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Antibacterial evaluation of Malaysian Kelulut, Tualang and Acacia honey against wound infecting bacteria

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Abstract. Bacterial infection is the most common contamination on wound. Honey is one alternative plant by-product that can be used as treatment to the bacterial infection. This study aims to evaluate the antibacterial properties of Malaysian honey represented by Kelulut, Tualang and Acacia against fourteen clinically isolated bacteria strains from wound. Agar well diffusion assay was utilised to measure the diameter of inhibition zone. Determination of minimum inhibitory concentration and minimum bactericidal concentration were performed to evaluate the bacteriostatic and bactericidal effects of the honey. The antibacterial properties of Malaysian honey were compared with manuka honey (UMF 18+). Kelulut, Tualang and Acacia have the diameter of inhibition zones that ranged from 10.7 to 24.5 mm, 9.2 to 17.7 mm and no inhibition to 15.3 mm, respectively. Kelulut, Tualang and Acacia showed bacteriostatic effect against the bacteria at concentration of 50% (w/v) and below. Kelulut was the only honey that owned bactericidal effect against the fourteen bacteria while the effect was absence in Tualang and Acacia on *E. coli*, *K. pneumonia*, *E. clocae* and *P. mirabilis*. The antibacterial properties of Kelulut was comparable to manuka honey since both honey demonstrated bacteriostatic and bactericidal effects against the fourteen clinically isolated bacteria.

1. Introduction

Bacterial infection is the most common contamination on wound [1,2] that delays the rate and reduce the quality of healing process by causing failure of grafts and flaps formation as a repair mechanism [3,4]. Due to its antibacterial properties, honey is one of the alternatives that can be used to prevent bacterial infection and improve the process of wound to heal. Based on its low pH, low water activity, and presence of antibacterial compounds i.e., peroxide and non-peroxide substances, honey provides a protective barrier which simultaneously treats and prevents microbial from infecting wound [5].

Honey has been proposed as antibacterial agent to treat bacterial infection. The advantages of using honey was due to its naturally available, non-toxic and most important, effective against resistant strains and does not reported to develop resistant strain [6,7]. Manuka was the honey that mostly used in clinical application in form of wound dressing and topical preparations such as hydrogel [8,9]. In recent years, there are numbers of preliminary studies conducted to evaluate the antibacterial properties of Malaysian honey against bacteria that associate with wound [10,11]. However, most studies have concentrated on the findings of Tualang despite other local honey such as Kelulut and Acacia.

Kelulut or stingless bee honey is produced by stingless bee from the genus *trigona* that was harvested from either meliponiculture or wild. The characteristics of this multiflora honey is depending mainly on the sources (nectar and pollen) collected by stingless bees and in Malaysia, the colour are ranged from light to dark amber [10,12] with identical sweet-sour taste. Tualang is a multiflora honey produced by the *Apis dorsata* which also known as Asian rock bees or the giant honey bee that collect pollen and nectar from various wild plants in Malaysia's rainforest jungle and stored at the hive which built at the branches of the tualang tree or scientifically known as *Koompassia excels* [13]. Tualang can be found in dark to light amber colour and it taste can be varied between bitter-sweet and sweet-sour. Usually tualang that harvested in Malaysia is dark amber in colour with slightly bitter-sweet taste [10]. Acacia is a monoflora honey derived from *Acacia mangium* produced either by *A. Mellifera* or *A. cerana* [14,15] that usually harvested from apiculture with modern moveable comb hives that built in farm using wood. As the source of acacia was from *Acacia mangium* flower nectar [16] or honeydew [14], the taste of the honey is sweet and flowery. The colour usually found between light and slightly dark amber.

Instead of being limitedly used as medicinal tonic [17], in this study, the implementation of Malaysian honey as an agent to eliminate the bacterial infection was studied by evaluating the antibacterial properties of Malaysian Kelulut, Tualang and Acacia against the fourteen clinical bacteria strains isolated from wound site of three patients. The antibacterial properties of the honey were evaluated through measurement of the diameter of inhibition zone, determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

2. Materials and methods

2.1. Preparation of honey

Three types of honey were used throughout this study known as Kelulut, Tualang and Acacia. For each type, three samples were obtained from the local suppliers with certificate of analysis accredited by several authorised institutions including Malaysian Agriculture Research and Development Institute (MARDI) and Food Quality and Safety Research and Development (UNIPEQ) for its authenticity and quality. The colour and pH of the honey was tabulated in Table 1. The commercially available medical-grade honey, Manuka (Comvita® Wound care UMF 18+, New Zealand) was used as a basis of comparison to verify the reliability of these studies.

A series of honey samples was prepared in different range of concentrations. For the determination of MIC and MBC, samples were prepared for each honey with dilution ranging from 5 to 90% (w/v) concentrations by using nutrient or soy broth as the diluents. In both evaluations, sugar-based honey (SB) were used as an artificial honey containing the major sugar compounds that commonly present in honey such as fructose, glucose, maltose and sucrose which mixed at proportion of 40%, 30%, 8% and 2% (w/v) respectively in sterile water [18].

2.2. Preparation of bacteria

Fourteen bacterial strains were kindly supplied by the Department of Pathology & Laboratory Medicine, International Islamic University Malaysia Medical Centre (IIUMMC) which were clinically isolated from tissues, pus or swabs of three different patients with infected foot ulcer and soft tissue. Among the fourteen clinical isolated strains, five were Gram-positive which were *Staphylococcus aureus, Staphylococcus hominis, Staphylococcus haemolyticus, Streptococcus pyogenes,* and *Streptococcus agalactiae.* The remaining nine bacteria strains were Gram-negative and known as *Acinetobacter baumannii, Proteus mirabilis, Proteus vulgaris, Enterobacter aerogenes, Enterobacter clocae, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella sp.,* and *Escherichia coli.* The isolation of bacteria was performed following a standard procedure of bacteria isolation and identification [19] with approval of the IIUM Research Ethics Committee (IREC) (Approval number: IREC 2019-062).

The isolated bacteria were inoculated into a sterile tube containing nutrient or soy broth (which is known as overnight culture) and incubated in a shaker incubator (Infors AG CH-4103 Bottmingen) at the temperature of 37 ºC, rotational speed of 120 rpm for 24 hours. Nutrient-based media (nutrient agar

and broth) was used to inoculate the bacteria except for *Streptococcus pyogenes* and *Streptococcus agalactiae* that were inoculated in the soy-based media (soy agar and broth). The inoculum was standardised at a final concentration of 1 x 10^8 colony forming units (CFU/ml) which is equal to 0.5 McFarland standard. The inoculum was prepared based on the optical density by diluting the overnight culture in fresh sterile broth to be in the absorbance range between 0.08 and 0.13 at 600 nm [20]. This culture served as the working bacteria for the subsequent assays during the determination of inhibition zone, MIC and MBC.

Table 1. The colour and pH of the honey samples.

Sample	Colour	pH
Kelulut 1	Dark amber	2.37 ±0.13
Kelulut 2	Dark amber	2.39 ±0.08
Kelulut 3	Dark amber	2.35 ±0.05
Tualang 1	Dark amber	3.88 ±0.04
Tualang 2	Dark amber	3.94 ±0.04
Tualang 3	Dark amber	3.92 ±0.05
Acacia 1	Light amber	4.17 ±0.01
Acacia 2	White	4.25 ±0.09
Acacia 3	Light amber	4.24 ±0.09

The symbol ± represents the standard deviation calculated between three biological replicates.

2.3. Measurement of inhibition zone through agar well diffusion assay

The measurement of inhibition zone was performed through the agar well diffusion assay which has been adapted from [21,22] with slight modifications. Agar was prepared according to the manufacturer's instructions. All honey samples were represented by three samples from each type of honey used. The inhibition zones of the honey were measured against fourteen clinically isolated bacteria. The working bacteria was inoculated using the pour plate technique by spreading 100 µL of the adjusted 0.5 McFarland culture on the agar surface. Each bacteria species was inoculated in a different plate. Upon inoculation, six mm diameter wells were cut on the agar surface to fill 80 µL of the test honey into the created well. Manuka was used as the positive control. Plates were incubated at 37ºC for 24 hours. The inhibition zone was determined by measuring the diameter of circular area that was formed on the surface of agar in millimetre (mm), including the diameter of well created. Each assay was carried out in triplicates. Based on the inhibition zone measured, the sensitivity of bacteria towards honey was categorised as not sensitive, sensitive, very sensitive and extremely sensitive as previously described [22]. The not-sensitive was denoted as the diameter of inhibition zone of lower than eight mm, sensitive for diameter from eight to fourteen mm, very-sensitive for diameter from fifteen to nineteen mm, and extremely-sensitive for diameter of twenty mm and above.

2.4. Minimum inhibitory concentration (MIC)

Determination of MIC and MBC were performed on one sample of Kelulut, Tualang and Acacia which the selection was based on the largest inhibition zone measured, representing higher antibacterial properties compared to the other samples. Based on the results obtained, Kelulut 1, Tualang 2 and Acacia 1 were selected. The MIC of each bacteria was determined by conducting the previously described methods with slight modifications [21]. This assay was performed in sterile 96 well round flat-bottom polystyrene microtitre plates Nunclon™ (Thermo Fisher Scientific, US). The honey samples which prepared as described in the previous section were dispensed into the test and control wells. It was performed by taken out 190 µl of the diluted honey sample from each concentration and aseptically transferred to the prepared 96-well plate containing 10 µl bacteria culture that has been adjusted to 0.5 McFarland standard. In this study, the antibacterial-free broth (without honey) served as viability

controls and bacteria-free broth (without honey and inoculum) served as sterility controls. The prepared microplate was then incubated in the shaker incubator (Infors AG CH-4103 Bottmingen) at 37 ºC, 120 rpm for 24 hours. The absorbance of the samples was quantified by using a microplate reader (Infinite® M200PRO, Tecan Group Ltd., Switzerland) at a time before incubation (known as t = 0) and at an elapsed time after 24 hours of incubation (known as t = 24) at a wavelength of 590 nm. The percentage of growth inhibition (PGI) was calculated using Equation (1). MIC value refers to the lowest concentration of a test material which results from 95% and above growth inhibition of the test organism.

$$PGI = \left[1 - \frac{Absorbance\ of\ the\ test\ well\ (at\ t{=}0) - Absorbance\ of\ test\ well\ (at\ t{=}24)}{Absorbance\ of\ viability\ control - Absorbance\ of\ sterility\ control}\right] \times 100\% \qquad (1)$$

2.5. Minimum bactericidal concentration (MBC)

The MIC determination provides insights about the concentration at which the bacteria growth is inhibited, the concentration at which it becomes suicidal to the bacteria was evaluated through the MBC determination. Based on MIC determination, the wells which resulted on 95% and above of growth inhibition were selected and one loopful suspension was retrieved and sub-cultured on freshly prepared Trypticase Soy Agar (TSA) using streak plate method [15] before being incubated at 37 ºC for 24 hours. These samples were then subsequently examined for any bacterial growth through formation of colony. Each test was performed in three biological replicates. The honey was considered as bacteriostatic if growth occurred and bactericidal if inhibition of growth persisted [11,15]. The lowest concentration with no growth of test organisms was considered as the MBC.

3. Results and discussion

3.1. Measurement of inhibition zone through agar well diffusion assay

The diameter of the inhibition zones on fourteen bacteria were measured and the results are tabulated in Table 2. The definition on sensitivity of bacteria toward honey was categorised as not sensitive (<8mm), sensitive (8 to 14 mm), very sensitive (15 to 19 mm) and extremely sensitive (20 mm and above) [22]. Kelulut has the diameter of inhibition zones ranged from 10.7 ±1.15 to 24.5 ±0.50 mm, indicating the susceptibility of bacteria toward Kelulut were varied between sensitive and extremely sensitive. Tualang recorded range of inhibition zone from 9.2 ±0.76 to 17.7 ±0.58 mm which the bacteria responded between sensitive and very sensitive toward Tualang. Acacia demonstrated the lowest antibacterial properties as it was the only honey that resulted with no inhibition on several bacteria including *P. aeruginosa*, *K. pneumonia*, *E. clocae* and *P. mirabilis*. The inhibition zone for Acacia ranged from no inhibition to 15.3 ±2.52 mm which the susceptibility of bacteria toward Acacia varied between not sensitive and very sensitive. The not sensitive response were demonstrated by nine bacteria including *S. aureus, S. hominis, P. aeruginosa, K. pneumonia, A. baumannii, E. clocae, P. mirabilis* and *P. vulgaris* towards Acacia.

In comparing between Malaysian honey, Kelulut showed the diameter of inhibition zones of more than 15 mm (very sensitive) on six bacteria - *S. hominis*, *S. haemolyticus*, *Salmonella* sp., *E. aerogenes*, *P. mirabilis*, and *P. vulgaris* while *S. haemolyticus* was the only bacteria that resulted more than 15 mm (very sensitive) toward Tualang and Acacia. Present study support the antibacterial properties possess by Acacia is likely caused by osmotic pressure due to presence of high sugar content as the diameter of inhibition zone measured for Acacia was similar to that of SB [23,24]. For Manuka, all bacteria were inhibited at diameter of inhibition zone of more than 15 mm (very sensitive and above), except on *P. aeruginosa* and *P. mirabilis* with inhibition zone of 9.0 ±0.00 and 10.8 ±0.29 mm, respectively. In comparing between Manuka and Malaysian honey, Kelulut was the only honey that demonstrated larger inhibition zone than Manuka. The effect was observed on *P. aeruginosa, P. mirabilis* and *P. vulgaris* with inhibition zone of 12.8 ±0.29 mm, 17.7 ±1.53 mm, and 20.7 ±1.15 mm, respectively and were 1.4, 1.6, and 1.2-fold larger than Manuka, respectively.

3.2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)
Further evaluation on antibacterial properties were done to identify the bacteriostatic and bactericidal effects owned by the honey. It was performed through determination of MIC and MBC, and the results are tabulated in Table 3. Kelulut demonstrated the MIC of below 20% (w/v) against the fourteen bacteria and the results were consistent with the previous study [15]. As compared to Tualang and Acacia, higher MIC were recorded which were below 40% and below 50% (w/v), respectively. For Manuka and SB, the MIC of not more than 15% and 60% (w/v) were recorded respectively. When compared between Manuka and Malaysian honey, Kelulut was the only honey that has lower MIC than Manuka which demonstrated on *E. coli, P. aeruginosa,* and *P. vulgaris* with the MIC of <5%, <5% and 7.5% (w/v), respectively as compared to manuka with 10%, 15%, and 10% (w/v), respectively. Also, similar MIC values were recorded on Kelulut and Manuka for *S. haemolyticus, A. baumannii, S. agalactiae* and *E. aerogenes* at <5%, <5%, 10% and 12.5% (w/v), respectively.

The results showed a different pattern for MBC evaluation. Manuka honey was observed to be generally stronger bactericidal agent as compared to Kelulut, Tualang and Acacia with the MBC ranged between 5% and 25% (w/v) to the tested bacteria, except on *P. aeruginosa.* Kelulut and Tualang has the MBC range from 12.5% to 40% (w/v) and from 30% to >90% (w/v), respectively. Acacia was the least strong bactericidal agent as the MBC were recorded to be generally >90% except for *P. aeruginosa* and *P. vulgaris* which were 50%, and 40% (w/v), respectively. MBC of Kelulut was recorded to be similar to Manuka which was at 20% (w/v) for *S. pyogenes* and *S. agalactiae* and 25% (w/v) for *E. aerogenes,* respectively. Interestingly, Kelulut has the lowest MBC of <5% (w/v) on *P. aeruginosa* and demonstrated as the most potent antibacterial agent to *P. aeruginosa* due to the lowest MIC and MBC recorded. The results were in a good agreement with the previous work that recorded the MIC and MBC of stingless bee honey on *P. aeruginosa* at 5% and 10% (w/w), respectively [25].

Among the tested Malaysian honey, Kelulut was the only honey that showed both bacteriostatic and bactericidal effects against the fourteen bacteria strains indicating potent antibacterial properties. The antibacterial action of Kelulut was reported to be due to presence of hydrogen peroxide (H_2O_2) [15], phenolics and flavonoids compounds [25–27] and acidic environment [26,28]. Among the factors, acidic environment was most prominent cause that influence the antibacterial properties of Kelulut [26] with the range of pH for between 2.4 to 3.4 [29]. In this study, the strong acidic environment was also recorded in Kelulut in which the average pH was 2.37 and was lowered by 1.78 and 1.65-fold as compared to Tualang and Acacia. This may explained the effectiveness of Kelulut to eliminate *P. aeruginosa* since its optimum pH for growth was 6.6 to 7.0 [30]. In addition, the physical appearance of dark amber in Kelulut (Table 1) indicate that the honey contains high phenolics and flavonoids compounds [25,27,31,32]. These compounds possess antimicrobial effect on its own and the effect may increase if combine with strong acidic environment [33]. This was expected to be the additional factors that enhanced the antibacterial properties of Kelulut as compared to Tualang and Acacia.

Table 2. Measurement of inhibition zone of honey on fourteen clinically isolated bacteria from wound site.

	Zone of inhibition (mm)										
	Kelulut Samples no.			Tualang samples no.			Acacia samples no.			Manuka	SB
	1	2	3	1	2	3	1	2	3		
Gram-positive											
S. aureus	14.3 ±0.58	13.7 ±1.53	13.7 ±1.15	9.3 ±0.58	12.2 ±0.29	12.3 ±0.58	13.3 ±0.58	9.0 ±1.00	7.8 ±0.29	23.8 ±0.29	7.7 ±0.29
S. hominis	17.8 ±1.26	18.0 ±1.00	17.7 ±1.53	14.5 ±1.32	15.0 ±0.00	13.5 ±1.32	12.3 ±1.53	8.0 ±0.00	7.8 ±0.29	21.3 ±0.58	-
S. haemolyticus	24.3 ±0.58	23.8 ±0.76	24.5 ±0.50	17.7 ±0.58	15.7 ±2.08	14.7 ±0.58	15.3 ±2.52	11.0 ±1.8	8.2 ±0.29	25.3 ±0.58	10.2 ±0.76
S. pyogenes	12.7 ±0.58	12.7 ±0.58	12.5 ±0.50	10.8 ±0.76	9.2 ±0.29	9.8 ±0.29	8.8 ±0.29	9.0 ±0.00	8.2 ±0.29	16.5 ±1.32	8.2 ±0.29
S. agalactiae	12.2 ±0.29	12.2 ±1.04	12.5 ±0.50	9.8 ±0.29	10.5 ±0.50	10.3 ±0.50	8.3 ±0.58	8.3 ±0.58	8.2 ±0.29	16.3 ±0.58	9.2 ±0.76
Gram-negative											
E. coli	11.3 ±3.21	11.0 ±1.00	11.3 ±1.15	10.5 ±0.50	11.3 ±0.29	11.0 ±0.00	8.7 ±0.58	8.2 ±0.29	7.8 ±0.29	18.5 ±0.50	-
P. aeruginosa	12.8 ±0.29	12.5 ±1.50	12.2 ±0.29	9.2 ±0.76	9.7 ±0.58	10.5 ±0.50	9.8 ±0.58	8.3 ±0.58	-	9.0 ±0.00	8.5 ±0.50
K. pneumonia	12.2 ±2.25	12.0 ±1.00	10.7 ±1.15	10.3 ±0.58	10.7 ±0.29	10.2 ±0.29	9.2 ±0.29	8.2 ±0.29	-	16.7 ±0.58	8.8 ±0.29
Salmonella sp.	16.8 ±1.26	15.7 ±0.58	16.5 ±1.32	11.0 ±0.00	13.0 ±0.50	10.7 ±0.76	9.8 ±0.29	8.3 ±0.58	8.0 ±0.00	18.0 ±1.32	8.7 ±0.58
A. baumannii	11.5 ±1.32	12.0 ±0.87	10.7 ±0.29	10.7 ±0.58	10.2 ±0.29	11.5 ±0.50	12.2 ±1.76	8.3 ±0.29	7.7 ±0.29	20.3 ±0.58	-
E. clocae	14.3 ±1.15	13.3 ±0.58	15.0 ±0.00	10.5 ±0.50	12.0 ±1.00	11.2 ±0.76	9.0 ±1.00	8.7 ±1.15	-	17.2 ±0.29	9.0 ±1.00
E. aerogenes	15.3 ±2.08	15.2 ±0.29	16.2 ±2.36	10.7 ±1.26	12.0 ±0.00	11.7 ±1.15	11.0 ±0.00	9.3 ±1.53	8.0 ±0.00	17.3 ±1.53	8.8 ±0.29
P. mirabilis	16.8 ±1.44	16.8 ±1.61	17.7 ±1.53	10.8 ±0.29	12.7 ±1.53	12.0 ±0.00	8.0 ±0.00	-	7.8 ±0.29	10.8 ±0.29	-
P. vulgaris	20.7 ±1.15	19.0 ±1.00	19.3 ±0.58	13.7 ±0.58	15.3 ±0.29	12.2 ±0.76	12.0 ±0.00	8.0 ±0.00	7.8 ±0.29	17.7 ±0.58	9.3 ±0.76

The symbol – represents no inhibition zone observed. The test was performed for three biological replicates. One sample t-test shown significant differences for the data collected (P-value < 0.05).

Table 3. MIC and MBC of tested honey against fourteen clinical isolated bacteria.

Bacteria	Kelulut		Tualang		Acacia		Manuka		SB	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive										
S. aureus	10%	30%	40%	50%	30%	>90%[b]	<5%[a]	5%	50%	>90%[b]
S. hominis	6.25%	25%	15%	30%	50%	>90%[b]	<5%[a]	7.5%	60%	>90%[b]
S. haemolyticus	<5%[a]	25%	12.5%	50%	40%	>90%[b]	<5%[a]	7.5%	40%	>90%[b]
S. pyogenes	20%	20%	30%	60%	30%	>90%[b]	10%	20%	40%	>90%[b]
S. agalactiae	10%	20%	30%	60%	40%	>90%[b]	10%	20%	40%	>90%[b]
Gram-negative										
E. coli	7.5%	40%	25%	>90%[b]	40%	>90%[b]	10%	12.5%	40%	>90%[b]
P. aeruginosa	<5%[a]	12.5%	20%	40%	30%	50%	15%	20%	40%	>90%[b]
K. pneumonia	10%	30%	40%	>90%[b]	30%	>90%[b]	6.25%	10%	40%	>90%[b]
Salmonella sp.	7.5%	25%	20%	60%	40%	>90%[b]	6.25%	10%	40%	>90%[b]
A. baumannii,	<5%[a]	12.5%	15%	60%	30%	>90%[b]	<5%[a]	7.50%	30%	>90%[b]
E. clocae	7.5%	20%	25%	>90%[b]	50%	>90%[b]	6.25%	10%	50%	>90%[b]
E. aerogenes	12.5%	25%	30%	60%	40%	>90%[b]	12.5%	25%	50%	>90%[b]
P. mirabilis	7.5%	25%	25%	>90%[b]	40%	>90%[b]	12.5%	15%	50%	>90%[b]
P. vulgaris	<5%[a]	20%	15%	30%	25%	40%	10%	12.5%	50%	>90%[b]

[a] The lowest concentration tested.
[b] The highest concentration tested.
One sample t-test shown significant differences for the data collected (P-value < 0.05).

Based on our observation from Quadrupole Time-of-Flight Mass Liquid Chromatography-mass Spectrometer (LCMS-QTOF), Kelulut had compounds such as reptoside, saikosaponin and 8-O acethylharpagide which were absence in Tualang and Acacia (data not shown) and these compounds have been associated with antibacterial properties and defend mechanism against bacterial infection [34–36]. The data may additionally explained the wide coverage of bactericidal effect in Kelulut against wound infectious bacteria.

In this study, *S. pyogenes* was inhibited at the highest concentration of Kelulut. This can be due to its ability to develop resistant mechanism such as biofilm formation to become less susceptible. Similar pattern was demonstrated by other types of honey in which higher concentration was require to inhibit *S. pyogenes*. However, once inhibited, *S. pyogenes* was simultaneously destroyed by Kelulut. The finding was in line with previous study that recorded simultaneous effects of both bacteriostatic and bactericidal at the same concentration of Kelulut on other bacteria species – *S. aureus, E. coli, P. aeruginosa* and *B. cereus* [15]. In contrast, *E. coli* was inhibited at low concentration of 7.5 (w/v) and require higher concentration to be killed by Kelulut which was at 40% (w/v). This could be due to the ability of cell wall peptidoglycan of *E. coli* to recover from disruption and resist penetration of compounds at low concentration of honey to promote cell lysis. Previous study has also reported a variation gap of bacteriostatic and bactericidal effects of Kelulut on *E. coli* with the MIC at 8% and MBC at 32%, respectively [28]. Similar condition were also notified on Tualang and Acacia which inhibit *E. coli* at low concentration (MIC of <40%), but refuse to abolish the bacteria even at highest concentration (90%).

In many types of acute and chronic wounds, *S. aureus* and *P. aeruginosa* are usually isolated from the infected wounds [37,38]. These bacteria often causes biofilm chronic infections which may suppress immune and antimicrobial activities, and promotes on the development of antibiotic resistance strain [38]. Similar to *S. aureus* and *P. aeruginosa*, other wound associate bacteria such as *S. pyogenes*, *P.*

mirabilis, and *E. clocae* can also develop biofilm as a resistance mechanism [39]. In this study, these bacteria that isolated from the infected wound site have been tested and found to be susceptible to the tested honey, especially on Kelulut. The antibacterial properties of Kelulut was demonstrated to be higher than Manuka on certain well-known biofilm-formation organisms – *P. aeruginosa* and *P. mirabilis*. Further evaluation to confirm on ability and factors that contribute to eradicate biofilm formation by Kelulut is necessary as the finding is beneficial in order to suppress the resistant mechanism owned by those infectious bacteria.

4. Conclusion

Malaysian Kelulut, Tualang and Acacia honey do possess antibacterial properties against the clinically isolated bacteria from wound which confirmed through manifestation of inhibition zone, bacteriostatic and bactericidal effects. Among the honey, Kelulut demonstrated the highest antibacterial properties since both bacteriostatic and bactericidal effects was owned by Kelulut against the fourteen clinical isolated bacteria. The similar effects was absence on Tualang and Acacia. Further evaluation on compounds that contribute to the antibacterial properties of Malaysian Kelulut, Tualang, and Acacia are necessary to potentially utilise the honey as an alternative to prevent bacterial infection.

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Ethical Approval Number

This project was performed under the ethical approval of IREC 2019-062. The IREC operates in accordance to the Declaration of Helsinki, International Conference of Harmonization Good Clinical Practice Guidelines (ICH-GCP), Malaysia Good Clinical Practice Guidelines and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.

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