Malaysian Journal of Pharmaceutical Sciences,
Vol. 3, No. 2, 1–10 (2005)

ANTIBACTERIAL ACTIVITY OF LOCAL MALAYSIAN HONEY

NURIZA TUMIN[1], N. ARSYIAH A. HALIM[1], M. SHAHJAHAN[2*], NOOR IZANI N. J., MUNAVVAR A . SATTAR[3], ABDUL HYE KHAN[3] AND S. S. J. MOHSIN

[1]School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia

[2]Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh

[3]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

The antibacterial activity of five different local honey brands viz. Tualang, Hutan, Gelang, Pucuk Daun and Ee Feng Gu honey obtained from different locations in Malaysia was investigated. Honeys were tested for putative antibacterial activity by disc diffusion assay and their inhibition of growth of six pathogenic bacteria in batch culture. Minimum inhibitory concentration (MIC) was determined for each of the honey tested using standard assay procedures. In terms of physicochemical properties, it was observed that the pH of these honeys was within 3.55–4.91, their specific gravity was 1.3–1.35, moisture content was 16–23.3% and dry matter content was 76.6–84%. Marked variations were observed in the antibacterial activity of these honey samples. Two honey brands, Hutan and Gelang did not produce any substantial antibacterial activity while other brands showed a spectrum of antibacterial activity with their growth inhibitory effect against at least three-four different bacterial species including S. typhi, S. aureus, S. sonnie and E. coli.

Keywords: *Malaysian honey, Antibacterial activity*

INTRODUCTION

Apitherapy or therapy with bee products is an age-old therapeutic practice as recorded by several ancient civilizations. Indeed, medicinal importance of honey has been documented in the world's oldest medical literatures (Eva 1976; Maryann 2000). In light of modern science, several important therapeutic effects of honey have been elucidated. Honey has been found to heal infected surgical wounds, burns and decubitus ulcer as mentioned by several researchers (Zumla and Lulat 1989; Cooper *et al.* 1999, 2002; Subrahmanyam *et al.* 2001). Honey has further been found to possess good antimycobacterial activity (Asadi-Pooya *et al.* 2003) and also inhibitory activity against *Helicobacter pylori* as reported by Ali and co-workers (Ali *et al.* 1991). Laboratory studies have revealed that honey is

* Corresponding author: Dr. M. Shahjahan

effective against Methicillin resistant *Staphylococcus aureus* (MRSA), β-hemolytic Streptococci and Vancomycin-resistant Enterococci (VRE) as reported in earlier studies (Allen *et al.* 2000; Kingsley 2001). It has further been reported that physical property along with geographical distribution and different floral sources may play important role in the antimicrobial activity of honey (Taormina *et al.* 2001).

In spite of a wide body of research on the antibacterial property of honey in different parts of the world, to the best of our knowledge systematic study on the possible antibacterial activity of Malaysian honey has not yet been done. In the present study, antibacterial activities of five different Malaysian honey collected from different localities were tested against six pathogenic bacteria. Physicochemical properties of these honeys were also studied to find whether any difference in their physicochemical properties could influence their antibacterial susceptibility, if there is any.

METHODS

Materials

Several local brands of honey collected from different sources were used in this study. These brands were "Tualang", "Hutan", "Gelang", "Pucuk Daun" and "Ee Feng Gu" brand. In the antibacterial study, several bacterial species known to be pathogenic to human such as *Escherichia coli* (ATCC25922), *Salmonella typhi*, *Shigella sonnei*, *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923) were used. These strains were obtained from the School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kelantan, Malaysia. Several commercially available (Oxoid Ltd., UK) antibiotic discs including Amoxycillin (10 μg), Chloramphenicol (30 μg), Ceftazidime (30 μg), Cefuroxime (30 μg), Colisitin (10 μg) and Gentamicin (30 μg) were also used as standard antibacterial agents for the MIC determinations.

Methods

Physicochemical study: Appearance of different honeys was observed. pH, moisture content, content of dry matter and specific gravity of these

honeys were also determined using conventional procedures. Total lipid content were measured as described by Bligh and Dyer (1959).

Antibacterial study: Antibacterial study was carried out in steps. In the first step, an *in vitro* screening was carried out using either disc diffusion (Fessia *et al.* 1988) or well diffusion (Georgii and Korting 1991) method. Disc diffusion was carried out using both plate diffusion and streak test.

Preparation of test materials: Test materials were prepared by diluting each honey at different dilutions, 20 µl/100 µl, 40 µl/100 µl, 60 µl/100 µl and 80 µl/100 µl. Moreover, net honey was also used as test material. All dilutions were carried out with double distilled and deionized sterilized water.

Disc diffusion method: In this approach, surface of Nutrient agar (Merck, Germany) plate or plates made with selective medium (Blood agar, Mac Conkey's agar, Muller Hinton agar and Todd Hewitt agar purchased either from Merck, Germany or Oxoid Ltd., UK) were uniformly inoculated in individual petri dishes with overnight stock cultures prepared in broth of either Luria Bertani or any selective media as mentioned earlier. In disc diffusion method, Whatman AA discs (Whatman International Ltd., UK), 6 mm in diameter were used. Test materials (20 µl of each dilution or net honey) were socked into the paper discs, dried at room temperature and carefully placed into the petri dishes seeded with inocula. Plates were kept at 4°C for four hours to provide sufficient time to diffuse the test material into the medium and finally incubated at 37°C for 8–12 hours. The diameter of the zone of inhibition produced around the discs was measured by Vernier calipers as index of putative antibacterial activity of test materials. The size of the inhibition zone further represented a quantitative measure of antibacterial activity of the test material. All experiments were performed in triplicate and the zone of inhibition was measured twice for each honey dilution and net preparation. Along with the test materials, standards antibiotic discs were also used to compare the antibacterial potency of the test materials.

Well diffusion method: In this approach, small circular holes were prepared using sterilized cork borer or glass rod on agar plates seeded with test organisms. These holes were filled with test materials followed by essentially the same steps as described for disc diffusion method.

Determination of minimum inhibitory concentration (MIC) of different brands of honey: In the second step of antibacterial study, MIC of different brands of honey were determined using serial dilution or turbidimetric assay (Fessia *et al.* 1988). MIC values were determined for all five different brands of honey against six different pathogenic bacteria, *Escherichia coli* (ATCC25922), *Salmonella typhi*, *Shigella sonnei*, *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923). In this method, graded doses (v/v) of different honey were used along with several standard antibiotics such as Ampicillin 100 µg/ml (Medochemie Ltd., Cyprus) and Cetfazidime 100 µg/ml (Glaxo Wellcome Ltd., UK) as controls. Overnight broth cultures of each bacterial strain were prepared in nutrient broth or using selective medium. The turbidity was visually compared with McFarland 0.5 standard (Becton, Dickinson and Company, MD, USA). McFarland standards are widely used as turbidity standards in the preparation of suspensions of microorganisms and have particular application in the preparation of bacterial inocula for performing antimicrobial susceptibility testing. These were prepared by adding sulfuric acid to an aqueous solution of barium chloride, which results in the formation of a suspended barium sulfate precipitate. According to the manufacturer the McFarland 0.5 standard corresponds approximately to a homogeneous *Escherichia coli* suspension of 1.5×10^8 cells per ml[3].

RESULTS AND DISCUSSION

Appearance of each of honey brand was examined and it was observed that "Tualang", "Hutan" and "Pucuk Daun" honey were brown to dark brown in color, whereas "Gelang" and "Ee Feng Gu" honey were golden yellow in color.

Physicochemical properties of five different honey

Several physical parameters such as pH, specific gravity, moisture content and dry matter content of these honeys were determined. The pH range of the five brands was from 3.55–4.91. Moreover, among the five different brands of honey, "Tualang" honey was most acidic as compared to others (Table 1). Specific gravity of five honeys were in between 1.300–1.350. The specific gravity of "Ee Feng Gu" honey was the highest at 1.350 and that of "Pucuk Daun" honey was the lowest at 1.300 (Table 1). The

moisture content was found to be varied between 16.0–23.3%. The results also revealed that the moisture content of honey varied with the types of honey.

Table 1: Physicochemical properties of five different Malaysian honey

Honey brand	Appearance	pH	Specific gravity	Moisture content (%)	Dry matter content (%)	Total lipid content (mg/100 g)
Tualang	Dark brown	3.55	1.335	23.3	76.6	100
Gelang	Golden yellow	4.15	1.335	16.9	83.1	100
Hutan	Brown	3.81	1.337	16.9	83.1	0
Ee Feng Gu	Golden yellow	4.17	1.300	21.9	78.1	100
Pucuk Daun	Brown	4.91	1.350	16.0	84.0	100

Standard procedures were used to determine pH, specific gravity, moisture and dry matter content and lipid content.

The moisture content of "Tualang" honey was the highest at 23.3% and that of "Ee Feng Gu" was the lowest at 16%. Moreover, dry matter content also varied depending on the types of honey. "Ee Feng Gu" honey contained the highest amount of dry matter with 84%. The dry matter of "Tualang", "Hutan", "Gelang" and "Pucuk Daun" honey were 76.6, 83.1, 83.1 and 78.1% respectively (Table 1). The amount of lipid in these honeys was negligible and the total lipid content of "Tualang", "Gelang", "Pucuk Daun" and "Ee Feng Gu" honey were the same, 100 mg/100 gm except that "Hutan" honey was lipid free (Table 1).

Antibacterial activity of five different Malaysian honeys

In the preliminary screening process it was observed that two honey brands, "Gelang" and "Hutan" did not have any antibacterial activity against any of the organisms tested. "Tualang" honey showed antibacterial effect against *E. coli, S. typhi* and *S. pyogenes*, with the strongest activity seen against *S. typhi*. Clear zones of inhibition were produced by its net preparation and all of its dilutions (20 µl/100 µl, 40 µl/100 µl, 60 µl/100 µl and 80 µl/100 µl). However, it was merely ineffective against the other three bacteria (Table 2).

Table 2: Preliminary antibacterial activity of different Malaysian honey brands

Bacterial strain	Honey dilution (µl/100 µl, v/v)					Control disc
	20	40	60	80	Net	
	Pucuk Daun honey Diameter of clearzone of Inhibition (mm)					
E. coli	20	25	30	30	30	CXM (30 µg) = 20
S. typhi	R	R	R	R	R	CXM (30 µg) = 27
S. sonnei	22	25	25	25	25	CXM (30 µg) = 23
P. aeruginosa	R	R	R	R	R	CL (10 µg) = 19
S. aureus	R	R	R	R	R	CXM (30 µg) = 30
S. pyogenes	R	R	R	R	R	CMC (30 µg) = 19
	Tualang honey Diameter of clear zone of Inhibition (mm)					
E. coli	R	R	18	21	25	AML (10 µg) = 28
S. typhi	26	30	31	36	34	AML (10 µg) = 28
S. sonnei	R	R	R	R	R	CAZ (30 µg) = 28
P. aeruginosa	R	R	R	R	R	CL (10 µg) = 15
S. aureus	R	R	R	R	R	CAZ (30 µg) = 20
S. pyogenes	R	20	23	25	15	AML (10 µg) = 32
	Ee Feng Gu honey Diameter of clear zone of Inhibition (mm)					
E. coli	15	18	25	26	20	CXM (30 µg) = 23
S. typhi	21	26	30	35	32	CXM (30 µg) = 22
S. sonnei	20	26	30	30	30	CXM (30 µg) = 23
P. aeruginosa	R	R	R	R	R	CL (10 µg) = 20
S. aureus	20	25	30	30	30	CXM (30 µg) = 20
S. pyogenes	R	R	R	R	R	CMC (30 µg) = 30

R = Resistant, AML = Amoxycillin, CAZ = Ceftazidime, CXM = Cefuroxime, CMC = Chloramphenicol, CL = Colistin. Either disc diffusion or well diffusion methods were used.

Preliminary antibacterial screening of "Pucuk Daun" honey showed that it was effective against *E. coli* and *S. sonnei* with a relatively strong potency against *S. sonnei*. This activity was seen with its net preparation and all dilutions (20 µl/100 µl, 40 µl/100 µl, 60 µl/100 µl and 80 µl/100 µl) produced clear zones indicated a potent antibacterial activity of all these honey against this bacterial species (Table 2).

However, "Ee Feng Gu" brand showed interesting results with antibacterial potency against four different bacterial strains, *E. coli*, *S. typhi*, *S. sonnei* and *S. aureus* in its net form and at all dilutions (20 µl/100 µl, 40 µl/100 µl, 60 µl/100 µl and 80 µl/100 µl) preparations (Table 2).

MICs of five different Malaysian honeys

MICs of all the five honey brands were tested against *E. coli*, *S. typhi*, *S. sonnei*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*. It was observed that all the honeys, except "Ee Feng Gu", were having substantial antibacterial activity at least against three bacterial species. Based on the MICs, it was also observed that all the honey was sensitive to *S. sonnei* with "Pucuk Daun" honey having the lowest MIC (Table 3).

Table 3: MICs of five different Malaysian honey

	Different honey brands				
	Tualang	**Gelang**	**Hutan**	**Ee Feng Gu**	**Pucuk Daun**
Bacterial strain	**Minimum inhibitory concentrations (μg/ml)**				
E. coli	195.0	140	230	270	160
S. typhi	97.5	35	115	135	80
S. sonnei	48.75	17.5	28.75	135	5
P. aeruginosa	48.75	70	57.50	135	80
S. aureus	97.5	70	230	270	160
S. pyogenes	97.5	140	230	135	80

Standard procedure was used to determine MIC. ATCC numbers of all the strains if available are provided in Methods section.

DISCUSSION

Physical characterization, biochemical and antibacterial analysis were performed on five selective brands of Malaysian honey. Physicochemical study (Table 1) revealed that all the honey brands were different in terms of their physicochemical properties except that all were acidic. Low pH or acidity of honey is believed to have important contribution in its antibacterial activity. Indeed, previous studies claimed that the antibacterial activity of honey is credited to its acidity along with osmolarity and other factors (Molan 1992; Melissa *et al.* 2004). Moreover, other physicochemical parameters, in particular the coloration of honey due to the presence of any particular chemical constituent(s) might also be contributed to its antimicrobial activity as reported by Taormina *et al.* (2001).

This preliminary antibacterial studies showed that "Ee Feng Gu" honey was effective against several pathogenic bacterial strains including *S. typhi*, *S. aureus, S. sonnei* and *E. coli* at all concentrations tested.

"Tualang" honey also exhibited substantial antibacterial activity but only against *E. coli, S. typhi* and *S. pyogenes* and that not all concentrations were effective as antibacterial. Another brand, "Pucuk Daun" honey, also found effective against only *E. coli* and *S. sonnei*. However, in this preliminary study two other honey brands, "Gelang" and "Hutan" did not show antibacterial activity against the organisms tested (Table 2).

In the second part of this study, it was observed that all the honey studied possess appreciable MIC against all the organisms tested (Table 3). It was further observed that as far as the MIC values are concerned, some bacterial strains, which were found insensitive in the preliminary antibacterial study, seemed to be sensitive to the honey brand used with appreciably good MIC values. It was further observed that two honey brand, which could not produce any antibacterial effect in the preliminary study has exhibited to have good MIC against most of the bacterial strains studied.

Almost all the honey brands used in this study were effective against *E. coli*, *S. aureus*, *S sonnei* and *S. typhi* and these findings concur with several earlier reports on antibacterial activity of honeys from other countries (Taormina *et. al.* 2001; Melissa *et al.* 2004). Further, this study also showed that like antibiotics, some organisms were sensitive to some brands of honey while others were insensitive. Same phenomenon was observed in terms of MIC values of the honey brands used as these values varied according to the bacterial strains. Moreover, in both experimental approaches employed in the antibacterial test, it was revealed that *S. sonnei* was the most vulnerable organisms to these honey brands.

The discrepancy in the observed antibacterial activity can be due to several reasons. One possibility might be related to the differences in susceptibility of each species of microorganism to the antibacterial activity of honey used. Similar observations are reported by others (Nzeako and Hamdi 2000; Ceyhan and Ugur 2001; Taormina *et al.* 2001). Moreover, the variable results observed between honeys could also possibly be due to the different floral sources utilized by the bees and the geographical factors like temperature, humidity where the honey was produced.

Other possible explanation for these observations could be the differences in putative antibacterial agent(s) present in these honeys. These agents may utilize hydrogen peroxide and non-peroxide antioxidant components. It could may well be that the organisms were

resistant to the diluted preparations (in the preliminary study the net preparation made with 20 µl/disc or well).

As reported by others (Melissa *et al.* 2004) dilution of honey enhances hydrogen peroxide mediated antibacterial activity may explain some of the discrepancies of observed with the antibacterial activity of these honey. The presence of unstable putative agents and/or thermolabile antibacterial agent(s) could also be inactivated during the experimental procedure and thus may be considered as possible explanation of the observed insensitivity of some honey samples found in the preliminary study. However, the present study is unable to clarify the possible causative agent(s) involved in the antibacterial activity of the honey used. Further in-depth studies are needed. Identification and characterization of the active principle(s) may provide valuable information on the quality and possible therapeutic potential of these Malaysian honeys.

ACKNOWLEDGEMENT

This research was partly supported by the short-term incentive grant, Universiti Sains Malaysia (Ref No. USM incentive grant, dt. 23/09/2003). We would also like to express our thanks to the School of Health Sciences, Health Campus, Universiti Sains Malaysia for chemicals and instrumental facilities.

REFERENCES

ALI, A. T., CHOWDHURY, M. N. & AL-HUMAYYAD, M. S. (1991) Inhibitory effect of natural honey on *Helicobacter pylori*, *Tropical Gastroenterology*, 12: 139–143.

ALLEN, K. L., HUTCHINSON, G. & MOLAN, P. C. (2000) The potential for using honey to treat wounds infected with MRSA and VRE. *First World Healing Congress*, *Melbourne, Australia*, 10–13 September 2000.

ASADI-POOYA, A. A., PNJEHSHAHIN, M. R. & BEHESHTI, S. (2003) The antimycobacterial effect of honey: An *in vitro* study, *Rivista di Biologia*, 96: 491–495.

BLIGH, E. G. & DYER, W. T. (1959) A rapid method of total lipid extraction and purification, *Canadian Journal of Biochemistry and Physiology*, 37: 911–917.

CEYHAN, N. & UGUR, A. (2001) Investigation of *in vitro* antimicrobial activity of honey, *Rivista di Biologia*, 94: 363–371.

COOPER, R. A., MOLAN, P. C. & HARDING, K. G. (1999) Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds, *Journal Royal Society of Medicine*, 92: 283–285.

COOPER, R. A., MOLAN, P. C. & HARDING, K. G. (2002) The sensitivity to honey of gram-positive cocci of clinical significance isolated from wounds, *Journal of Applied Microbiology*, 93: 857–863.

EVA, C. (1976) History of Honey. IN: EVA, C. *Honey a Comprehensive Survey*, pp. 454–465. (London: Morrison and Gibb Ltd.).

FESSIA, S., FAWCETT, P., MACVAUGH, C. & RYAN, S. (1988) Antimicrobial sensitivity testing. IN: *Diagnostic Clinical Microbiology: A Benchtop Perspective*, pp. 181–183 (USA: W. B. Saunders Company, Harcourt Brace Jovanovich, Inc.).

GEORGII, A. & KORTING, C. (1991) Antifungal susceptibility testing with dermatofit, *Mycoses,* 34: 193–199.

KINGSLEY, A. (2001) The use of honey in the treatment of infected wound, *British Journal of Nursing*, 10 (22 Suppl.): S13–6, S18, S20.

MARYANN, N. (2000) Honey as medicine has a long history – New Zealand honey is focus of intensive research, *Health Facts*, 25: 4–5.

MELISSA, A. M., OLGA, I. P-Z. & RANDY, W. W. (2004) Growth inhibition of food borne pathogens and food spoilage organisms by select raw honeys, *International Journal of Food Microbiology*, 97: 1–8.

MOLAN, P. C. (1992) The antibacterial activity of honey: 1. The nature of the antibacterial activity, *Bee World*, 73: 5–28.

NZEAKO, B. C. & HAMDI, J. (2000) Antimicrobial potential of honey on some microbial isolates, *Medical Sciences*, 2: 75–79.

SUBRAHMANYAM, M., HEMMADY, H. & PAWAR, S. G. (2001) Antibacterial activity of honey on bacteria isolated from wounds, *Annals of Burns and Fire Disasters*, XIV: 1.

TAORMINA, P. J., NIEMIRA, B. A. & BAUCHAT, 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: 217–225.

ZUMLA, A. & LULAT, A. (1989) Honey – A remedy rediscovered, *Journal of the Royal Society of Medicine*, 82: 384–385.