

## Antibacterial properties of Tualang, Acacia and Yemeni Sumur honey against selected food spoilage bacteria and foodborne pathogens

<sup>1</sup>Yousof, K., <sup>1,\*</sup>Nor-Khaizura, M.A.R., <sup>2</sup>Nur Hanani, Z.A. and <sup>2</sup>Ismail-Fitry, M.R.

<sup>1</sup>Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia

<sup>2</sup>Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia

### Article history:

Received: 5 August 2019  
Received in revised form: 5 October 2020  
Accepted: 28 January 2021  
Available Online: 13 February 2021

### Keywords:

Honey,  
Minimum Inhibitory Concentration,  
Minimum Bactericidal Concentration,  
pH,  
water activity

### DOI:

[https://doi.org/10.26656/fr.2017.5\(1\).270](https://doi.org/10.26656/fr.2017.5(1).270)

### Abstract

The antibacterial activity of honey is mainly credited to its acidity, osmolarity and enzymatic generation of hydrogen peroxide via glucose oxidase. Additional honey components, such as aromatic acids or phenolic compounds, also contribute to the overall antibacterial activity. The level of antibacterial activities found in honey varies with different types of honey, due to mainly the composition, percentage as well as the nature of the sugars present in the honey. This study aimed to evaluate the antibacterial activity of four types of honey, namely Tualang honey (TH<sub>1</sub>), Tualang honey (TH<sub>2</sub>), Acacia honey (AH) and Yemeni Sumur honey (YSH). Nine bacterial strains were used. Disc diffusion, well diffusion, minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC), and time-kill methods were performed to reveal the antibacterial potential of the selected honey. The MIC values ranged between 12.5 to 50% for both TH<sub>1</sub> and YSH while for TH<sub>2</sub> and AH it ranged between 25 to 50%. For MBC, it ranged from 25 to 50%. The time-kill in TH<sub>1</sub> *Staphylococcus aureus* (food isolate) showed total inhibition at 6 hrs in 2 X MIC, and for *Staphylococcus aureus* ATCC 29737 was 3.84 log CFU/g at the 6 hrs. Physicochemical quality of honey resulted as follows: the pH of the honey samples was acidic in nature ranging between 3.69 to 3.94, and the a<sub>w</sub> of the honey samples were between 0.53 to 0.69. For colour analysis, YSH was observed to have the maximum lightness and yellowness, and TH<sub>1</sub> has the maximum redness. While, AH had a minimum lightness, redness, and yellowness.

## 1. Introduction

Honey is characterised as a viscous, aromatic and sweet food substance that many people around the world enjoy its consumption. Since ancient times, honey has been used in medical treatment as an antiseptic until the antibiotics were invented. However, the treatment of diseases has become more difficult than ever due to the emergence of antibiotic-resistant bacteria. As a result, humans seek a natural alternative such as honey to treat diseases. Honey contains antibacterial compounds that are effective in killing or inhibiting a broad spectrum of bacteria (Boukraâ, 2013). Various studies have shown that honey is effective against a wide range of microorganisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

For all antibiotic classes, including the major last-

resort drugs, resistance is increasing worldwide and even more alarming, very few new antibiotics are being developed. The potent activity of honey against antibiotic-resistant bacteria resulted in renewed interest for its application. Several types of honey have been approved for clinical applications. The incomplete knowledge of the antibacterial compounds involved and the variability of antibacterial activity are, however, major obstacles for applicability of honey. Therefore, the objective of this work is to determine the *in vitro* antibacterial activity of Tualang Honey 1 (TH<sub>1</sub>), Tualang Honey 2 (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH) against foodborne pathogens and spoilage bacteria.

\*Corresponding author.

Email: [norkhaizura@upm.edu.my](mailto:norkhaizura@upm.edu.my)

## 2. Materials and methods

### 2.1 Honey samples

In this study, four honey samples were used. Tualang Honey 1 (TH<sub>1</sub>) is from Federal Agricultural Marketing Authority (FAMA), Malaysia. Tualang Honey 2 (TH<sub>2</sub>) is from MAM Industries, Malaysia. Acacia Honey (AH) is from Summer Pacific Sdn. Bhd., Malaysia. Yemeni Sumur honey (YSH) is from Rayyan Salsabila Sdn. Bhd., Malaysia. The differences between TH<sub>1</sub> and TH<sub>2</sub> were, therefore, just their brands.

### 2.2 Preparation of honey samples

Honey solutions were prepared by diluting honey to the required concentrations based on two-fold serial dilutions (0.195, 0.390, 0.781, 1.56, 3.125, 6.25, 12.5, 25, and 50% w/v) to check for the antibacterial activity of honey (CODEX STAN, 1981). The honey solutions were handled aseptically to avoid cross-contamination. All samples were then incubated for 30 mins at 37°C in a shaking water bath that allowed aeration of the solutions. Incubation was carried out in the dark because both hydrogen peroxide and glucose oxidase are light sensitive.

### 2.3 Test organisms

Nine bacterial strains were assayed; four were Gram-positive *Staphylococcus aureus* ATCC 29737, *Bacillus cereus* (food isolate), *Staphylococcus aureus* (food isolate) and *Listeria monocytogenes* ATCC 19112, and five Gram-negative *Escherichia coli* (food isolate), *Campylobacter jejuni* ATCC 29428, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 10536 and *Salmonella enterica* ser. Typhimurium ATCC 13311. Stock cultures of the ATCC were obtained from Thermo Fisher Scientific (US) while the remaining microorganisms were obtained from the culture collection of the Laboratory of Food Microbiology, Faculty of Food Science and Technology, University of Putra (UPM), Malaysia.

### 2.4 Preparation of test organisms

Stock cultures of the bacterial pathogens were subcultured in Nutrient agar (Oxoid, UK) at 37°C for 24 hrs. Colonies of fresh cultures of the different microorganisms from overnight growth were picked with a sterile inoculating loop and suspended in 3 to 4 mL of Nutrient broth (Oxoid, UK) in sterile test tubes and incubated for 2 to 3 hrs at 37°C. This was diluted with sterile distilled water to standardise the inoculum density used in this study.

### 2.5 Antibacterial activity of honey

#### 2.5.1 Disc diffusion assay

Disc diffusion assay was performed to demonstrate the sensitivity of microorganisms to the honey samples with a slight modification in which an overnight bacterial culture was suspended into 5 mL of 0.1% normal saline and adjusted with 0.5 Mc Farland standards (Reller *et al.*, 2009). A 50 µL of the bacterial suspension was spread well using sterile swabs on Mueller Hinton agar (Oxoid, UK). Sterile paper discs (Whatman AA discs, 6 mm in diameter) were used to impregnate nine different dilutions of each of the honey samples as follows (0.195, 0.390, 0.781, 1.56, 3.125, 6.25, 12.5, 25, and 50% w/v). Twenty microliters of each dilution of honey were soaked into the paper discs and allowed to dry at room temperature and placed aseptically on the surface of inoculated agar using sterile forceps and finally incubated for 16 to 24 hrs at 37°C. The assessment of antibacterial activity was based on the measurement of the diameter of the inhibition zone appearing around the disc. The sterile blank disc was used as a negative control. As a positive control, ciprofloxacin and gentamicin (Oxoid, UK) were used for Gram-negative bacteria, and for Gram-positive bacteria, ampicillin and penicillin G (Oxoid, UK) were used (Tumin *et al.*, 2005).

#### 2.5.2 Well diffusion assay

The procedure was similar to that of disc diffusion assay. Mueller Hinton plates were inoculated with 50 µL of 0.5 Mc Farland standard bacterial suspension. A hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a pipette tip, and 100 to 200 µL of honey of different concentrations (0.195, 0.390, 0.781, 1.56, 3.125, 6.25, 12.5, 25, and 50% w/v) was introduced into the well. Plates were incubated for 16 to 24 hrs at 37°C. The assessment of antibacterial activity was based on the measurement of the diameter of the inhibition zone appearing around the hole. Sterile distilled water was used as a negative control. As a positive control, ciprofloxacin and gentamicin (Oxoid, UK) were used for Gram-negative bacteria, and for Gram-positive bacteria, ampicillin and penicillin G (Oxoid, UK) were used (Tumin *et al.*, 2005).

#### 2.5.3 Minimum Inhibitory Concentration (MIC)

The MIC of different honey samples was determined using the two-fold dilution method (Balouiri *et al.*, 2016). Honey batches were investigated for their MIC against all the nine bacterial strains. About 1 mL of honey was transferred into each test tube followed by the addition of 1 mL of Mueller Hinton (Oxoid, UK) broth that contained standardised inoculum of 0.5 Mc Farland to achieve final dilutions of 0.195, 0.390, 0.781, 1.56,

3.125, 6.25, 12.5, 25, and 50% (v/v). The inoculated tubes were incubated at 37°C for 24 hrs. The highest dilution of the tested honey to inhibit growth (no turbidity in the tube) was considered as the MIC value of the honey against the tested bacterial species.

#### 2.5.4 Minimum Bactericidal Concentration (MBC)

MBC test was performed following the MIC test via a streak plate method. Each honey dilution with no bacterial growth (turbidity) from the MIC test was assayed. A loopful of bacterial strain was inoculated into sterile Mueller Hinton plates. The plates were then incubated at 37°C for 24 hrs. The least concentration that did not show any growth of tested organisms was considered as the MBC value of the tested honey against the tested bacterial species (Balouiri et al., 2016).

#### 2.5.5 Time-Kill

Time-kill assay was performed to determine the time taken for the microorganisms to be killed by the honey, and it was performed following the method of Rukayadi et al. (2010). Mueller Hinton broth was used, and bacterial inoculum was adjusted between 6 to 8 log CFU/mL. Final concentrations of honey were 0 MIC, ½× MIC, 1× MIC, and 2× MIC. Four bijoux bottles were added with the right amount of the inoculum and the honey respectively to produce the final concentrations of 0 MIC, ½× MIC, 1× MIC, and 2× MIC aliquots. These steps were performed for all the strains. The cultures were then incubated at 37°C with the agitation of 200 rpm. At predetermined intervals 0, ½, 1, 2, 4 and 6 hrs, 1 mL aliquots were serially diluted in 1% phosphate-buffered saline (PBS) and plated onto Mueller Hinton (Oxoid, UK). The plates were incubated at 37°C for 24 hrs, and the number of colonies was counted. The assays were carried out in duplicate. The graph of log<sub>10</sub> CFU/mL was plotted against time.

### 2.6 Physicochemical activity of honey

#### 2.6.1 Determination of pH

The pH values of the honey samples (10% aqueous honey solution) were measured at 28±2°C using a digital pH meter (Mettler Toledo) calibrated at pH 4.0, 7.0 and 9.0 using standard buffer solutions (Saxena et al., 2010).

#### 2.6.2 Determination of water activity

The water activity of honey solutions was determined at 25±0.2°C using an electronic dew-point water activity meter (Aqualab Series 3 model TE, USA) equipped with a temperature-controlled system which stabilised the temperature during measurement (Chirife et al., 2006).

#### 2.6.3 Colour analysis

The honey surface colour was measured using a colourimeter (HunterLab, model D25 L optical sensor, Hunter Associates Reston, VA, USA) calibrated with a white and a black standard plate. Honey solution (10 g of honey in 75 mL free CO<sub>2</sub> distilled water) was placed into a cylindrical (base diameter 11.3 cm and height 2 cm) optical cell. Reflectance values were obtained using a 45 mm viewing aperture. The results reported were the mean of five determinations (Shafiee et al., 2014).

#### 2.7 Statistical analysis

Experiments were performed in duplicate, and the results were expressed as mean values with standard deviations (SD). The significant differences were obtained by one-way analysis of variance (ANOVA, Tukey's test).

### 3. Results and discussion

#### 3.1 Disc diffusion assay

For the Disc Diffusion Assay, only *S. aureus* showed inhibition with a clear zone in all types of honey. For TH<sub>1</sub>, the inhibition zone at 25% was 10.08 mm, and at 50% the inhibition zone was 15.01 mm. For TH<sub>2</sub>, the inhibition zone at 25% was 6.25 mm, and at 50% it was 12.92 mm. For AH, the inhibition zone at 25% was 8.82 mm, and at 50% it was 13.06 mm. For YSH, the inhibition zone at 25% was 12.49 mm, and at 50% it was 20.35 mm, as shown in Table 1. The diameter of the inhibition zone of the positive control was between 22.00 – 24.24 mm, which was higher than the 50% concentration of treatments. The rest of the bacteria did not show any inhibition zone. There was a significant ( $p < 0.05$ ) difference between the samples.

Table 1. Diameter of inhibition zone (DIZ) of Tualang Honey (TH<sub>1</sub>), Tualang Honey (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH) against *Staphylococcus aureus* using Disc Diffusion Assay

Honey	Concentration (%)			Control
	12.5 mm	25 mm	50 mm	
TH <sub>1</sub>	6.00±0.00 <sup>c</sup>	10.08±0.20 <sup>b</sup>	15.01±0.35 <sup>a</sup>	P 22.40
TH <sub>2</sub>	6.00±0.00 <sup>c</sup>	6.25±1.21 <sup>b</sup>	12.92± 0.66 <sup>a</sup>	P 23.10
AH	6.00±0.00 <sup>c</sup>	8.82±0.54 <sup>b</sup>	13.06±0.41 <sup>a</sup>	P 24.24
YSH	6.00±0.00 <sup>c</sup>	12.49±0.38 <sup>b</sup>	20.35±1.07 <sup>a</sup>	P 22.00

Diameter of inhibition zone in mm (including disc). Values are expressed as mean±standard deviation. Values with different superscript within the row are significantly different ( $p < 0.05$ ).

The use of disc could lead to the exclusion of large molecules which are not properly absorbed by the paper disks and may contribute to inaccurate results as honey is

a complex solution consisting of different sizes of chemicals and compounds (Zainol et al., 2013). Kolayli et al. (2016) examined the antibacterial activities of chestnut honey using a disc, found that the highest inhibition was achieved against pathogenic bacteria such as *S. aureus*, *E. coli*, *Enterococcus faecalis* and *Yersinia pseudotuberculosis*.

### 3.2 Well diffusion assay

In the preliminary screening, all the honey showed various range diameter of inhibition zone (DIZ) against most of the tested bacteria. More than half of the isolates showed antibacterial activity in a concentration between 12.5, 25 and 50% (w/v). The inhibitory activity was significantly ( $p < 0.05$ ) affected by the type of bacteria used as well as the honey samples. TH<sub>1</sub> and YSH had the highest DIZ. For TH<sub>1</sub>, as shown in Table 2, the range was between 15.82 mm and 31.9 mm, lowest DIZ was seen on *S. aureus*, which was 15.82 mm at 25%. At 12.5 and 25%, both *E.* and *S. enterica* ser. Typhimurium ATCC 13311 almost showed the same DIZ, which was 18.13 mm and 18.37 mm, respectively. The DIZ was seen on *S. enterica* ser. Typhimurium ATCC 13311 at 50%, which was 31.95 mm. *S. aureus* ATCC 2973, *E. coli*, and *C.*

*jejuni* ATCC 29428 were almost in the same range 25.88, 25.23 and 25.44 mm, respectively. Only the positive control of *S. aureus* (food isolate) and *P. aeruginosa* ATCC 27853 had bigger DIZ. The rest of the controls showed lower DIZ in which 50% of honey was effective inhibiting than the antibiotic.

For TH<sub>2</sub>, DIZ was in the range of 13.91 mm and 27.16 mm as seen in Table 3 The highest DIZ was seen on *S. aureus* ATCC 29737 which was 27.26 mm at 50% and the lowest DIZ was seen on both *S. enterica* ser. Typhimurium ATCC 13311 and *E. coli* which were almost the same 13.91 mm and 13.93 mm at 12.5% and 25%, respectively. In the positive control, *S. aureus* (food isolate) had bigger DIZ of 2.29 mm. The rest of the controls showed lower DIZ. While for AH, the range was between 11.17 mm and 33.87 mm as shown in Table 4, *S. enterica* ser. Typhimurium ATCC 13311 showed bigger DIZ than TH<sub>1</sub>, which was 33.87 mm followed by both *S. aureus* and *S. aureus* ATCC 29737, which had 26.06 mm and 29.73 mm DIZ respectively, at 50%. The lowest DIZ was seen on *S. enterica* ser. Typhimurium ATCC 13311 same as TH<sub>2</sub> which was 11.1 mm at 12.5%. All positive control had smaller DIZ as compared to 50%

Table 2. Diameter of inhibition zone (DIZ) of Tualang Honey (TH<sub>1</sub>) against foodborne and pathogenic bacteria using Well Diffusion Assay

Bacteria	TH <sub>1</sub> (%)			
	12.5 mm	25 mm	50 mm	Control
<i>S. aureus</i>	6.00±0.00 <sup>c</sup>	15.82±0.57 <sup>b</sup>	23.66±0.23 <sup>a</sup>	P 25.36
<i>S. aureus</i> ATCC 29737	6.00±0.00 <sup>b</sup>	6.00±0.00 <sup>b</sup>	25.88±0.18 <sup>a</sup>	P 22.45
<i>E. coli</i>	18.13±0.18 <sup>b</sup>	6.00±0.00 <sup>c</sup>	25.23±0.18 <sup>a</sup>	CIP 7.47
<i>E. coli</i> ATCC 10536	6.00±0.00 <sup>b</sup>	6.00±0.00 <sup>b</sup>	23.38±0.40 <sup>a</sup>	CIP 7.73
<i>S. enterica</i> ser. Typhimurium ATCC 13311	6.00±0.00 <sup>c</sup>	18.37±0.19 <sup>b</sup>	31.95±1.27 <sup>a</sup>	CN 16.34
<i>P. aeruginosa</i> ATCC 27853	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	CN 12.56
<i>C. jejuni</i> ATCC 29428	6.00±0.00 <sup>b</sup>	6.00±0.00 <sup>b</sup>	25.44±0.16 <sup>a</sup>	CIP 0.00
<i>L. monocytogenes</i> ATCC 19112	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	CIP 0.00

Diameter of inhibition zone in mm (including disc). Values are expressed as mean±standard deviation. Values with different superscript within the row are significantly different ( $p < 0.05$ ).

Table 3. Diameter of inhibition zone (DIZ) of Tualang Honey (TH<sub>2</sub>) against foodborne and pathogenic bacteria using Well Diffusion Assay

Bacteria	TH <sub>2</sub> (%)			
	12.5 mm	25 mm	50 mm	Control
<i>S. aureus</i>	21.69±0.06 <sup>c</sup>	23.21±0.25 <sup>b</sup>	25.55±0.40 <sup>a</sup>	P 27.29
<i>S. aureus</i> ATCC 29737	6.00±0.00 <sup>c</sup>	20.27±0.25 <sup>b</sup>	27.16±0.86 <sup>a</sup>	P 25.76
<i>E. coli</i>	6.00±0.00 <sup>c</sup>	13.93±0.43 <sup>b</sup>	25.12±0.44 <sup>a</sup>	CIP 7.33
<i>E. coli</i> ATCC 10536	21.08±0.11 <sup>b</sup>	23.60±0.85 <sup>ab</sup>	25.11±1.04 <sup>a</sup>	CIP 8.10
<i>S. enterica</i> ser. Typhimurium ATCC 13311	13.91±0.47 <sup>c</sup>	20.53± 0.30 <sup>b</sup>	25.07±0.09 <sup>a</sup>	CN 18.22
<i>P. aeruginosa</i> ATCC 27853	6.00±0.00 <sup>b</sup>	6.00±0.00 <sup>b</sup>	24.64±0.81 <sup>a</sup>	CN 14.32
<i>C. jejuni</i> ATCC 29428	6.00±0.00 <sup>b</sup>	6.00±0.00 <sup>b</sup>	22.26±1.02 <sup>a</sup>	CIP 0.00
<i>L. monocytogenes</i> ATCC 19112	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	CIP 0.00

Diameter of inhibition zone in mm (including disc). Values are expressed as mean±standard deviation. Values with different superscript within the row are significantly different ( $p < 0.05$ ).

Table 4. Diameter of inhibition zone (DIZ) of Acacia Honey (AH) against foodborne and pathogenic bacteria using Well Diffusion Assay

Bacteria	AH (%)			
	12.5 mm	25 mm	50 mm	Control
<i>S. aureus</i>	18.14±0.17 <sup>b</sup>	18.94±1.02 <sup>b</sup>	26.06±0.08 <sup>a</sup>	P 23.50
<i>S. aureus</i> ATCC 29737	19.29±0.06 <sup>c</sup>	23.70±0.37 <sup>b</sup>	29.73±0.52 <sup>a</sup>	P 27.00
<i>E. coli</i>	6.00±0.00 <sup>c</sup>	22.97±0.32 <sup>b</sup>	25.29±0.33 <sup>a</sup>	CIP 7.44
<i>E. coli</i> ATCC 10536	6.00±0.00 <sup>c</sup>	21.81±0.61 <sup>b</sup>	25.08±0.60 <sup>a</sup>	CIP 7.10
<i>S. enterica</i> ser. Typhimurium ATCC 13311	11.17±0.77 <sup>c</sup>	20.61±0.31 <sup>b</sup>	33.87±0.36 <sup>a</sup>	CN 19.14
<i>P. aeruginosa</i> ATCC 27853	15.86±0.45 <sup>c</sup>	21.82±0.47 <sup>b</sup>	25.15±0.88 <sup>a</sup>	CN 16.50
<i>C. jejuni</i> ATCC 29428	6.00±0.00 <sup>c</sup>	16.89±0.39 <sup>b</sup>	22.18±1.05 <sup>a</sup>	CIP 0.00
<i>L. monocytogenes</i> ATCC 19112	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	CIP 0.00

Diameter of inhibition zone in mm (including disc). Values are expressed as mean±standard deviation. Values with different superscript within the row are significantly different ( $p < 0.05$ ).

Table 5. Diameter of inhibition zone (DIZ) of Yemeni Sumur Honey (YSH) against foodborne and pathogenic bacteria using Well Diffusion Assay

Bacteria	YSH (%)			
	12.5 mm	25 mm	50 mm	Control
<i>S. aureus</i>	15.13±0.09 <sup>c</sup>	19.87±0.13 <sup>b</sup>	29.89±0.46 <sup>a</sup>	P 28.11
<i>S. aureus</i> ATCC 29737	6.00±0.00 <sup>b</sup>	6.00±0.00 <sup>b</sup>	35.75±0.69 <sup>a</sup>	P 26.00
<i>E. coli</i>	6.00±0.00 <sup>c</sup>	25.19±0.63 <sup>b</sup>	27.71±0.77 <sup>a</sup>	CIP 8.20
<i>E. coli</i> ATCC 10536	6.00±0.00 <sup>c</sup>	22.84±0.49 <sup>b</sup>	29.01±1.30 <sup>a</sup>	CIP 7.24
<i>S. enterica</i> ser. Typhimurium ATCC 13311	6.00±0.00 <sup>c</sup>	20.80±0.55 <sup>b</sup>	24.99±1.15 <sup>a</sup>	CN 18.26
<i>P. aeruginosa</i> ATCC 27853	6.00±0.00 <sup>c</sup>	23.83±1.16 <sup>a</sup>	26.50±0.71 <sup>a</sup>	CN 15.00
<i>C. jejuni</i> ATCC 29428	6.00±0.00 <sup>c</sup>	17.58±0.74 <sup>b</sup>	21.96±1.30 <sup>a</sup>	CIP 0.00
<i>L. monocytogenes</i> ATCC 19112	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	6.00±0.00 <sup>a</sup>	CIP 0.00

Diameter of inhibition zone in mm (including disc). Values are expressed as mean±standard deviation. Values with different superscript within the row are significantly different ( $p < 0.05$ ).

honey concentration. YSH was in a range of 15.13 mm and 35.75 mm, as seen in Table 5. *S. aureus* ATCC 29737 showed the highest DIZ, 35.75 mm at 50%.

Meanwhile, at 12.5%, only *S. aureus* was inhibited with DIZ of 15.13 mm, and it was the lowest DIZ. All positive control had smaller DIZ as compared to 50% honey concentration. There was no inhibition of *B. cereus* and *L. monocytogenes* ATCC 19112 in all four types of honey.

Agar Diffusion Assay to assess the antibacterial activity is commonly performed in three different ways; well/cup diffusion, disc diffusion or agar dilution. The method usually depends on the nature of the antibacterial or the antibacterial to be tested, and on the kinetic properties of the molecules present in the honey. Agar well allows direct contact of honey components and bacteria immediately after application. The diffusion mechanism may also represent *in-vivo* conditions when honey is applied on infected wounds, and therefore, may provide information about the kinetic system of honey application (Zainol et al., 2013).

Both well diffusion and disc diffusion were

performed to compare between them, and there was a huge difference between the results which was obtained by both methods. Agar well exercise allows direct contact of honey components and bacteria immediately after application. In one study, it was shown that Tualang Honey could inhibit the growth of food spoilage bacteria which were *S. aureus*, *S. enterica* ser. Typhimurium, *E. coli* and *P. aeruginosa* as the DIZ was 24.50 mm, 18 mm, 16.50 mm and 16 mm, respectively (Aween et al., 2014). In the present work, for TH<sub>1</sub>, *S. aureus* had 15.82 mm DIZ at 25% which was less than they reported, but for TH<sub>2</sub> it was similar to the previous study for both *S. aureus* and *S. aureus* ATCC 29737 with DIZ of 23.21 mm and 20.27 mm. For *S. enterica* ser. Typhimurium ATCC 13311, both TH<sub>1</sub> and TH<sub>2</sub> had larger zones which were 18.37 mm and 20.53 mm, respectively.

Several researchers have tested the antibacterial activity of honey including Acacia, Tualang, and Manuka (UMF 18+) honey using well diffusion method against *S. aureus*, *E. coli*, *P. aeruginosa* and *B. cereus*; The highest inhibitory zone was obtained from Manuka honey against *S. aureus* with DIZ of 19.81 mm and in *E. coli* 14.04 mm. While in Tualang honey, *P. aeruginosa*

showed DIZ of 16.22 mm and in *B. cereus* 27.35 mm. Acacia honey showed the lowest inhibitory activity as compared to the other honey samples (Zainol et al., 2013). In this study, YSH showed bigger zones than Seder honey (Aween et al., 2014). Yemeni honey is well known for the antibacterial activity and the ability to fight bacteria. In *S. enterica* ser. Typhimurium ATCC 13311, the DIZ was 20.80 mm, and in *E. coli* it was 25.19 mm. In *P. aeruginosa* ATCC 27853, DIZ was 23.83 mm, and in *E. coli* ATCC 10536 22.84 mm (Aween et al., 2014). Furthermore, in this study, AH showed bigger DIZ when compared to another study which also used Acacia honey (Zainol et al., 2013). *S. aureus* ATCC 2937 showed DIZ of 23.70 mm, *E. coli* 22.97 mm and *P. aeruginosa* ATCC 27853 21.82 mm. Overall in the present study, both Tualang and Acacia honey showed an inhibitory effect against *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. enterica* ser. Typhimurium.

The antibacterial activity of *Tetragonisca angustula* honey has been well known and was found to possess good antibacterial activity against *S. aureus*. Another study revealed that *T. angustula* honey had significant antibacterial activity against several different bacterial strains, including *B. cereus* and *P. aeruginosa* (Miorin et al., 2003). A recent study has confirmed the antibacterial activity of *T. angustula* honey on Gram-positive bacteria such as *S. aureus*, and Gram-negative bacteria such as *P. aeruginosa* and *E. coli* (Rao et al., 2016). Wilkinson and Cavanagh (2005) studied the antibacterial activity of different types of honey against *E. coli* and *P. aeruginosa*. They found that all types of honey showed a zone of inhibition. A study was done using Nigerian honey against *S. aureus*, *E. coli*, and *P. aeruginosa*. It was found that 50% concentration could inhibit *P. aeruginosa* with DIZ of 8 mm and 100% could inhibit all the other bacteria with DIZ of 11 mm, 13.3 mm and 8 mm for *S. aureus*, *E. coli* and *P. aeruginosa*, respectively (Agbaje et al., 2006).

The inconsistency in the observed antibacterial activity could be due to a number of reasons. One of them could be related to the differences in susceptibility of each species of bacterium to the antibacterial activity of honey tested. Similar results were reported by Taormina et al. (2001). Furthermore, the different results observed between honey samples might also be due to the different floral sources used by the bees as well as the geographical factors like humidity, temperature, and where the honey was produced (Tumin et al., 2005). Previously, Ainul Hafiza et al. (2005) conducted a study of five types of local honey (*Belimbing*, *Gelam*, *Durian*, *Kelapa* and *Tualang*); using agar diffusion method for antibacterial activity against *S. aureus*. All samples had low colony counts ranging from 37 CFU/mL in *Kelapa*

honey to 161 CFU/mL in *Durian* honey. All samples showed a clear zone of inhibition, and there was a significant difference ( $p < 0.05$ ) among the samples.

Tumin et al. (2005) studied the antibacterial properties of several local Malaysian brands of honey which were *Tualang*, *Hutan*, *Gelang*, *Pucuk Daun* and *Ee Feng Gu* against six bacterial species in 20  $\mu\text{L}/100 \mu\text{L}$ , 40  $\mu\text{L}/100 \mu\text{L}$ , 60  $\mu\text{L}/100 \mu\text{L}$  and 80  $\mu\text{L}/100 \mu\text{L}$  concentrations. They found that Tualang honey exhibited antibacterial effect against *S. enterica* ser. Typhimurium, *Streptococcus pyogenes* and *E. coli*, with the highest activity seen against *S. enterica* ser. Typhimurium. In this finding, Tualang honey exhibited antibacterial properties against most of the bacteria with *S. aureus*, *E. coli*, and *S. enterica* ser. Typhimurium showed higher effects.

The antibacterial activity of chestnut honey at 100% concentration against *S. aureus* and *E. coli* were higher when compared to the flower honey at the same concentration. At 50% concentration, only chestnut honey had average inhibition against *S. aureus*. At 25% concentration, both of the honey had a slight inhibition against *S. aureus*. The diversity of flowers affect the composition of the honey produced. According to the literature, *S. aureus* is considered to be the most sensitive bacterium towards chestnut honey (Güneş et al., 2017). In the present work, all types of honey inhibited *S. aureus* and *E. coli* especially Tualang honey as it is well known for its antibacterial activity against *S. aureus*. Estevinho et al. (2008) studied the antibacterial activity of 20 honey samples from the nectar of *Lavandula*, *Echium* and *Erica* plants, and concluded that *S. aureus* was the most affected bacterium, followed by *B. subtilis*, *S. lentus*, *K. pneumoniae* and *E. coli* which were affected gradually.

Silici et al. (2010) examined the antibacterial activity of Rhododendron honey at concentrations of 10, 25, 50 and 75% against 13 different microorganisms. *P. aeruginosa* and *P. mirabilis* were the most sensitive microorganisms followed by *S. aureus*, *Aeromonas hydrophila*, *L. monocytogenes*, *B. subtilis*, *Mycobacterium smegmatis*, and *S. enterica* ser. Typhimurium while *B. cereus*, *E. coli* O157: H7 and *Y. enterocolitica* were resistant, and they did not show any DIZ. In this study, *S. aureus*, *E. coli*, *S. enterica* ser. Typhimurium and *P. aeruginosa* were inhibited by all types of honey, but Tualang and Yemeni Sumur honey showed higher effects. In another study, Nzeako and Hamdi (2000) investigated the antibacterial activity of six different honey samples in which Turkish honey showed higher antibacterial activity against *S. aureus*.

### 3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of honey samples

The disc and well diffusion methods are primarily a qualitative test for detecting the susceptibility of bacteria to antibacterial substances. However, both the MIC (the lowest concentration of the sample that will prevent at least 99% of the bacterial growth) and the MBC (the lowest concentration of honey required to kill at least 99% of the bacteria) reflects the quantity needed for bacterial inhibition (Balouiri *et al.*, 2016). The MIC and MBC of the four types of honey are shown in Tables 6 and 7. TH<sub>1</sub> and YSH had the most bacteriostatic effect as compared to other honey. They showed almost the same inhibitory concentration except for *P. aeruginosa* ATCC 27853, which was higher in TH<sub>1</sub> 12.5% MIC instead of 25% in YSH. In TH<sub>2</sub>, only *S. aureus* ATCC 29737, *S. enterica* ser. Typhimurium ATCC 13311, *S. aureus* (food isolate) and *L. monocytogenes* ATCC 1112 had 25% MIC. The rest of the bacteria did not show any inhibitory in the range which was developed. Also in AH only *S. aureus* ATCC 29737, *S. enterica* ser. Typhimurium ATCC 13311, *S. aureus* (food isolate) and *P. aeruginosa* ATCC 27853 showed a MIC of 25%, the rest of other bacteria exceeded the range.

A previous study which used Malaysian Tualang honey showed antibacterial activity against foodborne pathogen and food spoilage bacteria. The results were as follows; *S. aureus*, *E. coli*, *P. aeruginosa* and *S. enterica* ser. Typhimurium with a MIC of 12.5, 25, 12.5 and 3.125%, respectively (Shehu *et al.*, 2015). The result was in line with this study except for *S. enterica* ser. Typhimurium, which had a lower MIC. It was also supported by Tan *et al.* (2009) who reported that Tualang honey exhibited lower MIC against different microorganisms.

Tualang honey closely resembled New Zealand Manuka honey (Comvita UMF 18+) as found by a study in which Malaysian honey was used as a comparison (Zainol *et al.*, 2013). In that study the MIC of *S. aureus* was 10%, *E. coli* 20% and *P. aeruginosa* 12.5% for both Tualang and Manuka honey. Except for *B. cereus*, Manuka had a lower MIC of 10% and 15% for Tualang honey. While Kelulut honey also demonstrated constant MIC and MBC results at 20% for *S. aureus*, *E. coli*, *P. aeruginosa* and *B. cereus* (Zainol *et al.*, 2013).

The antibacterial activity of Tualang honey and Manuka honey was compared against the enteric microorganisms with *S. enterica* ser. Typhimurium having the highest MIC of 15%, and *P. aeruginosa*, *S.*

Table 6. Minimum Inhibitory Concentration (MIC) of Tualang Honey (TH<sub>1</sub>), Tualang Honey (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH) against foodborne and pathogenic bacteria

Bacteria	Types of honey (v/v)			
	TH <sub>1</sub>	TH <sub>2</sub>	AH	YSH
<i>S. aureus</i>	12.50%	25%	25%	12.50%
<i>S. aureus</i> ATCC 29737	12.50%	25%	25%	12.50%
<i>E. coli</i>	25%	>50%	>50%	25%
<i>E. coli</i> ATCC 10536	25%	>50%	>50%	25%
<i>S. enterica</i> ser. Typhimurium ATCC 13311	12.50%	25%	25%	12.50%
<i>P. aeruginosa</i> ATCC 27853	12.50%	>50%	25%	25%
<i>L. monocytogenes</i> ATCC19112	25%	25%	>50%	25%
<i>C. jejuni</i> ATCC 29428	>50%	>50%	>50%	>50%
<i>B. cereus</i>	>50%	>50%	>50%	>50%

Table 7. Minimum Bactericidal Concentration (MBC) of Tualang Honey (TH<sub>1</sub>), Tualang Honey (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH) against foodborne and pathogenic bacteria

Bacteria	Types of honey (v/v)			
	TH <sub>1</sub>	TH <sub>2</sub>	AH	YSH
<i>S. aureus</i>	25%	50%	50%	25%
<i>S. aureus</i> ATCC 29737	25%	50%	50%	25%
<i>E. coli</i>	50%	>50%	>50%	50%
<i>E. coli</i> ATCC 10536	50%	>50%	>50%	50%
<i>S. enterica</i> ser. Typhimurium ATCC 13311	25%	50%	50%	25%
<i>P. aeruginosa</i> ATCC 27853	25%	>50%	50%	50%
<i>L. monocytogenes</i> ATCC19112	50%	50%	>50%	50%
<i>C. jejuni</i> ATCC 29428	>50%	>50%	>50%	>50%
<i>B. cereus</i>	>50%	>50%	>50%	>50%

*aureus* and *E. coli* of 17.5%, 20% and 22.5%, respectively (Tan et al., 2009). The antibacterial activity of different botanical and floral honey was studied against *S. aureus* ATCC 43300, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 with Manuka honey having MIC of 7%, 6% and 14%, Acacia 10%, 12% and 13%, lavender 25%, 25%, and 21%, and for wild carrot honey 11%, 13% and 12%, respectively (Alzahrani et al., 2012).

TH<sub>1</sub> and YSH had the most bactericidal effect compared to other honey. These two honey showed almost the same inhibitory concentration except for *P. aeruginosa* ATCC 27853, which was higher in TH<sub>1</sub> 25% MBC instead of 50% in YSH. In TH<sub>2</sub>, only *S. aureus* ATCC 29737, *S. enterica* ser. Typhimurium ATCC 13311, *S. aureus* and *L. monocytogenes* ATCC 19112 showed MBC of 50%. In contrast, the rest of the bacteria did not show any bactericidal effect in the range which was developed. Also in AH only *S. aureus* ATCC 29737, *S. enterica* ser. Typhimurium ATCC 13311, *S. aureus* and *P. aeruginosa* ATCC 27853 showed an MBC of 50%, while the rest of the bacteria exceeded the range. Malaysian Tualang honey showed antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa* and *S. enterica* ser. Typhimurium with MBC of 25, 50, 25 and 12.5, respectively (Shehu et al., 2015). The results were in line with this study except for *S. enterica* ser. Typhimurium, which had lower MBC. The reaction of each bacterium towards honey is different depending on the bacterial growth and also the active compounds which are present in the honey.

In another study, Tualang honey had the same effect as Manuka honey (Comvita UMF 18+) against *S. aureus*, *P. aeruginosa* and *E. coli* with 15, 20 and 25% MBC. In *B. cereus*, Manuka honey showed lower MBC of 12.5%. In Acacia honey, *S. aureus* and *B. cereus* had the same MBC which was 25%, and *E. coli* and *P. aeruginosa* also had the same MBC of 50% (Zainol et al., 2013). Earlier in 2009, the effect of Tualang and Manuka honey was also studied against enteric microorganisms such as *S. aureus* and *E. coli* (>25% MBC), *P. aeruginosa* (25% MBC) and *S. enterica* ser. Typhimurium (20% MBC) (Tan et al., 2009).

### 3.4 Time-Kill

Time-Kill Kinetics test, also known as the "suspension tests or suspension time-kill analysis", determines the time required by the antibacterial agent to kill the challenged test microorganism. This test is utilised in microbiological studies to assess a test article's *in vitro* antibacterial activity in relation to time. In TH<sub>1</sub>, *S. aureus* (food isolate) (Figure 1) and *S. aureus* ATCC 29737 (Figure 2) showed 4.01 and 4.83 log CFU/g at 4 h

in 1× MIC. After 6 h, *S. aureus* (food isolate) showed complete inhibition and 3.84 log CFU/g for *S. aureus* ATCC 29737 at 2× MIC. After 6 h, *E. coli* (food isolate), *E. coli* ATCC 10536, *L. monocytogenes* ATCC 19112 showed 5.28, 5.34 and 5.03 log CFU/g at 1× MIC while for *S. enterica* ser. Typhimurium ATCC 1331, *P. aeruginosa* ATCC 27853 it was 4.46 and 4.30 log CFU/g at 2× MIC, respectively. For TH<sub>2</sub>, *S. aureus* (food isolate), *S. aureus* ATCC 29737, *S. enterica* ser. Typhimurium ATCC 13311, *P. aeruginosa* ATCC 27853 and *L. monocytogenes* ATCC 19112 were 5.25, 5.28, 4.38, 4.18 and 6.09 log CFU/g in 1× MIC concentration after 6 hrs, respectively. For AH, *S. aureus* (food isolate), *S. aureus* ATCC 29737 and *S. enterica* ser. Typhimurium ATCC 13311 showed 5.10, 5.18 and 5.53 log CFU/g in 1× MIC after 6 hrs. In YSH, *S. aureus* (food isolate), *S. aureus* ATCC 29737 and *S. enterica* ser. Typhimurium ATCC 13311 in 2× MIC showed 4.26, 3.68 and 3.83 log CFU/g after 6 h while *E. coli* (food isolate), *E. coli* ATCC 10536, *P. aeruginosa* ATCC 27853 and *L. monocytogenes* ATCC 19112 in 1× MIC showed 5.22, 4.80, 4.18 and 5.75 log CFU/g after 6 hrs.

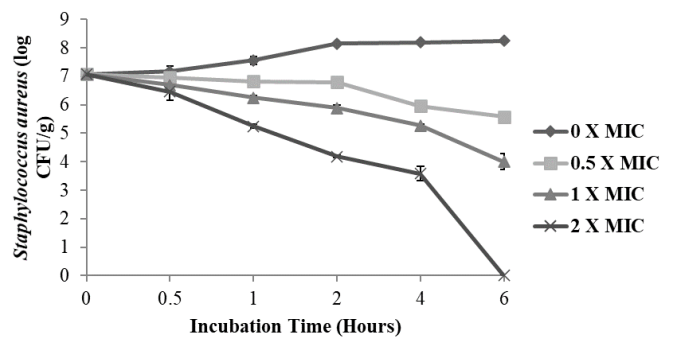


Figure 1. Time-kill plot of Tualang Honey (TH<sub>1</sub>) against *S. aureus*

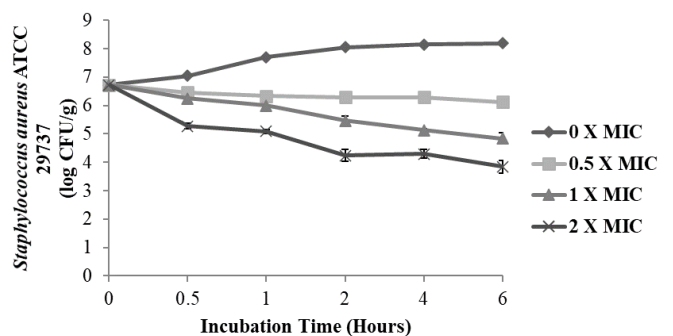


Figure 2. Time-kill plot of Tualang Honey (TH<sub>1</sub>) against *S. aureus* ATCC 29737

In one study, the growth of methicillin-resistant *S. aureus* (MRSA49) was entirely inhibited by 50% honey concentration after 8 hrs, whereas the growth of *S. aureus* was inhibited after 20 hrs. The examination of the killing kinetic of honey collected from the *longan* (*Dimocarpus longan*) flower at a concentration of 50% (v/v) showed a reduction in the survival of MRSA49 and



*S. aureus*, although, *S. aureus* showed more resistance to honey. Therefore, the potent activity of Thai honey against antibiotic-resistant pathogenic bacteria, MRSA, was the significant finding of that study (Jantakee and Tragoolpua, 2015). The antibacterial activity of honey is a property that is of significant interest in its function as an agent in treating bacterial infections that are resistant to the action of antibiotics.

By using the time-kill test, treatment with all stingless bee honey showed a significant reduction in viability of *S. aureus* ( $p < 0.001$ ) and *P. aeruginosa* ( $p < 0.001$ ), with a reduction in viability after 60 mins in the order of 1 to 3 log and  $>3$  log for each bacteria, respectively (Boorn et al., 2010). Moreover, results showed that stingless bee honey had a relatively rapid bactericidal action while the *Apis mellifera* honey did not. A previous study reported a similar trend where a little effect was seen after treatment with 25% (w/v) *A. mellifera* honey, whereas notable cell death was seen after treatment with 25% *Trigona biroi* honey. Although the majority of results showed clear overall trends, there were a few instances where outcomes were untypical.

The majority of constituents in a ripen honey are sugars accounting for 80% of the components which include glucose and fructose, sucrose and maltose are in a lesser amount, and less than 18% of water. The large percentages of sugars and low moisture content results in osmotic stress, hence, prevent the spoilage of honey by microorganisms. It has been observed that a slight increase in moisture content can result in yeast growth. However, the high percentage of sugar still prevents bacterial growth even after the honey is diluted to approximately 30 to 40% (Kwakman and Zaat, 2012). It is noteworthy that the antibacterial activity of honey after dilutions is due to other compounds found in honey, among which is the hydrogen peroxide ( $H_2O_2$ ). The production of  $H_2O_2$  is catalysed by a glucose oxidase enzyme, which is incorporated into collected nectar by the honey bees during honey production. Upon adequate dilution of honey, this enzyme is activated and in turn, catalyses the conversion of glucose to  $H_2O_2$  and gluconic acid in the presence of  $H_2O$  (Kwakman and Zaat 2012).

Although  $H_2O_2$  is the major antibacterial compound in honey, there are many honey varieties that have significant antibacterial activity resulting from non-peroxide compounds. Methylglyoxal and bee defensin-1 has been recently identified in Manuka honey and RS respectively as non-peroxide antibacterial compounds (Kwakman and Zaat 2012). The role of low pH (3.2 to 4.5) for the antibacterial activity of honey has been debatable in the world of science with a number of studies reporting opposing results with regards to the

antibacterial contribution of low pH (Adams et al., 2008). Furthermore, studies have suggested the presence of additional unknown antibacterial compounds in honey.

### 3.5 Physicochemical analysis

#### 3.5.1 Determination of pH

Overall, the pH values of the honey samples in this study were acidic ranging between 3.69 to 3.94 and within the recommended limit pH (3.4 to 6.1) (Moniruzzaman et al., 2013) as shown in (Figure 3). AH was the most acidic honey (pH 3.69), and the least acidic honey was YSH with pH 3.94. TH<sub>2</sub> and TH<sub>1</sub> had pH of 3.72 and 3.91, respectively. There was a significant difference ( $p < 0.05$ ) between the samples. Although honey is considered to be acidic, however, the high sugar content masks the acidity in the honey taste. The average pH of honey is pH 3.9. Formic acid and citric acid were originally believed to be the dominant acidic compounds in honey (Ball, 2007). However, it was recently discovered that gluconic acid is the predominant acid compound in honey, which is produced from bee secretions under the action of oxidase enzyme on glucose.

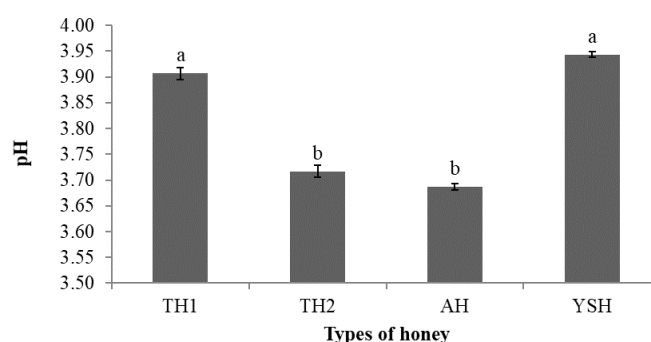


Figure 3. pH values of Tualang Honey (TH<sub>1</sub>), Tualang Honey (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH). Bars with different letters are significantly different ( $p < 0.05$ ).

A variation in the pH values of Brazilian, Spanish, Turkish and Algerian honey was found to be in the range of 3.10 to 4.05, 3.63 to 5.01, 3.67 to 4.57 and 3.49 to 4.53 respectively (Amri and Ladjama, 2013). A pH value between 3.5 to 5.0 for honey from Northwest Spain was also reported (Olga et al., 2012). Similarly, pH values between 3.8 to 4.5 for Moroccan honey were also reported (Karabagias et al., 2014). The acidity is likely to contribute to the antibacterial potency of the honey (Almasaudi et al., 2016). Properties and the composition of honey depend on its geographical floral origin, season, environmental factors as well as treatment of beekeepers (El Sohaimy et al., 2015).

#### 3.5.2 Determination of water activity

The water activity ( $a_w$ ) of honey samples were in

between 0.53 to 0.62 (Figure 4). The  $a_w$  of honey should be below 0.60 (Chirife *et al.*, 2006). TH<sub>2</sub> exceeded the level with 0.62  $a_w$ ; the rest of the honey was within the limit. TH<sub>1</sub> and YSH had the same  $a_w$  of 0.53, and for AH it was 0.60. There was a significant difference ( $p < 0.05$ ) in  $a_w$  between the samples. These results are quite similar to those of Greek honey for which the  $a_w$  ranged from 0.53 to 0.67 (Lazaridou *et al.*, 2004). The  $a_w$  is an important factor which governs the food stability by preventing or limiting microbial growth. The increase in  $a_w$  influences the shelf life of honey and supports the growth of undesirable microflora, especially osmotolerant yeasts. The osmotolerant yeasts are able to grow at a minimal  $a_w$  of 0.60 (Saxena *et al.*, 2010). Although osmolality plays a significant role in the antibacterial activity of honey, there are other factors that influence the antibacterial effect of honey. The presence of such factors in honey depends to a greater extent on the source of nectar, location of the flowers, the storage time, preservation method and related water conditions (Almasaudi *et al.*, 2016). Some of the quality problems found in honey such as stability, viscosity and crystallisation are due to  $a_w$  (Zamora *et al.*, 2006). During glucose crystallisation, glucose monohydrate is formed, and other water molecules are dehydrated, resulting in a decrease in solute concentration in the liquid phase and increase in  $a_w$ .

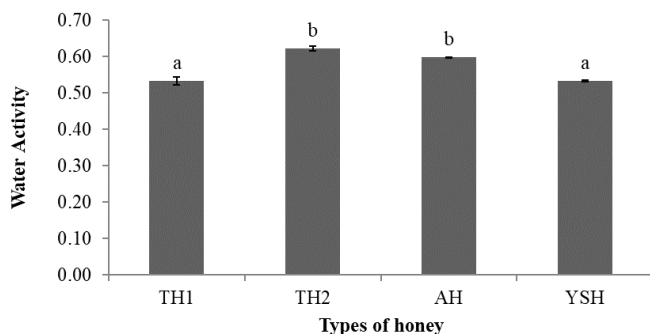


Figure 4. Water activities of Tualang Honey (TH<sub>1</sub>), Tualang Honey (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH). Bars with different letters are significantly different ( $p < 0.05$ ).

### 3.5.3 Colour analysis

Colour is the first attractive attribute of honey, and as such, it is very important for commercialisation. It is a significant parameter in the quality, acceptance and preference of consumers (Missio *et al.*, 2016). As shown in Table 8, the  $L^*$  parameter ranged from 2.27 to 13.63. The  $a^*$  parameter ranged between 1.44 to 1.62. The  $b^*$  parameter ranged from 12.09 to 2.27. There was a significant ( $p < 0.05$ ) difference between the samples. The maximum and minimum lightness was seen in YSH and AH, respectively. The maximum and minimum redness was seen in TH<sub>1</sub> and AH, respectively. The

maximum and minimum brightness was seen in YSH and AH, respectively. Honey's colour normally differs over a wide range of tones, ranging from light yellow to amber, dark amber and, in rare cases, black. Occasionally, even green or red hues may occur.

The colour of unprocessed honey depends on its botanical or floral origins. For this reason, colour is significant in the classification of monofloral honey for commercial activities (Moniruzzaman *et al.*, 2013). It also depends on its ash content, temperature and time of storage (Gámbaro *et al.*, 2006). The Codex Alimentarius Committee on Sugars (2001) stipulates that the colour of honey should be nearly colourless to dark brown. In one study, seven reputed commercial Indian honey brands showed  $L^*$ ,  $a^*$  and  $b^*$  values ranging from 26.3 to 36.8, 0.12 to 4.9 and 0.7 to 14.4, respectively (Saxena *et al.*, 2010). Boussaid *et al.* (2014) evaluated six samples of honey which were collected from different regions of Tunisia and found that  $L^*$  values ranged from 36.64 to 51.37. The lightness of honey plays an important role as it is what the consumers prefer. The analysed honey also contains other colours orange, green and yellow. Rosemary honey showed green colour, which is the negative value of  $a^*$  while mint honey had the highest redness followed by eucalyptus honey. The orange, thyme eucalyptus horehound and mint honey showed values between 10 and 20 for  $b^*$  (Boussaid *et al.*, 2014).

Table 8. Color analysis of Tualang Honey (TH<sub>1</sub>), Tualang Honey (TH<sub>2</sub>), Acacia Honey (AH) and Yemeni Sumur Honey (YSH)

Honey	$L^*$	$a^*$	$b^*$
TH <sub>1</sub>	3.93±0.62 <sup>b</sup>	1.62±0.17 <sup>a</sup>	2.61± 0.10 <sup>b</sup>
TH <sub>2</sub>	2.97±0.21 <sup>b</sup>	1.58±0.12 <sup>a</sup>	3.16±0.07 <sup>b</sup>
AH	2.27±0.04 <sup>b</sup>	1.44±0.09 <sup>a</sup>	2.27±0.05 <sup>b</sup>
YSH	13.63±0.54 <sup>a</sup>	1.52±0.10 <sup>a</sup>	12.09±0.62 <sup>a</sup>

Diameter of inhibition zone in mm (including disc). Values are expressed as mean±standard deviation. Values with different superscript within the row are significantly different ( $p < 0.05$ ).

## 4. Conclusion

In disc diffusion, TH<sub>1</sub> and YSH showed higher DIZ of 10.08 mm and 12.49 mm at 25%, and 15.01 mm and 20.35 mm at 50% against *S. aureus* (food isolate), respectively. While in well diffusion, TH<sub>1</sub> and AH showed the highest DIZ of 31.95 mm and 33.87 mm by *S. enterica* ser. Typhimurium ATCC 13311 at 50%. Meanwhile, in TH<sub>2</sub> and YSH, *S. aureus* ATCC 29737 showed the highest DIZ of 27.16 mm and 35.75 mm at 50%. In TH<sub>1</sub>, positive control of *S. aureus* (food isolate) and *P. aeruginosa* ATCC 27853 showed bigger DIZ than 50% honey concentration while TH<sub>2</sub> on *S. aureus* (food isolate) positive control had bigger DIZ. In both AH and

YSH, 50% of honey had bigger DIZ than the positive control. The lowest MIC was obtained by both TH<sub>1</sub> and YSH against *S. aureus* (food isolate), *S. aureus* ATCC 29737, *P. aeruginosa* ATCC 27853 and *S. enterica* ser. Typhimurium ATCC 13311 with 12.5% MIC concentration, and the lowest MBC was also by TH<sub>1</sub> and YSH against *S. aureus* (food isolate), *S. aureus* ATCC 29737 and *S. enterica* ser. Typhimurium ATCC 13311 with 25% MBC concentration. In the time-kill test, TH<sub>1</sub> on *S. aureus* (food isolate) showed a complete reduction at 6 hrs and in TH<sub>2</sub> on *S. enterica* ser. Typhimurium ATCC 13311 showed the highest reduction of 2.21 log CFU/g at 1× MIC. For AH, *P. aeruginosa* ATCC 27853 showed the highest reduction of 2.48 log CFU/g at 1× MIC, and for YSH, *S. aureus* (food isolate) and *S. enterica* ser. Typhimurium ATCC 13311 showed 2.74 and 2.73 log CFU/g at 2× MIC, respectively. The highly acidic honey was AH with pH of 3.69, and the lowest water activity was measured in TH<sub>1</sub> and YSH at 0.53. Maximum L\* and b\* was by YSH, and maximum a\* was by TH<sub>1</sub>.

### Conflict interests

The authors declare that they have no competing interests.

### Acknowledgement

We gratefully acknowledge the financial support from Universiti Putra Malaysia under IPS (*Insentif Putra Siswazah*) Research Grant (GP-IPS/2016/9488200).

### References

- Adams, C.J., Boulton, C.H., Deadman, B.J., Farr, J.M., Grainger, M.N., Manley-Harris, M. and Snow, M.J. (2008). Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research*, 343(4), 651-659. <https://doi.org/10.1016/j.carres.2007.12.011>
- Agbaje, E.O., Ogunsanya, T. and Aiwerioba, O.I.R. (2006). Conventional use of honey as antibacterial agent. *Annals of African Medicine*, 5(2), 78-81.
- Ainul Hafiza, A.H., Yusof, N. and Maimon, A. (2005). Potential of Malaysian local honey as an antibacterial agent. *Sains Malaysiana*, 34(1), 17-20.
- Almasaudi, S.B., Al-nahari, A.A.M., El-ghany, E.S.M.A., Muhayawi, S.M.A., Al-jaouni, S., Azhar, E. and Harakeh, S. (2017). Antimicrobial effect of different types of honey on *Staphylococcus aureus*. *Saudi Journal of Biological Sciences*, 24(6), 1255-1261. <https://doi.org/10.1016/j.sjbs.2016.08.007>
- Alzahrani, H.A., Alsabehi, R., Boukraâ, L., Abdellah, F., Bellik, Y. and Bakhotmah, B.A. (2012). Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. *Molecules*, 17(9), 10540-10549. <https://doi.org/10.3390/molecules170910540>
- Amri, A. and Ladjama, A. (2013). Physicochemical characterisation of some multifloral honeys from honeybees *Apis mellifera* collected in the Algerian northeast. *African Journal of Food Science*, 7(7), 168-173. <https://doi.org/10.5897/AJFS13.0986>
- Aween, M.M., Hassan, Z., Huda-Faujani, N., Emdakim, M.M. and Muhiyaldin, B.J. (2014). Potency of honey as antibacterial agent against multiple antibiotic resistant pathogens evaluated by different methods. *American Journal of Applied Sciences*, 11(10), 1773-1783. <https://doi.org/10.3844/ofsp.9491>
- Ball, D.W. (2007). The chemical composition of honey. *Journal of Chemical Education*, 84(10), 1643. <https://doi.org/10.1021/ed084p1643>
- Balouiri, M., Sadiki, M. and Ibensouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71-79. <https://doi.org/10.1016/j.jppha.2015.11.005>
- Boorn, K.L., Khor, Y.Y., Sweetman, E., Tan, F., Heard, T.A. and Hammer, K.A. (2010). Antimicrobial activity of honey from the stingless bee *Trigona carbonaria* determined by agar diffusion, agar dilution, broth microdilution and time-kill methodology. *Journal of Applied Microbiology*, 108(5), 1534-1543. <https://doi.org/10.1111/j.1365-2672.2009.04552.x>
- Boukraâ, L. (Ed.). (2013). Honey in traditional and modern medicine. USA: CRC Press. <https://doi.org/10.1201/b15608>
- Boussaid, A., Chouaibi, M., Rezig, L., Hellal, R., Donsi, F., Ferrari, G. and Hamdi, S. (2018). Physicochemical and bioactive properties of six honey samples from various floral origins from Tunisia. *Arabian Journal of Chemistry*, 11(2), 265-274. <https://doi.org/10.1016/j.arabjc.2014.08.011>
- Chirife, J., Zamora, M.C. and Motto, A. (2006). The correlation between water activity and % moisture in honey: Fundamental aspects and application to Argentine honeys. *Journal of Food Engineering*, 72(3), 287-292. <https://doi.org/10.1016/j.jfoodeng.2004.12.009>
- Codex Alimentarius Committee. (2001). Codex Alimentarius Committee on Sugars 2001. Rome: FAO. Retrieved from [www.fao.org/input/download/standards/338/CXS\\_212e\\_u.pdf](http://www.fao.org/input/download/standards/338/CXS_212e_u.pdf)
- El Sohaimy, S.A., Masry, S.H.D. and Shehata, M.G.

- (2015). Physicochemical characteristics of honey from different origins. *Annals of Agricultural Sciences*, 60(2), 279-287. <https://doi.org/10.1016/j.aas.2015.10.015>
- Estevinho, L., Pereira, A.P., Moreira, L., Dias, L.G. and Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology*, 46(12), 3774-3779. <https://doi.org/10.1016/j.fct.2008.09.062>
- Güneş, M.E., Şahin, S., Demir, C., Borum, E. and Tosunoğlu, A. (2017). Determination of phenolic compounds profile in chestnut and floral honeys and their antioxidant and antimicrobial activities. *Journal of Food Biochemistry*, 41(3), 12345. <https://doi.org/10.1111/jfbc.12345>
- Jantakee, K. and Tragoolpua, Y. (2015). Activities of different types of Thai honey on pathogenic bacteria causing skin diseases, tyrosinase enzyme and generating free radicals. *Biological Research*, 48(1), 4. <https://doi.org/10.1186/0717-6287-48-4>
- Karabagias, I.K., Badeka, A., Kontakos, S., Karabournioti, S. and Kontominas, M.G. (2014). Characterisation and classification of Greek pine honeys according to their geographical origin based on volatiles, physicochemical parameters and chemometrics. *Food Chemistry*, 146, 548-557. <https://doi.org/10.1016/j.foodchem.2013.09.105>
- Kwakman, P.H. and Zaat, S.A. (2012). Antibacterial components of honey. *IUBMB Life*, 64(1), 48-55. <https://doi.org/10.1002/iub.578>
- Lazaridou, A., Biliaderis, C.G., Bacandritsos, N. and Sabatini, A. G. (2004). Composition, thermal and rheological behaviour of selected Greek honeys. *Journal of Food Engineering*, 64(1), 9-21. <https://doi.org/10.1016/j.jfoodeng.2003.09.007>
- Miorin, P.L., Levy Junior, N.C., Custodio, A.R., Bretz, W.A. and Marcucci, M.C. (2003). Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. *Journal of Applied Microbiology*, 95(5), 913-920. <https://doi.org/10.1046/j.1365-2672.2003.02050.x>
- Missio, P., Gauche, C., Gonzaga, L.V., Carolina, A. and Costa, O. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, 196, 309-323. <https://doi.org/10.1016/j.foodchem.2015.09.051>
- Moniruzzaman, M., Khalil, M.I., Sulaiman, S.A. and Gan, S.H. (2013) Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. *BMC Complementary and Alternative Medicine*, 13(1), 43. <https://doi.org/10.1186/1472-6882-13-43>
- Nzeako, B.C. and Hamdi, J. (2000). Antimicrobial potential of honey on some microbial isolates. *Sultan Qaboos University Medical Journal*, 2(2), 75-79.
- Olga, E., María, F.G. and Carmen, S.M. (2012). Differentiation of blossom honey and honeydew honey from Northwest Spain. *Agricultural*, 2(1), 25-37. <https://doi.org/10.3390/agriculture2010025>
- Rao, P.V., Krishnan, K.T., Salleh, N. and Gan, S.H. (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. *The Brazilian Journal of Pharmaceutical Sciences*, 26(5), 657-664. <https://doi.org/10.1016/j.bjp.2016.01.012>
- Rukayadi, Y., Han, S., Yong, D. and Hwang, J.K. (2010). In vitro antibacterial activity of panduratin A against enterococci clinical isolates. *Biology and Pharmaceutical Bulletin*, 33(9), 1489-1493. <https://doi.org/10.1248/bpb.33.1489>
- Saxena, S., Gautam, S. and Sharma, A. (2010). Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chemistry*, 118(2), 391-397. <https://doi.org/10.1016/j.foodchem.2009.05.001>
- Shafiee, S., Minaei, S., Moghaddam-Charkari, N. and Barzegar, M. (2014). Honey characterisation using computer vision system and artificial neural networks. *Food Chemistry*, 159, 143-150. <https://doi.org/10.1016/j.foodchem.2014.02.136>
- Shehu, A., Mohd, A.K.R., Aniza, A. and Salwani, I. (2015). Antibacterial activity and antioxidant capacity of Malaysian tualang honey. *International Journal of Science and Research*, 4(4), 1758-1762.
- Silici, S., Sagdic, O. and Ekici, L. (2010). Total phenolic content, antiradical, antioxidant and antimicrobial activities of *Rhododendron* honeys. *Food Chemistry*, 121(1), 238-243. <https://doi.org/10.1016/j.foodchem.2009.11.078>
- Tan, H.T., Rahman, R.A., Gan, S.H., Halim, A.S., Asma'Hassan, S., Sulaiman, S.A. and Kirnpal-Kaur, B.S. (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complementary and Alternative Medicine*, 9(1), 34. <https://doi.org/10.1186/1472-6882-9-34>
- Taormina, P.J., Niemira, B.A. and Beuchat, L.R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International Journal of Food Microbiology*, 69(3), 217-225. [https://doi.org/10.1016/S0168-1605\(01\)00505-0](https://doi.org/10.1016/S0168-1605(01)00505-0)

- Tumin, N., Halim, N.A., Shahjahan, M., Noor Izani, N.J., Sattar, M.A., Khan, A.H. and Mohsin, S.S.J. (2005). Antibacterial activity of local Malaysian honey. *Malaysian Journal of Pharmaceutical Sciences*, 3(2), 1-10.
- Wilkinson, J.M. and Cavanagh, H.M. (2005). Antibacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. *Journal of Medicine Food*, 8(1), 100-103. <https://doi.org/10.1089/jmf.2005.8.100>
- Zainol, M.I., Yusoff, K.M. and Yusof, M.Y.M. (2013). Antibacterial activity of selected Malaysian honey. *BMC Complementary and Alternative Medicine*, 13 (1), 129. <https://doi.org/10.1186/1472-6882-13-129>
- Zamora, M.C., Chirife, J. and Roldán, D. (2006). On the nature of the relationship between water activity and % moisture in honey. *Food Control*, 17(8), 642-647. <https://doi.org/10.1016/j.foodcont.2005.04.002>