variable was the inhibition zone of MRSA and VRSA in Hinton Muller` media containing ethanol extract of propolis and honey of Trigona spp in some different concentrations measured from the formed diameter of inhibition zone. Data were analyzed by One Way ANOVA test and Kruskal-Wallis test at 95% confidence level. The results of the research showed that there were differences in MRSA and VRSA inhibition zone against various concentrations of Trigona spp honey. The strength of Trigona spp honey which was in inhibiting the growth of MRSA was 1000 mg/ml concentration with a diameter of 25.2 mm. VRSA at 1000 mg/ml concentration with a diameter of 26.6 mm. The strength of Trigona spp propolis extract which is useful in inhibiting the growth of MRSA is at 1000 mg/ml concentration with a diameter of 17.8 mm. VRSA at 1000 mg/ml concentration with a diameter of 16.4 mm.

**Keywords:** Propolis; Honey; Trigona spp; Staphylococcus aureus; Resistant

## INTRODUCTION

The case of Methicilin-Resistant Staphylococcus Aureus (MRSA) in 1961 was found in England while in the USA was discovered in 1968<sup>1</sup>. In Asia, the prevalence of infection of MRSA reaches 70%. While in Indonesia in 2006 the incidence is 25,5%. The first clinical isolate of Vancomycin-Resistant Staphylococcus aureus (VRSA) reported in the USA in 2002<sup>2</sup>. In Medical College and Hospital, Midnapore, West Bengal, India recovered from 100 isolates of Staphylococcus aureus strains to 70% into MRSA, 54.3% as (VRSA), and 54.3% for both MRSA and VRSA<sup>3</sup>. At the Teaching Hospital, in Sari, Iran of Staphylococcus aureus isolates were 31.31% and 16.1%, indicating MRSA is vancomycin-intermediate

Staphylococcus aureus (VISA)<sup>4</sup>. Hospital Khartoum, Sudan found 41% of MRSA, 12% VISA<sup>5</sup> MRSA in poultry samples in Serdang, Malaysia was found 9 out of 30 isolates studied. The spread of pathogens and not only in hospitals but can also spread in poultry<sup>6</sup>.

In Indonesia, research of Vancomycin Resistant Staphylococcus Aureus (VRSA) found in 10 out of 64 isolates (15,6 mg/ml) and the stethoscope membrane in Margono Soekarjo hospital, Purwokerto<sup>7</sup>. A study on steteskop at a regional hospital in South Kalimantan also showed the presence of Staphylococcus aureus<sup>8</sup>. The strain of Staphylococcus aureus which is resistant towards Methicilin and Vancomycin found in the surgery room and ICU at RSUD Ratu Zalecha Martapura<sup>9</sup>.

One of the natural substance that was believed empirically has a lot of benefits and relatively safe is Propolis and honey from the bee. Various bee species produce propolis for self-defense. Propolis mostly used to cure various disease in the past last year<sup>10</sup>. The type of bee beside *Apis* spp is *Trigona* spp, this bee produces honey than other and rarely farmed. The estimate contains propolis from this species is more than *Apis* spp<sup>11</sup>.

Propolis has some benefits as anticancer, antiviral, antifungal and antibiotics<sup>11</sup>. Research about the advantages of *Trigona* sp's propolis from Kabupaten Bulukumba, South Sulawesi can inhibit the *S. mutant* growth. In vitro, research of Agustina<sup>12</sup> shows the propolis extract from a bee in Malang can give an impact and inhibit the positive gram bacteria *Staphylococcus epidermidis* growth in a concentration 60 mg/ml and negative gram bacteria *Pseudomonas aeruginosa* growth in concentration 70 mg/ml. This research about the effectivity of Propolis and *Trigona* spp hone bee from south Kalimantan antibacterial effect has proven that have resistivity towards *Salmonella typhi* and *Staphylococcus aureus*<sup>13</sup>.

The aim of this research was to determine the inhibitory zone of MRSA, VRSA to the propolis extract and honey of *Trigona* spp at the concentration of 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml and 1000 mg/ml. Also to know the effective concentration of the propolis extract and honey of *Trigona* spp in inhibiting the growth of MRSA, VRSA.

## MATERIALS AND METHOD

The type of research that used is True Experiment Method with Posttest only control design, which is having a resistivity test for Propolis extract and *Trigona* spp honey in a concentration 200 mg/ml, 400 mg/ml,

600 mg/ml, 800 mg/ml and 1000 mg/ml. The object in this research is Propolis and Honey from *Trigona* spp beehive from Barabai.

Independent variable in this research is Propolis extract and *Trigona* spp honey bee with a concentration 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml and 1000 mg/ml. The dependent variable in this research is resistivity of MRSA and VRSA in Muller Hinton media (Merck) which contain the ethanol extract of propolis and *Trigona* spp honey bee in some variant concentration which measured from the diameters of the resistivity that form.

About 20 grams of Propolis extracted with 200 ml ethanol 70% using maceration technique in 3 days filtered with filter paper and the propolis filtrate concentrated which evaporated the ethanol in 50°C using a water bath, until obtaining the concentrated extract. This thick propolis extract being added with Propylene glycol with the same weight. This concentration 1000 mg/ml will be diluted using sterile aqua dest to obtain a concentration 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml. It also should be done with bee materials. Antimicrobial test for Propolis and bee using a diffusion method well technique. Measure the diameters of the transparent circle in mm using a ruler around the well which contain material test to determine the resistivity of Propolis/*Trigona* spp honey bee towards bacteria and retested in 5 times<sup>12</sup>.

## RESULTS AND DISCUSSION

Result of Antimicrobial Test for Honey and Propolis *Trigona* spp

Antimicrobial test for honey and propolis extract *Trigona* spp towards isolate MRSA and VRSA shows the variation diameters of inhibition. Inhibition data of honey and propolis extract shows in table I-4.

**Table 1. Inhibition zone honey *Trigona* spp towards MRSA, VRSA**

Concentration Honey <i>Trigona</i> spp	Inhibition Zone Honey <i>Trigona</i> spp (mm)									
	MRSA					VRSA				
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
200mg/ml	0	0	0	0	0	0	8	0	0	0
400mg/ml	9	8	16	10	13	15	15	15	15	10
600mg/ml	15	19	25	17	21	23	21	18	21	18
800mg/ml	17	22	27	21	21	25	25	25	23	22
1000mg/ml	22	24	29	25	26	28	27	26	27	25

Rep = Repetition

**Table 2. Inhibition zone Propolis Extract *Trigona spp* towards MRSA, VRSA**

Concentration Propolis Extract <i>Trigona spp</i>	Inhibition Zone Propolis Extract <i>Trigona spp</i> (mm)									
	MRSA					VRSA				
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
200mg/ml	8	8	8	9	9	10	11	11	11	11
400mg/ml	10	10	11	9	11	12	12	12	12	13
600mg/ml	12	13	14	13	14	13	13	13	14	14
800mg/ml	16	15	15	15	15	15	16	16	15	15
1000mg/ml	18	18	18	18	17	16	17	17	16	16

Rep = Repetition

**Table 3. Inhibition Honey and Propolis Extract *Trigona spp* towards MRSA, VRSA**

Concentration	Isolate	Substance	Mean	SD
			200mg/ml	Isolate MRSA
N = 5	Isolate MRSA	Honey	0	0
		Isolate VRSA	Propolis	10,8
	Isolate VRSA	Honey	1,6	1,600
		Isolate MRSA	Propolis	10,2
400mg/ml	Isolate MRSA	Honey	11,2	1,463
		Isolate VRSA	Propolis	12,2
N = 5	Isolate VRSA		Honey	14
		600mg/ml	Isolate MRSA	Propolis
N = 5	Isolate MRSA			Honey
		Isolate VRSA	Propolis	13,4
	Isolate VRSA		Honey	20,2
		800mg/ml	Isolate MRSA	Propolis
N = 5	Isolate MRSA			Honey
		Isolate VRSA	Propolis	15,4
	Isolate VRSA		Honey	24
		1000mg/ml	Isolate MRSA	Propolis
N = 5	Isolate MRSA			Honey
		Isolate VRSA	Propolis	16,4
	Isolate VRSA		Honey	26,6

**Table 4. The Result Statistic Test Inhibition of Honey and Propolis Extract *Trigona spp* towards MRSA, VRSA**

Subject	Propolis				Honey			
	MRSA		VRSA		MRSA		VRSA	
Kontrol	0	P value <sup>a</sup>	0	P value <sup>b</sup>	0	P value <sup>a</sup>	0	P value <sup>b</sup>
200mg/ml N=5	8,4	0,00*	10,8	0,00*	0	0,00*	1,6	0,00*
400mg/ml N=5	10,2		12,2		11,2		14	
600mg/ml N=5	13,2		13,4		19,4		20,2	
800mg/ml N=5	15,2		15,4		21,6		24	
1000mg/ml N=5	17,8		16,4		25,2		26,6	

<sup>a</sup> Anova Test<sup>b</sup> Kruskal Wallis Test

\* &lt; 0,05 there is the significant different

The antibacterial mechanism in honey according to Suganda<sup>14</sup> affected to a high level of Glucose and Fructose in honey, the acidity of honey and also hydrogen peroxide composition. According to Hamad<sup>15</sup>, the formation of glucose and fructose in honey through the osmotic process can cause dehydration to bacteria cell since a lot of water comes out and in this situation, the bacteria can quickly become lysis. The high acidity level of honey with pH 3,2-4,5 can cause the bacteria cell metabolism process to become slower when the compounds that bacteria need for a living are unavailable therefore it can cause the cell lysis easily.

According to Sulaiman<sup>16</sup> the composition of hydrogen peroxide which is cytotoxic with the free radical formation that comes out will destruct the bacteria cell structure including the cell wall and cell membrane, this thing also can make the bacteria cell lysis so that it can decrease the bacterial growth,

The study result of Hijriah et al.<sup>17</sup>, shows that the Minimum Inhibit Concentration (MIC) *Trigona spp* honey bee towards *Staphylococcus aureus* in concentration 37,5 mg/ml and Minimum Bactericidal Concentration (MBC) in concentration 50 mg/ml. Results of research on *Trigona carbonaria* honey bee towards *Staphylococcus aureus* with minimum bactericidal concentrations 1.2-1.8 mg/mL<sup>18</sup>. The study of MRSA towards honey already done by Molan P.C<sup>19</sup> and shows that honey has antimicrobial activity towards MRSA.

Extraction process for propolis that chosen for this study is doing maceration using organic diluents ethanol 70 mg/ml. maceration aim itself to give some time for propolis and diluents to have an interaction so that the diluents can dilute the compound inside. According to Hasan et al.<sup>20</sup>, using ethanol 70 mg/ml better than ethanol absolute (95mg/ml) because it can dilute more active material such as flavonoid more.

Gould<sup>21</sup> said that some factors that affect the antibacterial potency of some material are concentration,

amount, and type of bacteria that will test. Related to factor type of bacteria that will be tested, MRSA VRSA is positive gram group. Propolis has some lower activity towards negative gram bacteria than a positive one.

This thing could be possible because the cell wall structure negative gram bacteria relatively complex consist of three-layer that is the outer layer is a polysaccharide, in the middle that is lipoprotein, and the inner layer is peptidoglycan so that antimicrobial compound will be hard to enter the cell and find the target. Other study shows propolis activity lower towards negative gram bacteria, was done by Agustina<sup>12</sup> what the best concentration of propolis extract from Malang to inhibit the negative gram bacteria growth (*Pseudomonas aeruginosa*) is 700 mg/ml while towards positive gram bacteria (*Staphylococcus epidermidis*) is 600 mg/ml.

The study result of Novilla et al.<sup>22</sup> *Apis mellifera* propolis extract can inhibit MRSA growth in vitro. The resistivity that form is 2 mm in a concentration of 2 µg. The study from Nori E.B<sup>23</sup> also shows that the ethanol extraction from propolis sensitive in 2 µg. Research results, Inhibition zone of olive oil extracts of propolis on *Staphylococcus aureus* was higher (22.4 mm) than Ethanolic extracts and Water Extracts<sup>24</sup>.

This research resulted in greater inhibition zone on the material honey bee *Trigona* spp. Research AL-Waili, N. et al., 2012 showed that the extract of propolis and honey bees have synergy in inhibiting the growth of *Staphylococcus aureus*<sup>25</sup>.

## CONCLUSION

The inhibition *Trigona* spp honey bee in a concentration 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml and 1000 mg/ml towards MRSA averagely (mm) 0; 11,2; 19,4; 21,6; 25,2; VRSA 1,6; 14; 20,2; 24; 26,6. Inhibition propolis extract *Trigona* spp bee in concentration 200 mg/ml, 400 mg/ml, 600 mg/ml, 800 mg/ml and 1000 mg/ml towards MRSA averagely (mm) 8,4; 10,2; 13,2; 15,2; 17,8 VRSA 10,8; 12,2; 13,4; 15,4; 16,4

Minimum concentration that will form the biggest inhibition of MRSA and VRSA towards propolis extract and *Trigona* spp honey bee is 1000 mg/ml.

## Gratitude

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**Ethical Clearance:** Taken From *Health Research Ethics Committee* Politeknik Kesehatan Banjarmasin

**Conflict of Interest:** Nil

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