A Comparative Study of the Antimicrobial Activity of Wild (Tualang) Honey and Artificial Honey Against *Methecillin-resistant Staphylococcus Aureus, Streptococcus Pneumoniae* and *Klebsiella Pneumoniae*

May Florence Dela Cruz Bacayo[1]*, Shazwan Fahmi Shafi[1], Wong Charng Choon[1], Santosh Fattepur[1], Kiran Chanabasappa Nilugal[1], Jiyauddin Khan[1], Fadli Asmani[1], Eddy Yusuf[1]
[1]School of Pharmacy, Management and Science University, 40100 Shah Alam Malaysia

Keywords: *Tualang* honey, Artificial honey; Antimicrobial activity.

Abstract : Background: Antimicrobial agents are becoming less effective due to the emergence of antibiotic-resistant microbes, thus the discovery of alternative antimicrobial agents are urgently needed. Honey is believed to have antimicrobial activity and are used in traditional medicine as skin healing dressing for wound. Objectives: To find an alternative substance as a substitute for the current antimicrobial agent for the three strains of microbes and to make a comparison between the antimicrobial activity of the wild (*Tualang*) honey and the artificial honey. Method: The 50 % (w/v) of honey were diluted using double dilution method and are tested for its antimicrobial activity using disc diffusion method with Ampicillin as a standard antibiotic for *Streptococcus pneumoniae,* Gentamycin for *Klebsiella pneumonia* and Vancomycin for *Methicillin-resistant Staphylococcus aurues* as positive control. The MIC and MBC were determined. Results: Based on the results obtained from the disc diffussion assay, it shows that wild (*Tualang)* honey has antimicrobial activity at a concentration of 10 % (w/v) for *MRSA* and *S. pneumoniae* while at 25 % (w/v) for *K. pneumoniae*. Meanwhile it need 100% (w/v) concentration of artificial honey to shows its antimicrobial activity. Wild *(Tualang)* honey was recorded as the most potent honey against *S. pneumoniae*, in which a dilution of 10% (w/v) was required to inhibit the growth and kill *S. pneumoniae* colony at 20% (w/v). The concentration of 20% (w/v) was required to inhibit *MRSA* and 25% (w/v) to kill *MRSA*. Meanwhile, the highest concentration were required to inhibit *K.pneumonia* at 50% (w/v) and no bactericidal effect were recorded. Conclusion: The comparative study between *Tualang* honey and Artificial honey gives a promising result that the *Tualang* honey has a highest antimicrobial activity against *MRSA, Klebsiella pneumoniae, Sterptococcus pneumoniae* compared to the Artificial honey.

1 INTRODUCTION

Honey is a natural syrup which have a complex function of physicochemical properties (color, flavor and texture), mainly determined by their botanic and geographic origins. Honey is a concentrated aqueous solution of glucose (31%) and fructose (39%). It contains free amino acids at a level of 1%, pollen being one of their sources. Proline, which might originate from bees, is the prevalent amino acid and makes up 50–85% of the amino acid fraction (White, 1975). Organic acids are present in honey at low concentrations (<0.5%) and it is related to the color, flavor and physico-chemical properties of the honey, such as pH, acidity, and electrical conductivity. Organic acids chelate metals can synergistically enhance the antioxidant action of phenolic compounds (Gheldof, Wang & Engeseth, 2002). Moreover, acetic acid and ethanol can be used as fermentation indicators and formic acid as an indicator for the treatment of Varroa infestation (Calderone, 2000).

It was used as a wound treatment that are non-responsive to conventional therapies, such as diabetic ulcers, and wounds infected with antibiotic-resistant bacteria. There were countless studies that recount the antimicrobial activity of honey against microbes. (Dunford C, Cooper R, Molan P, et al, 200).

With the emergence of antibiotic-resistant microbial strains, such as *Methicillin-resistant S. aureus* that can cause difficult-to-treat wound infections, honey has again caught the attention of medical researchers. Scientists had first reported the ability of honey to treat infections caused by microbes in the late 1800s,

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but with the advent of antibiotics in the early 1900s, the scientific interest in honey decreased. (Fry DE, Barie PS, 2001)
The aim and objective of this study is to determine the antimicrobial activity of honey against *MRSA, Klebsiella pneumoniae, Streptococcus pneumoniae* which can be an alternative to the existing antimicrobial agent. It is so that the case of antimicrobial resistance will reduce significantly. This study is also to compare antimicrobial activity of the wild (*Tualang*) honey and artificial honey. Thus comparing also the antimicrobial activity of wild (*Tualang*) honey, artificial honey and the standard antibiotics.

2 METHODOLOGY

2.1 Preparation of Media

38g of MHA is dissolved into 1 liter of saline water and the solution is stirred homogenously. The agar solution is then autoclaved for 2 hours and poured into the petridish until the height is not more than 5mm keeping the petridish dry. The pH of the Mueller-Hintom medium should be maintained at 7.2 to 7.4 at room temperature and keep in the incubator set to 37°C prior to use . (Mm & Fatema, 2009)

2.2 Minimum Inhibitory Concentration (MIC)

The 50% w/v was prepared by diluting 10 g of honey in 20 ml of Mueller Hinton broth and the two-fold dilution of the honey solution in Mueller Hinton broth were prepared. Ten sterile tubes were labeled each and placed in a test tube rack. Tube 1 with 1 ml of honey solution was labeled as the honey control and tube 8 was labeled as growth control. 1 ml of Mueller Hinton broth was added to test tube 1 to 4. Using a sterile micropipette, take 1 ml from tube 1 and transfer to tube 2 and thoroughly mixed. 1 ml again was transferred from tube 2 to tube 3. This process was repeated until tube 4 except for growth control tube. Using fresh micropipette for each dilution. The last tube received no honey solution and was served as growth control then add 20% (4g in 20 ml), 15% (7.5g in 50 ml), 10% (1g in 10 ml), and 5% (2.5 in 50 ml) in test tube 5 to 8 respectively. Each tube was inoculated including the growth control except honey control with 1 ml of the culture of respective organism. The tubes were incubated at 37°c for 24 hours. The tubes were examined for growth and were determined the MIC of the tested antibiotics, which is bacteriostatic for the test organism. The tubes were examined for visible growth (cloudy) and was recorded growth as (+) and no growth as (-).

2.3 Minimum Bactrialcidal Concentration (MBC)

The MBC test was used to determined the lowest concentration needed to kill the bacteria. MBC was perform after MIC test. Each honey dilution with no bacteria growth from the mic test was determined. For each honey solution that has no growth was incubated onto Mueller Hinton agar plate. It was spread evenly and incubated at 37°c for 24 hours. MBC were determined by minimum concentration that allowed less than 1% of bacterial growth.(Fakruddin,M., 2013)

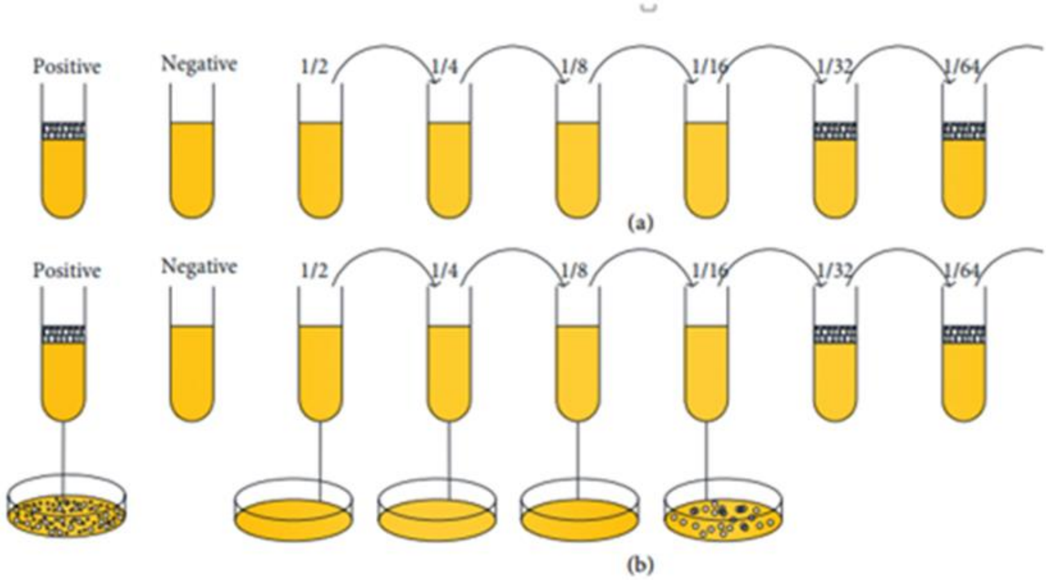

Figure 3: (a) Minimum inhibitory concentration (MIC) and (b) Minimum Bactericidal Concentration (MBC) (Yilmaz, 2012)

2.4 Disc Diffusion Assay for Determination of Zone of Inhibition

The antimicrobial activity of the diluted honey concentration is tested by disc diffusion susceptibility method . The Mueller-Hinton Agar (MHA) plate is used as the agar plate for the bacterial culture because it gives a good result in batch to batch reproducibility, it is low in of sulfonamide, trimethoprim and tetracycline inhibitors. (Zainol, Mohd Yusoff, & Mohd Yusof, 2013) Tualang and artificial honey were prepared by double dilution method from 100% concentration until 6. 25% of the concentration is diluted with distilled water. A sterile swab is used to spread the suspension of the pure culture on one side face of the agar plate. The honey solution and standard antibiotic is applied to the opposite side face of the agar plate and distilled water was employed as negative control. The agar plate was incubated for 24 hours at a temperature of 37°C. The size of the zone of inhibition were measured using a metric ruler. The larger zone of inhibition represents the antimicrobial activity.

3 DATA ANALYSIS

The data obtained from different formulations will be analyzed by Mean, Standard deviation and one-way analysis of variance (ANOVA) procedure using the Statistical Package for the Social Science (SPSS) program (IBM SPSS Statistics 22.0).

When there is a statistically significant difference, a post-hoc Tukey test will be then conducted to detect the differences among the pairs. A statistically significant difference is considered at $p < 0.05$.

4 RESULTS

Several evaluation tests were conducted in order to determined the effectiveness of Tualang honey and artificial honey against sample bacteria which is disc diffussion test, Minimum Inhibition Test and Minimum Bactericidal Test.

From the result obtain, it shows that Tualang Honey has antimicrobial activity against *MRSA* and *S. pneumoniae* at lowest concentration of 10 %(w/v), while against *K. pneumoniae* at concentration 25 %(w/v). It means that Tualang honey has quite potent antimicrobial activity against *MRSA* and *S. pneumoniae* as it only need 10 %(w/v) concentration. Meanwhile, *K. pneumoniae* has higher resistance compare to *MRSA* and *S. pneumoniae* as it need 25 %(w/v) of Tualang honey to shows its antimicrobial activity.

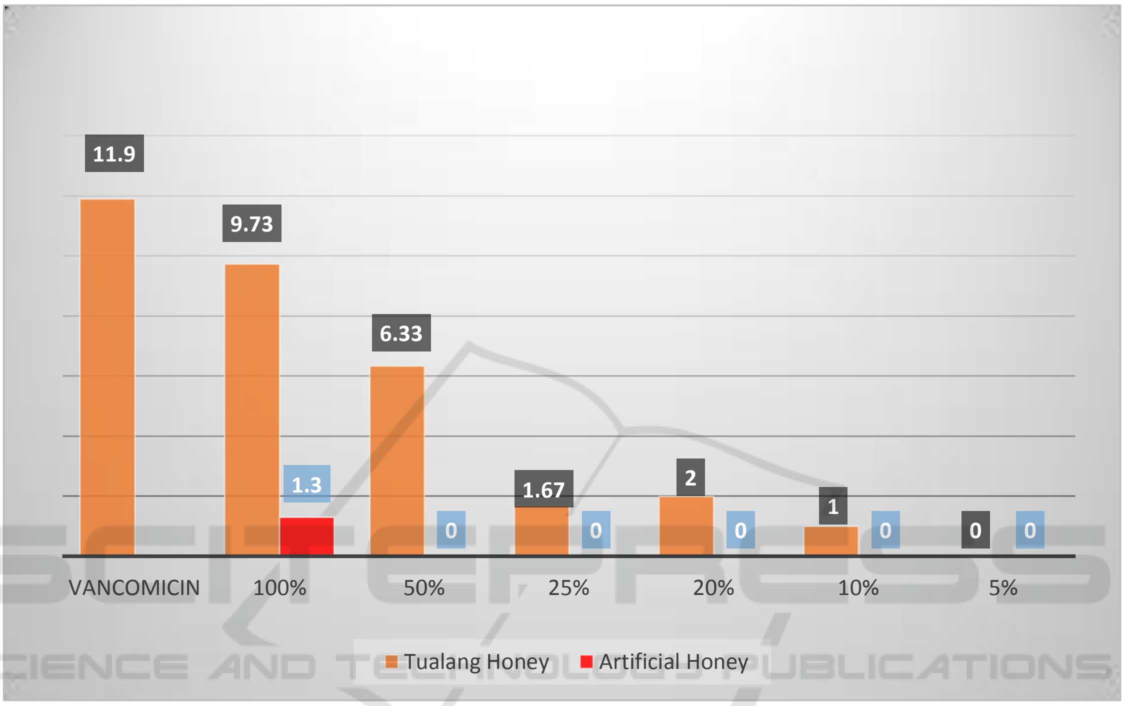

Figure 2: Zone of Inhibition for MRSA against Tualang Honey, Artificial Honey and Positive Control.

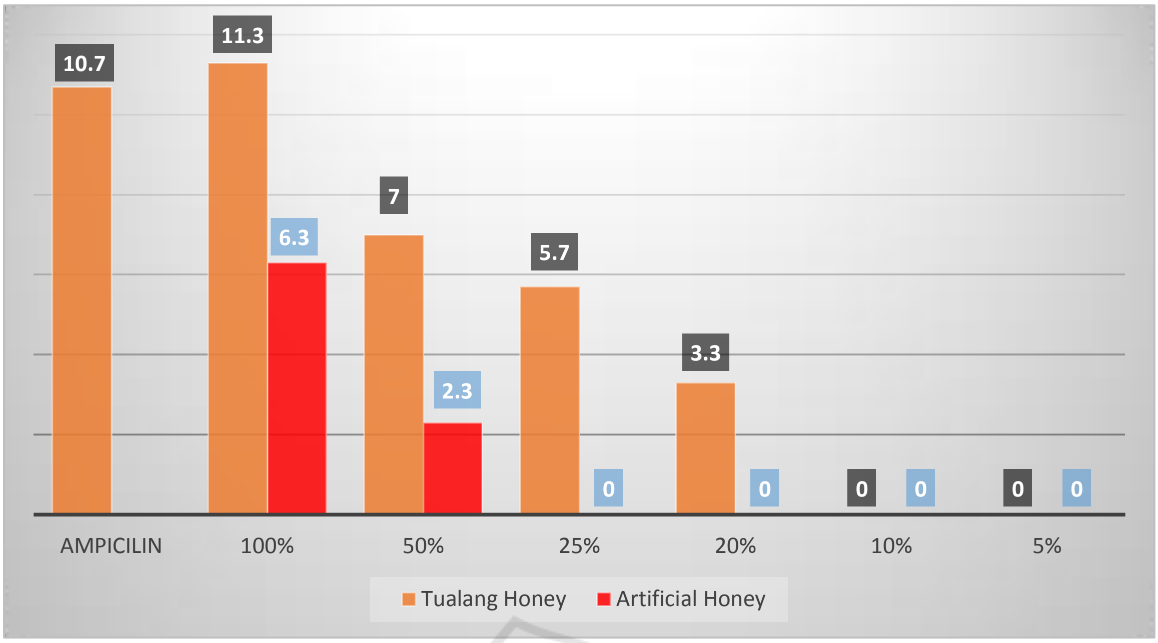

Figure 3: Zone of Inhibition for *Streptococcus pneumoniae* against Tualang Honey, Artificial Honey and Positive Control.

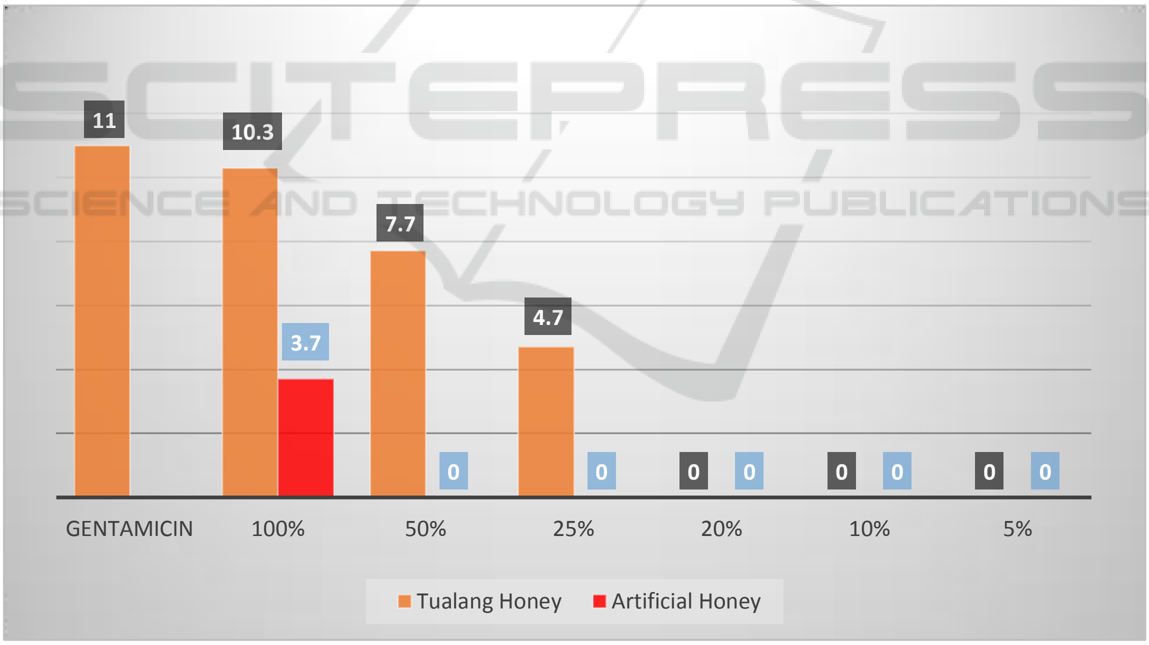

Figure 4: Zone of Inhibition for Klebsiella pneumoniae against Tualang Honey, Artificial Honey and Positive Control.

Table 1: MIC and MBC values of Tualang honey and artificial honey.

Honey Sample	MIC & MBC %(w/v)					
	Methicillin Resistence Streptococcus Aerus (MRSA)		Streptococcus pneumoniae		Klebsiella pneumoniae	
	MIC	MBC	MIC	MBC	MIC	MBC
Tualang Honey	20	25	10	20	50	>50
Artificial Honey	0	0	50	>50	0	0

5 DISCUSSION

This study was about investigating the antimicrobial activity of Tualang and artificial honey against *MRSA*, *S.pneumoniae* and *K. pneumoniae* as there are limited studies available comparing different type of honey. Based on the results obtained from the disc diffussion test, it shows that Tualang honey has antimicrobial activity at concentration of 10 % (w/v) for MRSA and *S. pneumoniae* while at 25% (w/v) for *K. pneumoniae*. Meanwhile it need 100% (w/v) concentration of artificial honey to shows its antimicrobial activity. The results shows that Tualang honey has high antimicrobial activity against the selected bacteria as its only need as low as 10%(w/v) to 25%(w/v) concentration to inhibit the growth of *MRSA*, *S.pneumoniae* and *K pneumoniae*. These result were due to the two important enzymes known to contribute to the major biological activities of honey which are bee-origin glucose oxidase and floral-origin catalase. These enzymes will determine the antimicrobial activity of Tualang honey. (Zainol, Mohd Yusoff, & Mohd Yusof, 2013).

For MIC and MBC values, Tualang honey was recorded as the most potent honey against *S. pneumoniae*, in which a dilution of 10% (w/v) was require to inhibit and kill them at 20% (w/v). The concentration of 20% (w/v) are require to inhibit MRSA and 25% (w/v) to kill them. Meanwhile, the highest concentration are require to inhibit *K. pneumoniae* at 50% (w/v) and no bactericidal effect were recorded. Overall, the bactericidal activities of Tualang honey were recorded to be one reading higher than their inhibitory effect. Results of artificial honey showed that only *S. pneumoniae* show were inhibited at concentration 50% (w/v) and no bactericidal value. It also shows no MIC and MBC value against other bacteria.The statistical analysis showed that the antimicrobial activity of the Tualang honey and artificial honey were significant when compared with positive control with the P value of <0.05 against *MRSA*, *S.pneumoniae* and *K.pneumoniae*.

6 CONCLUSION

The in-vitro comparative study of antimicrobial activity between Tualang honey and artificial honey against the selected gram-negative bacteria concludes that the Tualang honey has highest antimicrobial activity against *MRSA*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae* compared to artificial honey.

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