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Anti-Inflammatory and Anti-Microbial Activities of Selected Honey Samples

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ABSTRACT

The medical property of honey in wound healing was reported due to its anti-inflammatory and anti-microbial activities. It is believed that polar compounds including sugars and polyphenols in honey could contribute to the biological activities. In this study, crude honey samples of Tualang, Gelam and Acacia were fractionated into polar fractions using a C18 reversed-phase extraction column. The polar fractions were found to be more effective than crude honey samples because of lower effective concentration (0.04-0.37 g mL^{-1}) at 50% of inhibition. However, the polar fractions did not improve the selectivity of inhibition against cyclooxygenase-2. Polar fractionation also did not improve the anti-microbial activities of crude honey samples. The improvement was only showed by Tualang honey fraction (~70%) with the minimum inhibitory concentration (3.1% (w/v)) against *Candida albican*. The potential bioactive compounds were putatively identified as hydroxymethylfurfural, ethyl glucuronide, myricetin and apigenin glycoside based on the results of LC-MS/MS.

Key words: Tualang honey, gelam honey, acacia honey, cyclooxygenases, pathogenic microbes, solid phase extraction

INTRODUCTION

Honey is a nutritious, high sugar content and ready to eat food. Honey has been used for medical purposes, particularly for wound treatment since ancient time as firstly documented by the Egyptians in 2000 BC (Dunford *et al*., 2000). This traditional medication was then displaced by the discovery of antibiotics (Khoo *et al*., 2010). Recently, the medical usage of honey for wound, burn and skin ulcer treatment has been rediscovered, partly due to the development of antibiotic-resistant microbes (Khoo *et al*., 2010).

The wound healing property of honey has been extensively investigated for various honey samples by researchers. The findings found that this property was mainly attributed to the anti-microbial activity of honey. Recent studies demonstrated the anti-bacterial effect of honey on approximately 60 species of bacteria, including aerobes and anaerobes, gram-positive and gram-negative bacteria (Molan, 2002). Honey has also been shown to have anti-fungal activity against some yeasts and molds (Molan, 2002; Wilkinson and Cavanagh, 2005).

The anti-microbial property was likely due to the high viscosity and high osmolarity activities resulted from high sugar content in honey (Osato *et al*., 1999; Khoo *et al*., 2010). The other

explanation for the anti-microbial property includes honey acidity due to the presence of gluconic acid, low protein content, low redox potential because of high reducing sugars, high carbon-to-nitrogen ratio, hydrogen peroxide as a byproduct of glucose oxidase and other non-peroxide compounds such as lysozymes, phenolic acids and flavonoids from floral sources (Snowdon and Cliver, 1996; Osato *et al*., 1999; Weston, 2000). Based on previous studies on anti-bacterial activity of various honey samples, Brudzynski and Kim (2011) concluded that the phytochemical composition in honey was responsible for the bacteriostatic and bactericidal action in a certain extent. The variance in the anti-bacterial activity could be attributed to their phenolic profiles (Aljadi and Yusoff, 2003). The major antibacterial compounds such as methyl-4-hydroxy-3,5-dimethoxy benzoate and methyl-3,4,5-trimethoxy benzoate were detected in honey from New Zealand (Russel, 1983). However, Weston (2000) reported that the contribution of flavonoids for anti-bacterial activity of honey was smaller than the contribution from hydrogen peroxide, catalase and glucose oxidase. It is believed that the anti-microbial activity of honey was attributed to the synergistic action of these factors.

There are also evidences to show that prolong infection by microorganisms would stimulate inflammation. The infection of bacteria such as *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Acinobacter baumannii* was found to cause inflammation on full-thickness burn wounds in rats (Khoo *et al*., 2010). Inflammation is simulated by the activity of cyclooxygenase, which is a biocatalyst of prostaglandin synthesis. There are two cyclooxygenases namely: Cyclooxygenase-1 (COX-1) which is expressed in the gastrointestinal tract for homeostasis and cyclooxygenase-2 (COX-2) which is induced at the site of inflammation. Therefore, many studies have extensively been focused on the discovery of compound with higher selectivity against COX-2 than COX-1 (Cho *et al*., 2004). This is because COX-1 inhibitor might cause unfavorable side effects such as mucosal damage, ulceration and other complication as reported for the existing non-steroidal anti-inflammatory drugs (NSAIDs). It is hypothesized that selective COX-2 inhibitor exhibits an improved safety profile. Truly selective COX-2 inhibitors (rofecoxib and celecoxib) have been shown to have no effect on gastric mucosal prostaglandin synthesis, no acute injury and no chronic ulceration compared to placebo (Hawkey, 2001).

Honey that commonly consumed by local people, namely Tualang, Gelam and Acacia honey samples were collected from reliable sources for this study. Solid phase extraction was carried out in order to collect highly polar extracts from the honey samples for anti-microbial and anti-inflammatory assays. The biological activity of crude honey samples and their polar fractions on pathogenic microbes and inflammatory enzymes (cyclooxygenase-1 and 2) were compared and related to their chemical compositions.

MATERIALS AND METHODS

Honey samples and chemicals: Honey samples of Tualang and Gelam were purchased from the major honey distributor in Malaysia, namely Federal Agricultural Marketing Authority (FAMA). Acacia honey samples were harvested directly from a bee farm located at Kota Tinggi, Johor. Manuka honey (UMF 10+) was purchased from a local pharmacy in Johor Bahru. All the honey samples were placed in a tight sealed glass bottle and stored at 20°C before analysis.

Cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and standard prostaglandin D (PGD) were bought from Sigma-Aldrich (St. Louis, USA). Arachidonic acid, L-epinephrine and hydrochloric acid were purchased from Fischer Scientific (Waltham, USA). Formic acid was obtained from J.T. Baker (New Jersey, USA). All solvents such as methanol and ethyl acetate were

of HPLC grade. Barnstead NANO pure diamond water purification system (State of Illinois, USA) with 18.2 MΩ resistivity was used to generate deionized water. Tris base was sourced from Whitehouse Station, New Jersey. Microbiological growth media such as Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Mueller-Hinton Agar (MHA) and Agar-Agar granulated were purchased from Merck (Darmstadt, Germany).

Extraction of honey samples: The fractionation of honey samples was carried out by using C18 column (500 mg, 10×12 mm) in a Solid Phase Extraction (SPE) system. A 0.5 g of honey was dissolved in 1 mL of acidified water (pH 2) and loaded onto the column for fractionation. The pH of the acidified water was adjusted by using hydrochloric acid (1 M). Before sample elution, the C18 column was pre-conditioned by rinsing with 5 mL of acidified water. Only polar honey fractions were collected by eluting 20 mL of acidified water through the sample loaded column. The eluent was then collected and vacuum dried for anti-inflammatory and anti-microbial assays.

Cyclooxygenase assay for anti-inflammatory activity: The anti-inflammatory activities of honey and its polar fraction were analyzed by cyclooxygenase assay based on the method described by Cao *et al*. (2011). Aliquots of cyclooxygenase were prepared by transferring 2 μL of COX-1 or 6.67 μL of COX-2 into 20 μL of Tris-HCl buffer (pH 8). A 146 μL of 100 mM Tris-HCl buffer (pH 8), 2 μL of 100 μM hematin and 10 μL of 40 mM (L)-epinephrine were added and mixed into a tube. An aliquot of cyclooxygenase and 2 μL honey solution (0.5 g mL^{-1}) were added into the tube containing co-factors in Tris-HCl buffer solution. The mixture was left for 10 min and then 20 μL of 5 μM arachidonic acid in 100 mM Tris-HCl buffer (pH 8.0) was added to initiate the reaction of prostaglandin formation. After 2 min, 20 μL of 2.0 M HCl was added to terminate the reaction. After 30 min, prostaglandin was extracted from the solution by adding 800 μL of hexane/ethyl acetate (50:50, v/v). The organic layer was pipetted and evaporated to dryness. The sample was restored in 1 mL of methanol for quantitative analysis by UPLC-MS/MS. A serial concentration of standard prostaglandin D ranging from 0.2-1.0 ppm was prepared for calibration curve construction. Distilled water was used to replace honey solution in control experiment. This assay was carried out in triplicate for each honey and its fraction.

Well diffusion technique of anti-microbial activity: The anti-microbial activities of honey and its fraction were carried out by using well diffusion method. The assay was determined against four microorganisms that commonly found in wound, namely *Salmonella typhimunum* (gram-negative bacteria, ATCC 14028), *Staphylococcus aureus* (gram-positive bacteria, ATCC 25923), *Candida albican* (ATCC 10231) and *Fusarium oxysporium*. The microbial strains were inoculated in the specific growth media at the different incubation time and temperatures. The MHA agar was used for bacteria at 37°C for 24 h, SDA was incubated for yeast at 28°C for 48 h, whereas PDA was prepared for fungi incubation at 28°C for 48 h. One loop of individual microbe was suspended in 5 mL of sterile distilled water and well mixed the stock suspension. A 100 μL of the stock suspension was then added into 20 mL of specific nutrient agar and poured into a plate for solidification. Holes with 1 cm in diameter were made using a sterile cork borer on the agar plate containing microbes. A 100 μL of honey sample with different concentrations by two fold serial of dilution was added into individual well for the assay of microbial inhibition. The inhibition activity was expressed as Minimum Inhibitory Concentration (MIC) of samples.

LC-MS/MS and LC-PDA: The formation of prostaglandin as a result of cyclooxygenase catalysis on arachidonic acid was analyzed by a hyphenated system of Ultra Performance Liquid Chromatography (UPLC, Waters Acquity Milford, MA) coupled with a triple quadrupole-linear ion tandem mass spectrometer (Applied Biosystem 4000 Q Trap; Life technology Corporation, Carlsbad, CA) and an Electrospray Ionization (ESI) source. A binary solvent system consisted of 0.1% formic acid in deionized water as solvent A and 0.1% formic acid in acetonitrile as solvent B. A C18 Acquity column (150×4.6 mm, 1.7 μm) was used for separation at the flow rate of 0.2 mL min^{-1}. The gradient was the 0-8 min, 10-90% B; 8-10 min, 90% B; 10-12 min, 90-10% B and 12-15 min, 10% B. A single negative ion transition of multiple reaction monitoring (m/z 351>271) approach was used to quantify prostaglandin in the samples. Nitrogen was used as ion source gas and the ion source was set at temperature 400°C and voltage -4.5 kV. Other parameters were 40 psi for curtain gas, 40 psi for drying solvent and 25 psi for collision gas. The declustering potential was -40 V and collision exit energy was -10 V with the scan rate of 1000 amu sec^{-1}.

A capillary liquid chromatography (Dionex Corporation Ultimate 3000; Sunnyvale, CA) system was integrated with a diode array detector (Dionex Ultimate 3000) to fingerprint the samples. A C18 reversed phase XSelect HSS T3 column (2.1×100 mm, 2.5 μm) with a flow rate of 150 μL min^{-1} was used for separation and detected at 290 nm. A binary gradient system consists of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile). The LC gradient was 0-10 min, 10% B, 10-25 min, 10-80% B, 25-30 min, 80% B, 30-30.1 min, 80-10% B and 30.1-35 min, 10% B. The injection volume was 5 μL. All samples were filtered with 0.2 μm nylon membrane filter prior to injection.

RESULTS AND DISCUSSION

Anti-inflammatory activity of crude honey samples and their acidified water fractions: The anti-inflammatory assay was carried out by using the optimized parameters reported by Cao *et al*. (2011). It was about half of the theoretical concentration of prostaglandin D (30.45 ppb) extracted from the assay using a mixture of organic solvents consisted of hexane and ethyl acetate in a ratio of 50:50 (v/v). The reduction of prostaglandin concentration was monitored to determine the percentage of inhibition by honey samples and their fractions. When the concentration of honey was increased, the production of prostaglandin was further inhibited as presented in Fig. 1.

The value of EC_{50} was calculated to determine the required concentration of sample for 50% of the inhibition. It means that the lower EC_{50} value, the sample has better inhibitory activity against the enzymatic production of prostaglandin. Based on the results of Table 1, the acidified water fractions of honey samples exhibited better activity in the inhibition of prostaglandin production. Most probably, the acidified water fraction of honey consisted of sugars, organic acids and phenolic acids.

Table 1: Effective concentration at 50% of inhibition by crude honey samples and their acidified water fractions for prostaglandin production

	Crude honey			Acidified water fraction		
	EC_{50} (g mL^{-1})		Selectivity	EC_{50} (g mL^{-1})		Selectivity
Honeys	COX-1	COX-2	COX-1/COX-2	COX-1	COX-2	COX-1/COX-2
Tualang	3.535	0.333	10.62	0.04	0.30	0.13
Gelam	6.130	3.050	2.00	0.14	0.37	0.38
Acacia	1.660	1.159	1.43	0.13	0.10	1.30
Manuka	1.285	0.671	1.92	0.04	0.16	0.25

COX-1: Cyclooxygenase-1, COX-2: Cyclooxygenase-2

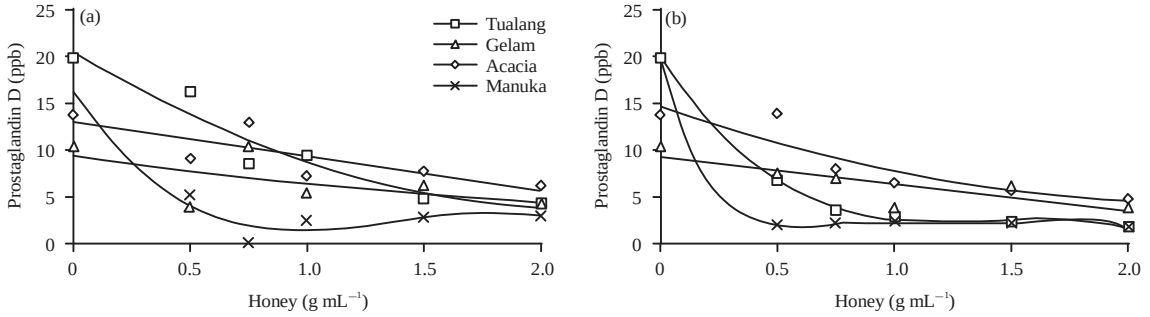

Fig. 1(a-b): Inhibition of prostaglandin D produced by (a) COX-1 and (b) COX-2 using crude honey samples

However, the selectivity of acidified water fractions for anti-inflammatory activity was lower than crude honey samples as presented in Table 1. The selectivity is defined as the ratio of EC_{50} for COX-1 and COX-2. The compounds in the acidified water fraction are not selective enough to inhibit the activity of COX-2 over COX-1. High selectivity of honey fraction is important to avoid undesirable effect caused by the inhibition of COX-1. Previous investigators reported the inhibition of COX-1 could provoke undesirable side effects such as ulcerogenic and nephrotoxic activities (Cho *et al*., 2004). This is because, COX-1 is responsible for the production of basal level of prostaglandin for gastrointestinal tract homeostasis. Therefore, researchers are looking for high selectivity nonsteroidal anti-inflammatory drugs on COX-2 to avoid any consecutive side effect. The crude honey sample of Tualang showed the highest selectivity ratio and could be a potential anti-inflammatory agent. This is because the crude honey sample of Tualang could selectively inhibit inducible COX-2 better than its isoform COX-1. Interestingly, the selective inhibition of Tualang honey was found to be better than Manuka honey as presented in Table 1. Likewise, the performance of Gelam honey was also comparable to Manuka honey.

Many studies have been carried out on the structure-activity relationship and anti-inflammatory activity using pure compounds of flavonoids such as chrysin, apigenin, wogonin, catechin and tectorigenin (Cho *et al*., 2004). Their findings indicated that flavonoids exerted potent inhibitory activity against pro-inflammatory enzymes, COX-2 and iNOS (inducible nitric oxide synthase). Therefore, the increase of the inhibitory activities of honey fractions could be due to the increase in the concentration of flavonoids such as quercetin, myricetin and apigenin glycoside in the honey fractions as tabulated in Table 2. Somehow, the flavonoids did not exhibit high selectivity against COX-2 compared to COX-1. Therefore, the acidified water fractions of honey samples improved the anti-inflammatory activities, but did not improve the selectivity against COX-2.

Anti-microbial activity of crude honey samples and their acidified water fractions: The microbes in honey are primarily yeasts and spore-forming bacteria (Snowdon and Cliver, 1996). Some bacteria can survive in honey, but bacteria are unlikely to grow in honey. Therefore, the total plate count could be ranged from zero to tens of thousands per gram of honey sample. In the present study, the total plate counts for Tualang, Gelam and Acacia honey were found to be 1300, 470 and 520 CFU g^{-1}, respectively. This standard plate count value just provides general information about microbial quality of honey. The microbial quality of honey samples were acceptable in this study. No pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* (Enterobacteriaceae family) and *Escherichia coli* were detected in the honey samples.

Table 2: Putative compounds in the acidified water fractions of honey samples

					Acidified water honey fraction				
Compounds	Rt (min)	m/z	Mode	MS/MS	Tualang	Gelam	Acacia	Manuka	Reference
Hydroxymethylfurfural	10.2	127	Positive	109/99/91/81	✓[na]	✓[a]		✓[na]	Gokmen and Senyuva (2006)
Dihydroxymethylfurfural	10.4	144	Positive	144/127/109/90		✓[b]		✓[na]	Gokmen and Senyuva (2006)
Unknown	1.0	156	Positive	132/115/112			✓[na]		
Ethyl glucuronide	2.9	221	Negative	129/85	✓[na]				Politi *et al.* (2005)
Coumaroyl putrescine	18.7	235	Positive	217/127/109	✓[na]			✓[na]	Felipe *et al.* (2014)
2-methoxy-5-(methylthiolamide) cinnamic acid	18.8	252	Positive	235/207/177/161/109	✓[na]			✓[na]	Liu *et al.* (2004)
Psilocybin	21.5	285	Positive	240/183/95	✓[na]	✓[na]		✓[na]	Van Orden (2008)
Hexosyl hydroxymethylfurfural	13.8	289	Positive	271/253/235/207/127/109		✓[na]		✓[na]	Gokmen and Senyuva (2006)
Quercetin	11.7	301	Negative	283/255/151		✓[na]			Jia *et al.* (2015)
Unknown	2.0	305	Negative	175/147	✓[na]				
Myricetin	6.3	317	Negative	273/151	✓[na]				Gates and Lopes (2012)
3-hydroxyphenyl-2,2'-methylenebis-(cyclohexane-1,3-dione)	10.3	328	Positive	310/292/264/178/166	✓[na]		✓[na]		Ali *et al.* (2009)
Dihydroxylphenyllactoyltartaric acid	10.6	329	Negative	311/283	✓[na]	✓[na]			Chen *et al.* (2012)
Caffeoylmalonyltartaric acid	16.7	415	Negative	371/355/311		✓[na]			Chen *et al.* (2012)
Apigenin glycoside	6.0	431	Negative	413/333/269	✓[na]				Krasteva and Nikolov (2008)

na: Not available, a: Retention time at 12.1 min, b: Retention time at 12.2 min

The total yeast and mold counts were found to be 620 CFU g^{-1} for Tualang honey, but not detected in Gelam and Acacia honey sample. Snowdon and Cliver (1996) reported that most honey samples contained detectable level of yeast and yeast could grow well in acidic condition of honey to a high number colony resulted from secondary contamination of poor handling technique. Furthermore, yeast also could not be inhibited by high sugar content of honey Snowdon and Cliver (1996). The osmophilic or sugar tolerant yeasts could ferment honey even under low water content in honey. Fermentation by osmophilic yeasts would occur with a water activity higher than 0.65 (Piana *et al.*, 1991) or water content higher than 17.1% (w/v) in honey (Iurlina and Fritz, 2005).

In the present study, the anti-microbial activity of honey samples was increased with the increase of honey concentration. The finding was in line with Tumin *et al*. (2005) who reported that concentrated honey could produce larger inhibitory zone than diluted honey samples. The crude honey sample of Tualang was found to exhibit higher anti-microbial activity at the concentration 75 than 100% (w/v). The inhibitory activity of Tualang crude honey might be attributed to hydrogen peroxide which was produced during dilution. Somehow, the presence of hydrogen peroxide might not be the single factor contributing to the anti-microbial activity of Tualang honey samples. The Minimum Inhibitory Concentration (MIC) of Tualang crude honey was found to be 50% (w/v) which was lower than the MIC, 97.5% (w/v) reported by Tumin *et al*. (2005). The variance could be explained by the difference in the biochemical composition of Tualang honey samples collected at different harvesting time. Previously, Nasir *et al*. (2010) also reported that Tualang honey demonstrated the substantial bactericidal (bacteria killing) and bacteriostatic (bacteria inhibiting) effects. However, Mundo *et al*. (2004) reported that hydrogen peroxide was not the factor inhibiting the growth of several pathogenic bacteria including *Salmonella.* Gelam honey was one of the honey samples and they revealed that the inhibition was caused by high sugar content.

Based on the observation on the microbial inoculated plates, the crude honey samples could inhibit the growth of *Salmonella typhimurium* and *Candida albican,* but showed no inhibition activity for *Staphylococcus aureus* and *Fusarium oxysporium*. Tumin *et al*. (2005) also reported no inhibition exhibited by Tualang honey samples at the concentration 20-80% against *S. aureus*. The inhibitory activities of honey samples were tremendously increased at the concentration of 50% for both *S. typhimurium* and *C. albican* (Fig. 2a). In comparison, the inhibition against *S. typhimurium* was found to be higher than *C. albican* at the concentration higher than 50% of honey samples. At the concentration lower than 50%, the honey samples showed no inhibitory activity against *S. typhimurium*, but low activity against *C. albican*. The MICs of honey samples against *S. typhimurium* and *C. albican* were 50% and 3.1-6.3%, respectively as shown in Table 3. Although the honey samples exhibited higher inhibitory activities against *S. typhimurium*, their MICs were found to be higher than *C. albican*. This finding explains that the compound(s) in the honey samples are active for the inhibition of *C. albican* even at low concentration, but no more active against *S. typhimurium* at low concentration.

Interestingly, the inhibitory activities of the honey samples were significantly higher than the anti-microbial activity exhibited by crude honey sample of Manuka (Fig. 2a). In the present study, the MIC of Manuka honey, 12.5% (w/v) was close to the MIC, 15% (w/v) of Manuka honey reported by Tan *et al*. (2009) for the inhibition of *S. typhimurium*. On the other hand, a higher MIC of Manuka honey (25% w/v) was reported for *C. albican* inhibition (Table 3). The low activity of Manuka honey against *C. albican* was also reported by Patton *et al*. (2006) in their disc and well diffusion techniques. Somehow, Malaysian honey samples showed higher activities in Fig. 2a and

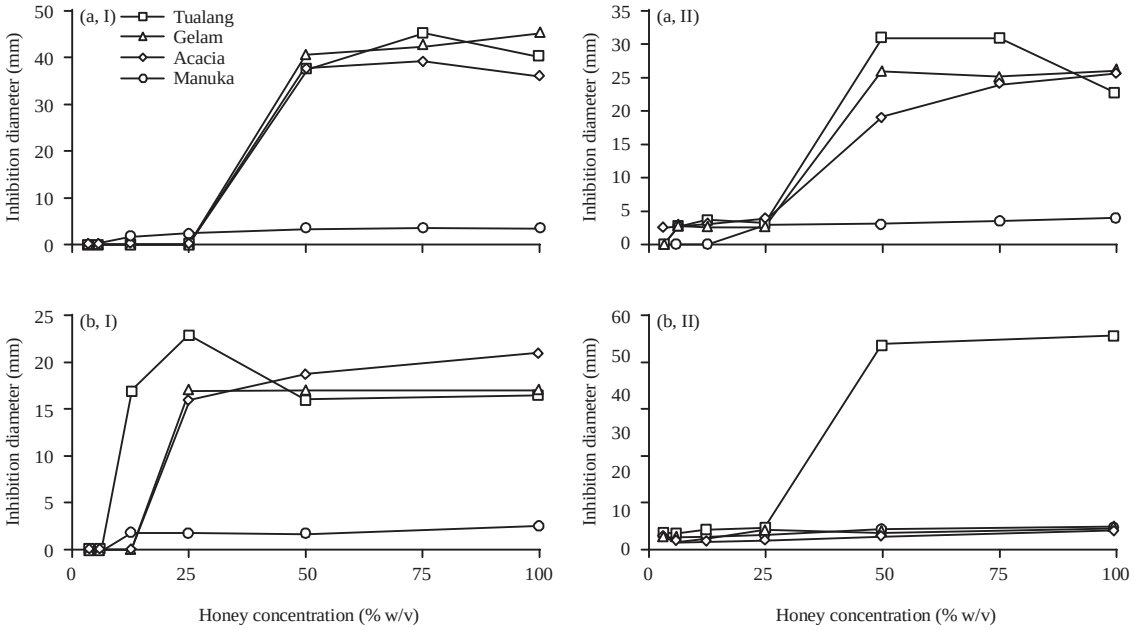

Fig. 2(a-b): (a) Diameter of inhibition zones exhibited by crude honey samples and (b) Polar fractions at different concentrations I: *Salmonella typhimurium* and II: *Candida albican*

lower MIC values (Table 3) than crude honey of Manuka against *C. albican.* This indicates that Malaysian honey samples are more effective than Manuka honey sample to suppress the growth of *C. albican*. Therefore, the honey samples could be better inhibitors for *C. albican* than Manuka honey.

After fractionation, the inhibitory activities of honey fractions were decreased, particularly the activity against *C. albican* as presented in Fig. 2b. Surprisingly, only the Tualang honey fraction showed a sharp increase in the inhibitory activity against *C. albican.* The active compound for the inhibition of *C. albican* might present in the acidified water fraction of Tualang honey samples. Tentatively, the active compounds could be hydroxymethylfurfural, ethyl glucuronide, myricetin and apigenin glycoside which were detected in the Tualang honey fraction (Table 2).

It is also interesting to highlight that the MICs of honey fractions were improved after fractionation, even though the activity of inhibition was lower than crude honey samples (Table 3). The lower MIC after fractionation could be due to the higher concentration of active compounds in the honey fractions. The improvement was also reported by Kirnpal-Kaur *et al.* (2011) who found that the acidified water fraction of Tualang honey samples exhibited higher anti-microbial activity against *Salmonella* sp. than its ethanolic fraction. The presence of active compound(s) in the polar fraction of honey samples might contribute to the partial activity against the microorganisms. Previously, Weston *et al.* (1999) also reported that the anti-bacterial activity of honey was associated with its carbohydrate fraction. The removal of non-polar compounds had improved the microbial inhibition in term of MIC values. There were also researchers who reported sugars and phenolic compounds could contribute to the activity of microbial inhibition (Havsteen, 1983).

Chromatographic fingerprints of acidified water fractions of honey samples: Figure 3 is an overlaid chromatogram for the acidified water fractions of honey samples. It is clear that

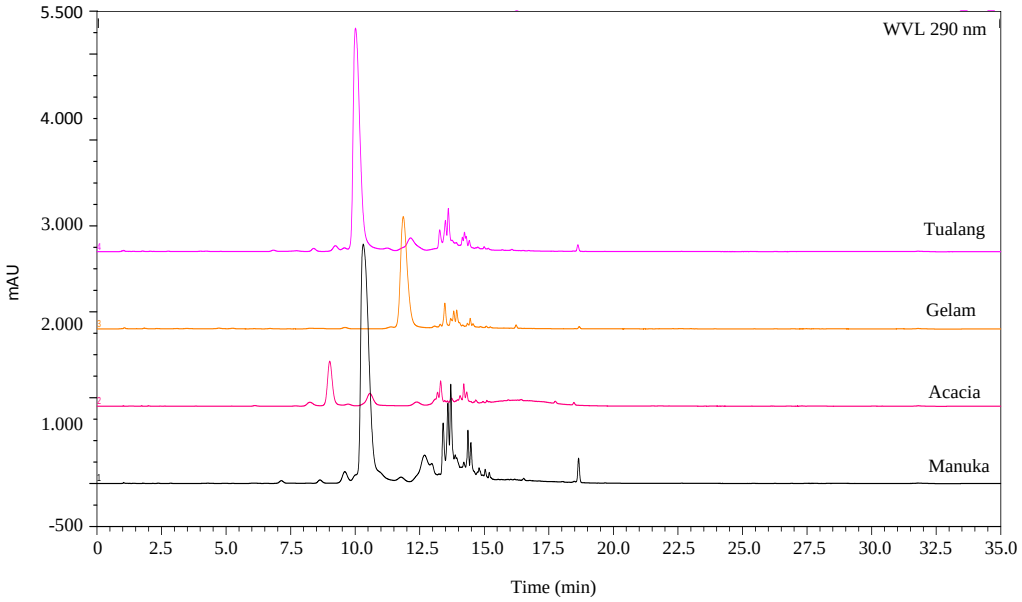

Fig. 3: Chromatogram of acidified water fractions of honey samples at 290 nm

Table 3: Minimum inhibitory concentration of crude honey samples and their acidified water fractions

	Minimum inhibitory concentration (% w/v)			
	Salmonella typhimurium		*Candida albican*	
Samples	Crude honey	Honey fraction	Crude honey	Honey fraction
Manuka	12.5	12.5	25.0	3.1
Tualang	50.0	12.5	6.3	3.1
Gelam	50.0	25.0	6.3	0.8
Acacia	50.0	25.0	3.1	3.1

Manuka honey fraction contains the highest number of peaks and the highest accumulated concentrations of all the peaks compared to the other honey fractions. Most probably, the peaks belong to organic acids and phenolic acids because they are highly polar compounds which tend to be firstly eluted from the C18 reserved phase column. The presence of organic acids could be contributed by the enzymatic reaction in honey. Acid hydrolysis would also release phenolic acids from complex structure of compounds and produce simple sugars from polysaccharides. Even though sugars could be eluted together with the acids, sugars do not absorb UV light at a wavelength of 290 nm (Karkacier *et al.*, 2003). There were also researchers who used a VIS/NIR spectroscopic system (446-1125 nm) to detect sugars from food based products (Rady and Guyer, 2015). Therefore, the UV scan at 290 nm could not detect sugars, but other polar compounds that could not bind to the non-polar reversed phase C18 column. Acacia honey fraction appears to have the lowest number and concentration of peaks in its acidified water fraction. This might explain the lower anti-inflammatory and anti-microbial activities of Acacia honey sample compared to Tualang and Gelam honey samples.

The putative compounds identified from the mass spectra are presented in Table 2. Based on the retention time of the peaks, the most intense peak for Tualang, Gelam and Manuka are putatively identified as hydroxymethylfurfural (m/z 127) and its derivative,

dihydroxymethylfurfural (m/z 144). However, the retention time of these compounds are different for Gelam honey fraction as presented in Fig. 3. Hydroxymethylfurfural in Gelam honey fraction might be geometrically different in chemical structure from other honey fractions. Indeed, hydroxymethylfurfural could be spontaneously formed as a result of fructose dehydration under acidic condition in honey samples. Most probably, the inhibitory activities of honey polar fractions could be due to the presence of hydroxymethylfurfural. This is because hydroxymethylfurfural has been proven to have high antioxidant capacity as reported by Zhao *et al*. (2013). This study also reported hydroxymethylfurfural could be developed as a novel natural antioxidant with potential application in cancer chemoprevention.

CONCLUSION

Honey has been used as food and medicine since ancient time. The medical property of honey in wound and burn healing was reported due to its anti-inflammatory and anti-microbial activities. However, the bioactive compounds that attributed to the biological activities are still unknown. Therefore, a C18 reversed phase solid-liquid extraction was used to fractionate the honey samples into polar fractions. It is believed that the polar compounds including sugars and polyphenols in honey are the major contributors to the biological activities, even though some investigators reported that the formation of hydrogen peroxide as a result of honey dilution could contribute to the activities significantly. Interestingly, the polar fractions of honey samples could improve the anti-inflammatory activity, but could not increase the selectivity against COX-2. However, the microbial inhibition of crude honey samples was higher than their polar fractions, particularly for *S. typhimurium* and *C. albican*. The performance of the honey fractions was also higher than Manuka honey fraction for the inhibition of *S. typhimurium*, but comparable with Manuka fraction for the inhibition of *C. albican*. Fractionation was found to improve Tualang honey for the inhibition of *C. albican* significantly. The increment in the anti-microbial activity of Tualang honey fraction was about 70% and the minimum inhibitory concentration was improved from 6.3-3.1% (w/v).

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