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IN-VITRO SCREENING OF MALAYSIAN HONEY FROM DIFFERENT FLORAL SOURCES FOR ANTIBACTERIAL ACTIVITY ON HUMAN PATHOGENIC BACTERIA

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Abstract

Background: Different researches on therapeutic effects of honey have been conducted in different regions; however the study on the potential antibacterial activity of Malaysian honey is still limited. In this study, antibacterial activities of different monofloral honey samples were tested against several common human pathogenic bacteria.
Materials and Methods: The well-diffusion method, minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) techniques were employed to investigate the putative antibacterial activity of Malaysian monofloral honey from *Koompassia excelsa* (Becc.) Taub (Tualang), *Melaleuca cajuputi* Powell (Gelam) and *Durio zibethinus* Murr. (Durian). Honey samples were tested against *Staphylococcus aureus* ATCC6518 and ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Enterococcus faecium* LMG16192, *Enterococcus faecalis* LMG16216 and ATCC29212, *Escherichia coli* ATCC25922, *Salmonella enterica* serovar Typhimurium ATCC14028 and *Klebsiella pneumoniae* ATCC13883.
Results: Marked variations were observed in the antibacterial activity of these honey samples. Durian honey failed to produce substantial antibacterial activity, whereas Tualang and Gelam honey showed a spectrum of antibacterial activity with their growth inhibitory effects against all of the tested bacterial species including vancomycin-resistant enterococci (VRE).
Conclusion: Present findings suggested Gelam honey possesses highest antibacterial effect among the tested Malaysian honey samples.

Keywords: Honey; monofloral; antibacterial; well-diffusion method; VRE

Introduction

Honey is a viscous, sugary, translucent, yellowish brown or light yellow liquid, where it deposited in the honey comb. Honey bees (*Apis* spp.) suck out the nectar from flowers and deposit in the stomach where the nectar blends with protein and enzymes of the bee, which is then being converted into honey. The antibacterial activity of honey was first recognized in 1892 by van Ketel (Dustmann, 1979). Recent years, honey has been selected for the treatments of bacterial infections by medical profession, especially with the emergence and continuous development of antibiotic resistance of pathogenic bacteria, where modern therapeutic agents failed to treat (Molan, 2001). The antibacterial activity of honey has been attributed to high osmolarity, acidic pH, hydrogen peroxide generation, and presence of other phytochemical constituents such as aromatic acids and phenolic compounds (Molan, 1992a,b). According to Molan (1992a), hydrogen peroxide is the major contributor to the antibacterial activity of honey, and the different levels of hydrogen peroxide in honey from different sources are responsible for their varying antibacterial effects. However, the presence of non-peroxide compounds in the honey also is believed to inhibit an extensive range of bacteria. Although all honey consists of similar nutritional profile but Taormina et al. (2001) reported that honey from different sources contain different levels of antibacterial activity, may due to varied geographical distribution and floral content. In spite of a vast research on the antibacterial property of honey in various parts of the world (Al-Namma, 2009), to the best of our understanding the study on the potential antibacterial activity of Malaysian honey has not yet been properly documented. In this study, antibacterial activities of three different Malaysian monofloral honey samples were tested against nine strains of common human pathogenic bacteria.

Materials and Methods

Honey samples and bacterial strains

Monofloral honey of different floral sources namely *Koompassia excelsa* (Becc.) Taub (Tualang), *Melaleuca cajuputi* Powell (Gelam) and *Durio zibethinus* Murr. (Durian) were obtained from several geographical locations in Malaysia. Human pathogenic bacteria species such as gram-positives: *Staphylococcus aureus* (ATCC6518 and ATCC25923), *Staphylococcus epidermidis* (ATCC12228), *Enterococcus faecalis* (ATCC12228), vancomycin-resistant enterococci (VRE) species: *Enterococcus faecium* (LMG16192) and *Enterococcus faecalis* (LMG16216); gram-negatives: *Escherichia coli* (ATCC25922), *Salmonella enterica* serovar Typhimurium (ATCC14028) and *Klebsiella pneumoniae* (ATCC13883) were provided by Faculty of Science, Universiti Tunku Abdul Rahman (UTAR), Malaysia.

In-vitro antibacterial activity tests
Agar well-diffusion method

Three to five bacterial colonies of 24-hour-old pure culture were suspended in 10 ml nutrient broth. The turbidity of the suspension was adjusted to achieve 0.5 McFarland (equivalent to that of 1.5 X 10^8 CFU/ml) with the absorbance range of 0.08 to 0.13 by spectrophotometer at wavelength of 625 nm (Andrew, 2009). The bacterial suspension was then seeded evenly onto the surface of Mueller Hinton agar plates with a sterile

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swab. Each honey type was diluted in sterile distilled water to different concentrations of 20%, 40%, 60%, 80% (v/v) and 100% undiluted honey. Wells were cut using 6 mm diameter cork borer to which appropriate concentrations of honey and sterile distilled water (sterility control) were added. The plates were incubated at 37°C and examined after 24 hours incubation. All the tests were carried out in triplicate and the mean values were obtained.

Broth dilution method

The bacterial strains which were successfully inhibited by the tested honey in well-diffusion method were further tested for minimum inhibitory concentration (MIC). Appropriate volume of honey was added into nutrient broth and then serially twofold diluted to obtain varying concentrations of 2000 mg/ml, 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, 15.63 mg/ml and 7.81 mg/ml respectively. Then the adjusted 0.5 McFarland bacterial suspension was added to each honey sample and incubated at 37°C for 24 hours, after which the tubes were checked macroscopically and compared with negative control to determine the lowest concentration of honey sample with no visible growth is determined as MIC (Agbeje et al., 2006). Tubes without visible growth or turbidity in MIC were then tested for minimum bactericidal concentration (MBC). The nutrient agar plates evenly seeded with 50 µl of the culture from the tubes with no visual growth shown in the MIC test were incubated at 37°C for 24 hours. Lowest concentration without any visible growth of bacterial colony on plate was determined as MBC (Mohapatra et al., 2011). The assays were duplicated.

Results

From the preliminary screening, it was observed all tested Malaysian honey exhibited various degrees of inhibitory effect with the well-diffusion method. Formation of clear zones indicated the presence of potent antibacterial activity. Generally, more concentrated honey demonstrated higher antibacterial potency than the diluted honey. *Koompassia excelsa* (Tualang) honey showed antibacterial effect against all the tested bacteria including VRE (*E. faecalis* LMG16216 and *E. faecium* LMG16192) from 80% (v/v) onwards with the strongest activity seen against *S. enterica* ser. Typhimurium ATCC14028 even in the lowest concentration 20% (v/v) (Figure 1). *Melaleuca cajuputi* (Gelam) honey showed that it was more potent than Tualang honey as its inhibition of most of the tested bacteria started from 40% (v/v). It was effective against *K. penumoniae* ATCC13883, *S. aureus* ATCC6518 and *S. epidermidis* ATCC12228 and with a relatively strong potency against VRE and the rest (Figure 2). *K. pneumoniae* ATCC13883 was the most susceptible to the *Durio zibethinus* (Durian) honey followed by *S. epidermidis* ATCC12228 among the tested strains. However, it was merely effective against *S. aureus* ATCC25923, *E. coli* ATCC25922, *E. faecalis* ATCC29212 and *S. enterica* ser. Typhimurium ATCC14028 and totally ineffective against the rest (Figure 3). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the honey sample are shown in Table 1. It was observed that all the honey types, except Durian honey, exhibited substantial bactericidal activity to all the bacterial species. Based on the outcome, it was also observed that *S. aureus* ATCC6518 was the most sensitive to Gelam honey with the lowest MIC and MBC.

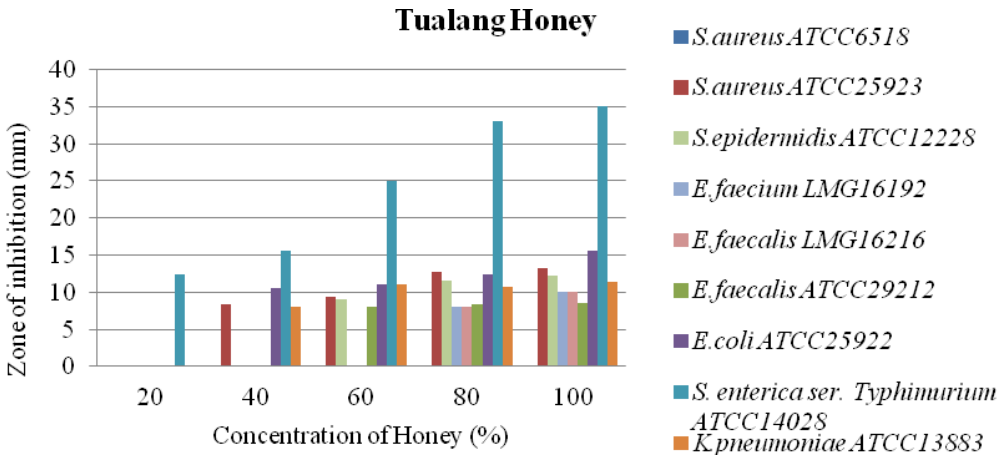

Figure 1: Zone of inhibition produced by Tualang honey against bacterial strains.

Discussion

In present study, Gelam honey from the source of *Melaleuca cajuputi* was able to exert inhibition and bactericidal effect against most bacterial strains and species including antibiotic-resistant strains, this proven its strongest antibacterial potential compare to honey from *Koompassia excelsa* (Tualang) and *Durio zibethinus* (Durian). From the outcome, concentration of honey used is directly proportional to inhibitory effect has indicated that antibacterial effect of honey works best in its undiluted form, where the conditions of antibacterial properties like acidity, osmolarity, and phytochemical components including flavonoids and phenolic content are well preserved (Badawy et al., 2004; Molan, 2001). However, as

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reported by Mundo et al. (2004), dilution of honey activates the activity of glucose oxidase which enhances hydrogen peroxide-mediated activity and this may be a possible explanation of the inhibitory potency of diluted honey on certain bacteria. The range of MIC and MBC values of honey correlated well with the results obtained using well-diffusion method that showed the strongest antibacterial potency of Gelam honey followed by Tualang and Durian honey.

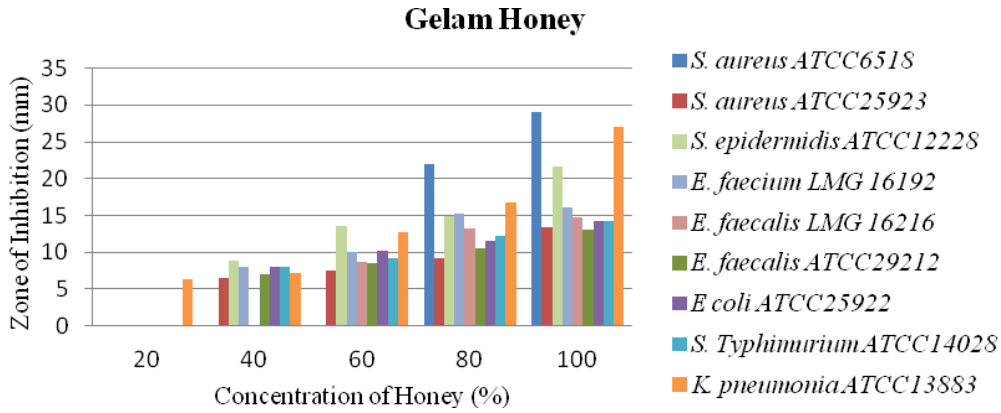

Figure 2: Zone of inhibition produced by Gelam honey against bacterial strains.

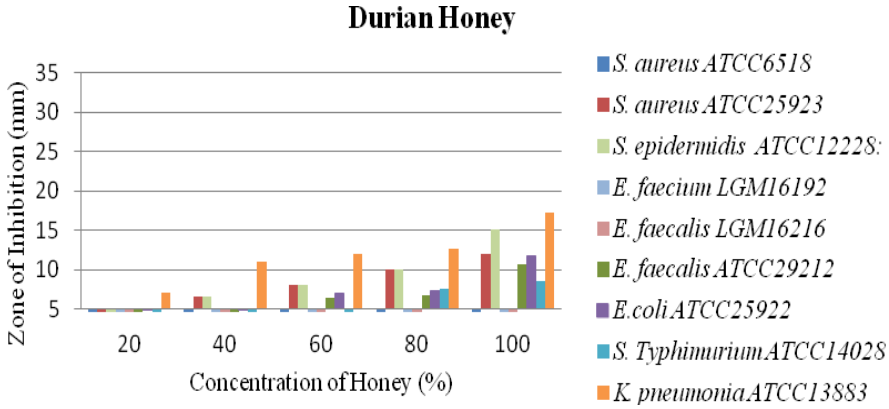

Figure 3: Zone of inhibition produced by Durian honey against bacterial strains.

Table 1: MIC and MBC (mg/ml) of three Malaysian honey.

Bacterial strains	Tualang honey		Gelam honey		Durian honey	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC6518	500	2000	125	125	500	1000
S. aureus ATCC25923	250	500	1000	1000	250	500
S. epidermidis ATCC12228	250	1000	250	250	250	1000
E. faecium LMG16192	250	2000	500	2000	500	2000
E. faecalis LMG16216	250	2000	500	2000	500	2000
E. faecalis ATCC12228	250	2000	1000	1000	500	2000
E. coli ATCC25922	250	500	500	500	500	NA
S. enterica ser. Typhimurium ATCC14028	125	500	250	500	1000	NA
K. pneumoniae ATCC13883	500	1000	125	250	125	250

NA: No activity seen against the tested bacteria

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In the whole, higher susceptibility of gram-negative bacteria to honey was seen, in which *S. enterica* ser. Typhimurium inhibited the most by Tualang honey while *K. pneumoniae* was highly susceptible to the action of Durian honey. This indeed supported by Al-Namma (2009) and El-sukhon et al. (1994) who also observed that honey has a greater inhibitory effect on gram-negative bacteria compared to gram-positive bacteria. According to Taormina et al. (2001), the antibacterial activity of honey on gram-negative bacteria was attributed to the presence of several factors such as: high content of tetracycline derivatives, hydrogen peroxide and powerful antioxidants. On the other hand, the cell wall of gram-negative bacteria is more prone to mechanical breakage because of the low amount of peptidoglycan compared to gram-positives (Tortora et al., 2013).

In overall, observed inconsistent pattern of antibacterial potency in this study can be due to several reasons. One possibility might be related to the differences in sensitivity of each bacterial species to the inhibitory activity of honey used that reported by others (Ceyhan and Ugur, 2001; Taormina et al., 2001). In addition, the discrepancy of the antibacterial activity between honeys could due to the difference of chemical composition including sugar profile, glycerol, ethanol, as well as other physicochemical parameters which closely related to the variation of floral origin and geographical provenience (Molan, 1992b). Previous study also showed human pathogens including gram-positives, gram-negatives and fungi exhibited diverse sensitivities towards honey sample from different sources (Mercan et al., 2007).

The excellent antibacterial activity of Malaysian honey especially honey from *M. cajuputi* (Gelam) against these human pathogens indicates the usefulness of honey as an antibacterial agent. These honey samples could have potential applications in foods to spoilage microorganisms or pathogens to enhance the safety of foods. Nevertheless, further in-depth studies are necessary including the identification and characterization of the related active components that may suggest any possible therapeutic potential.

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