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Shaoka and Sidr Honeys Surpass in Their Antibacterial Activity Local and Imported Honeys Available in Saudi Markets Against Pathogenic and Food Spoilage Bacteria

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Abstract: The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of nine widely used honeys in Saudi Arabia (Yemeni Sidr, Taify Sidr, Kashmiri Sidr, Shaoka, Somra, Black Seed, Black Forest, and Clover honeys), and Manuka honey were estimated against 5 pathogenic Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella* Enterica *serovar typhimurium, Shigella flexneri* and *Klebsiella pneumoniae*), 2 pathogenic Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*), a food spoilage Gram-positive bacterium (*Bacillus subtilis*) and an acid fast bacterium (*Mycobacterium phlei*). Results revealed that *P. aeruginosa* was the most sensitive Gram-negative bacterium and *St. pyogenes* was the most sensitive Gram-positive bacterium. The MIC of the tested bacteria ranged between 5 and 20% honey (w/v) and the MBC ranged between 5 and 30% (w/v). Shaoka had lower MICs and MBCs against Gram-negative bacteria and Yemeni Sidr had lower MICs and MBCs against Gram-positive bacteria. It could be concluded that Shaoka and Sidr honeys surpass other honeys in their antibacterial activity and therefore, could be used for treatment of bacterial infections and for prevention of food spoilage

Key words: Antibacterial, Food spoilage bacteria, pathogenic bacteria, Saudi honeys.

INTRODUCTION

Since ancient times honey has been used in many cultures as an effective remedy (Majno, 1975; Krell, 1996; Smith, 2009). The fact that honey has antibacterial properties was recognized for more than a century because it cures infections (Dustmann, 1979; Subrahmanyam, 2001). There are numerous reports of the antimicrobial activity of honey against a wide range of bacterial and fungal species (Molan, 1992; Tumin, 2005; Chute, 2010; Kwakman, 2010)The antimicrobial activity of honey could be attributed to several factors (Wahdan, 1998). These factors are, osmotic effect of honey (6), the low pH of honey being between 3.2 and 4.5 (Molan, 1992; Cooper, 2002), hydrogen peroxide (13), defensin-1(9), as well as the presence of phytochemical factors (Frankel, 1998; Allen, 1991).

Honey has a well established usage as a wound dressing in ancient and traditional medicines (Zumla, 1989; Fakoor, 2007). In recent times this has been re-discovered, and honey is widely used as a topical antibacterial agent for the treatment of wounds, burns and skin ulcers (Molan, 2001; McInerney, 1990). Several types of bacteria, commonly involved in wound infections like *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Klebsiella* spps., *Streptococcus faecalis* and *Pseudomonas aeruginosa*, are susceptible to the antibacterial activity of honey regardless to their resistance to antibiotics (Effem, 1988; Lusby, 2005; George, 2007; Cooper, 2008).

Honey is a traditional remedy for dyspepsia and peptic ulcers (Yoirish, 1977; Kandil, 1987). *Helicobacter pylori*, the causative agent in many cases of dyspepsia and peptic ulcers was found to be sensitive in an agar well diffusion assay to a 20% (v/v) solution of honey (Al-Somai, 1994; Ali, 1991). Also, honey is effective in treating bacterial gastroenteritis (Haffejee, 1985). Pure honey has bacteriostatic and bactericidal activity against many enteropathogenic organisms, including *Salmonella* spps., *Shigella* spps. and enteropathogenic *E. coli* at 4-8% (v/v) and 5 -10% (v/v) respectively (Jeddar, 1985; Jeffrey, 1996).

A large number of honeys are available in the Saudi market. These honeys are either locally produced or imported from different countries. Some of these honeys are traditionally used as remedy for several ailments. In a previous study, the antibacterial activity of 24 types of honeys available in the Saudi market were evaluated and compared to Manuka honey and several types of the tested honeys were recognized as potent antimicrobials (Halawani, 2011).

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In this study nine types of honeys, locally produced or imported as well as Manuka honey, were evaluated for their antibacterial activity against several pathogenic and food spoilage bacteria.

MATERIALS AND METHODS

Bacteria:

Clinical isolates of Escherichia coli, Pseudomonas aeruginosa, Salmonella Enterica serovar *typhimurium, Shigella flexneri, Klebsiella pneumoniae, Staphylococcus aureus,* and *Streptococcus pyogenes* were obtained from the stock culture of the Department of Biology, Faculty of Science, Taif University. *Mycobacterium phlei* and *Bacillus subtilis* were obtained from the Department of Microbiology, Faculty of Pharmacy, Tanta University.

***Honey Samples*:**

Nine honey samples were used, eight honeys purchased from the local markets of Taif and Manuka honey was purchased from Superbee factory, New Zealand (Table 1). All honeys were kept at room temperature in dark glass containers.

Minimum Inhibitory and Minimum Bactericidal Concentration of Honey:

Each honey sample was serially diluted with nutrient broth to give series of concentrations in sterile micro-titration plates. Each series of dilutions was inoculated with 10^4 CFU/ml of the tested bacteria and incubated at 37^{o}C for 18 hours before determining the least concentration that inhibited the appearance of visible growth.

***Minimum Bactericidal Concentration (MBC)*:**

The minimum bactericidal activity was determined by sub-culturing inhibitory concentrations of each tested honey after reading the MIC in micro-titration plates.

***Sporicidal Activity of Honeys*:**

Twenty-five and 50% concentrations of Yemeni Sidr, Taify Sidr, Kashmiri Sidr, Shaoka, Somra, Manuka, Black Seed, Black Forest, and Clover honeys were inoculated with 10^5 CFU/ml and incubated at 37^{o}C for 2, 4, 8, 24 h. Samples were withdrawn at time intervals and the spores were counted by plating onto the surface of Muller-Hinton agar plates.

Statistical Analysis:

Comparison between means was conducted using Analysis Variance (ANOVA), minitab software.

Table 1: Honeys used in the study and their source and floral origin..

Local honeys		Imported honeys		
Name	Floral source	Name	Country of origin	Floral source
Taify Sidr	*Zizphus spina-christi*	Sidr	Yemen	*Zizphus spina-christi*
Somra	*Acacia tortilis*	Sidr	Kashmir	*Zizphus spina-christi*
Shaoka	*Fagonia cretica*	Clover	Egypt	*Trifolium alexandrinum*
Black Seed	*Nigella sativa*	Black Forest	Germany	(Honey due) Sweet secretions of aphids
		Manuka	New Zealand	*Leptospermum scoparium*

Results:

Antibacterial Activity of Honeys Against Different Types of Pathogenic Bacteria:

The antibacterial activity of 9 selected honeys, against 7 clinical bacterial isolates (*E. coli*, *Sh. flexneri*, *Sl. Enterica* serovar *typhimurium*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *St. pyogenes*), a food spoilage bacterium (*B. subtilis*) and an acid fast bacterium (*M. phlei*) was evaluated (Tables 2 and 3).

MIC for honeys against the tested bacteria ranged between 5 and 20 % w/v. On the other hand the MBC for the tested honeys ranged between 5 and 30 % w/v (Table 2 and 3), usually the lethal honey concentrations did not exceed double the bacteriostatic concentrations (Tables 2 and 3).

While, Gram-positive bacteria were inhibited by 5-10 % (w/v) honey and killed by 5-15 % (w/v) honey, Gram-negative bacteria except for *P. aeruginosa* were inhibited by concentrations ranging between, 10 to 20% (w/v) and killed by 15-20 % (w/v) honey . *P. aeruginosa* was the most sensitive Gram-negative bacteria and was inhibited by 5-15 % honey and killed by 7.5-15% (w/v) honey (Table 2).

K. pneumoniae was the most resistant Gram-negative bacteria. Its MIC and MBC ranged between 15-20 and 15-30% (w/v), respectively (Table 2). On the other hand, the most sensitive Gram positive bacteria was *St. pyogenes*. It was inhibited by a concentration ranging between 5 and 10 % honey and killed by a concentration ranging between5and15 % honey (Table 3).

The susceptibility of *M. phlei* was intermediate between that of Gram-positive and Gram-negative bacteria (Tables 2 and 3). Yemeni Sidr, Taify Sidr, Shaoka, and Manuka honeys were relatively more powerful antibacterial than other honeys (Tables 2 and 3). Shaoka had lower MICs and MBCs against Gram-negative bacteria, which ranged between 10-15% (w/v) honey and 12.5-20 % (w/v) respectively (Table 2). On the other hand Yemeni Sidr had lower MICs and MBCs against Gram-positive bacteria ranging between 5-10 and 5-15, respectively.

Sporicidal Activity of Honeys:

Two dilutions (25 and 50%) of Yemeni Sidr, Taify Sidr, Kashmiri Sidr, Shaoka, Somra, Manuka, Black Seed, Black Forest, and Clover honeys were tested for their ability to kill spores of *B. subtilis*. Honeys inoculated with 10^5 CFU/ml were incubated for 2, 4, 8, 24 h and the count of spores was estimated at time intervals. There was no decrease in the count of spores by any of the tested honeys (Data not shown).

Table 2 : Antibacterial activity of nine selected honeys against five Gram-negative bacteria.

Honey	*E. coli*		*Sh. Flexneri*		*Sl. typhimurium*		*K. pneumoniae*		*P. aeruginosa*	
	MIC*	MBC**	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Taify Sidr	10 ± 0.0	15 ± 0.0	10 ± 0.0	15 ± 0.0	15 ± 0.0	15 ± 0.0	15 ± 0.0	17.5 ± 3.5	7.5 ± 3.5	7.5 ± 3.5
Shokah	10 ± 0.0	12.5 ± 3.5	17.5 ± 3.5	20 ± 0.0	10 ± 0.0	15 ± 0.0	12.5 ± 3.5	15 ± 3.5	5 ± 0.0	7.5 ± 3.5
Somrah	15 ± 0.0	22.5 ± 3.5	15 ± 0.0	22.5 ± 10.6	12.5 ± 3.5	17.5 ± 3.5	15 ± 0.0	17.5 ± 3.5	5 ± 0.0	10 ± 0.0
Yemeni Sidr	10 ±0.0	17.5 ± 3.5	10 ±0.0	17.5 ±3.5	12.5 ± 3.5	17.5 ± 3.5	15 ± 3.5	20 ± 0.0	7.5 ± 3.5	7.5 ± 3.5
Kashmiri Sidr	15 ± 0.0	22.5 ± 3.5	17.5 ± 3.5	25 ± 7.1	15 ± 0.0	22.5 ± 10.6	15 ± 0.0	25 ± 7.1	7.5 ± 3.5	10 ± 0.0
Black Seed	12.5 ± 3.5	15 ± 0.0	15 ± 0.0	15 ± 0.0	15 ± 0.0	17.5 ± 3.5	15 ± 0.0	17.5 ± 3.5	7.5 ± 3.5	7.5 ± 3.5
Clover	15 ± 0.0	17.5 ± 3.5	15 ± 0.0	20 ± 7.1	20 ± 0.0	22.5 ± 10.6	20 ± 0.0	30 ± 0.0	15 ± 0.0	15 ± 0.0
Black Forest	12.5 ± 3.5	17.5 ± 3.5	12.5 ± 3.5	20 ± 7.1	15 ± 0.0	22.5 ± 10.6	15 ± 0.0	17.5 ± 3.5	7.5 ± 3.5	1 0± 0.0
Manuka	12.5 ± 3.5	12.5 ± 3.5	15 ± 0.0	25 ± 7.1	17.5 ± 3.5	22.5 ± 7.1	17.5 ± 3.5	20 ± 0.0	7.5 ± 3.5	10 ± 0.0

*Minimum inhibitory concentration
**Minimum bactericidal concentration

Table 3: Antibacterial activity of nine selected honeys against three Gram-positive bacteria and an Acid-fast bacterium

Honey	*M. pheli*		*S. aureus*		*B subtilis*		*St. pyogenes*	
	MIC*	MBC**	MIC	MBC	MIC	MBC	MIC	MBC
Taify	10 ± 0.0	12.5 ± 3.5	7.5 ± 3.5	7.5 ± 3.5	10 ± 0.0	12.5 ± 3.5	7.5 ± 3.5	10 ± 0.0
Shokah	7.5 ± 3.5	10 ± 0.0	5 ± 0.0	7.5 ± 3.5	10 ± 0.0	7.5 ±	7.5 ± 3.5	10 ± 0.0
Somrah	15 ± 0.0	17.5 ± 3.5	10 ± 0.0	15 ± 0.0	10 ± 0.0	12.5 ± 3.5	10 ± 0.0	12.5 ± 3.5
Yemeni	7.5 ± 3.5	15 ± 7.5	5 ± 0.0	7.5 ± 3.5	10 ± 0.0	10 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Kashmiri	12.5 ± 3.5	17.5 ± 3.5	10 ± 0.0	15 ± 0.0	10 ± 0.0	10 ± 0.0	7.5 ± 3.5	12.5 ± 3.5
Black Seed	7.5 ± 3.5	7.5 ± 3.5	10 ± 0.0	10 ± 0.0	10 ± 0.0	7.5 ± 3.5	7.5 ± 3.5	10 ± 0.0
Clover	15 ± 0.0	20 ± 7.5	15 ± 0.0	15 ± 0.0	15 ± 0.0	25 ± 7.5	10 ± 0.0	15 ± 0.0
Black Forest	12.5 ± 3.5	15 ± 0.0	12.5 ± 3.5	15 ± 0.0	7.5 ± 3.5	12.5 ± 3.5	5 ± 0.0	10 ± 0.0
Manuka	15 ± 0.0	22.5 ±	7.5 ± 3.5	20 ± 0.0	12.5 ±	15 ± 0.0	5 ± 0.0	10 ± 0.0

*Minimum inhibitory concentration
**Minimum bactericidal concentration

Discussion:

The MIC and MBC of Yemeni Sidr, Taify Sidr, Kashmiri Sidr, Shaoka, Somra, Manuka, Black Seed, Black Forest, and Clover honeys against 5 Gram-negative bacteria (*E. coli*, *Sh. flexneri*, *Sl. Enterica* serovar *typhimurium*, *K. pneumoniae*, *P. aeruginosa*), 3 Gram-positive bacteria (*S. aureus*, *St. pyogenes*), a food spoilage Gram-positive bacterium (*B. subtilis*) and an acid-fast bacterium (*M. phlei*) were evaluated.

While the MIC for honeys against the tested bacteria ranged between 5 and 20 % w/v, the MBC ranged between 5 and 30 % w/v. Generally speaking, Gram-positive bacteria were more sensitive than Gram-negative bacteria. Amongst the tested Gram-negative bacteria *P. aeruginosa* was the most sensitive and *K. pneumoniae* was the least sensitive. The significant sensitivity of *P. aeruginosa* to honeys compared to other gram-negative bacteria is interesting because this organism is known to be intrinsically resistant to antimicrobials like antibiotics, preservatives and disinfectants (Giamarellou, 2001; Harris, 1999). *St. pyogenes* was the most sensitive Gram-positive bacterium and *M. phlei* was intermediate in its susceptibility to honeys between that of Gram-positive and Gram-negative bacteria.

The concentration required to inhibit the growth of the spore forming *B. subtilis* ranged between 7.5 and 12.5. This result agreement with other studies shown that a heat-resistant spoilage bacterium as *B. stearothermophilis* were highly sensitive to honey (Natarajan, 2001).

Yemeni Sidr, Taify Sidr, Shaoka, and Manuka honeys were relatively more powerful antibacterials than other honeys. Shaoka had lower MIC and MBC against Gram-negative bacteria and Yemeni Sidr had lower MIC and MBC against Gram-positive bacteria. Peroxide activity in Taify Sidr, Yemeni Sidr and Shaoka honeys ranges between 8.3 and 15.6 (Halawani, 2011), therefore, the main antibacterial factor in these honeys is their phytochemicals. Identification of antimicrobial phytochemicals in honeys has gained the interest of several research workers (Adams, 2008; Atrott, 2009). It would be interesting to identify the antibacterial phytochemicals in Taify Sidr, Yemeni Sidr and Shaoka honeys.

E. coli, *K. pneumoniae*, and *Sh. flexneri* used in this study were resistant to several antibiotics (Data not shown), therefore, our data are in agreement with other studies which demonstrated the activity of different honeys against clinical bacteria isolates regardless to their resistance to antibiotics (Hsu, 2005; Allen, 2000; Natarajan, 2001).

Vegetative cells of *B. subtilis* , which is a food spoilage bacterium was inhibited and killed at 7.5-15 % (w/v) honey, therefore, the tested honeys could be used to preserve food from spoilage (Mundo, 2004). Peroxides are known to have sporicidal activity (Turner, 1983). Honeys used in this study had levels of peroxide (Halawani, 2011), therefore, we tested them for possible sporicidal activity. None of the investigated honeys affected the number of spores of *B. subtilis* after, 2-24 h. This suggests that neither, the amount of peroxide produced nor the phytochemicals present in the investigated honeys were effective in eradication of spores. This explains the presence of bacterial spores of *Bacillus* and *Chlostridium* species as contaminants of honeys (Iurlina, 2005).

It may be concluded from this study that Shaoka and Sidr honeys available in Saudi market are potent antibacterial against pathogenic and food spoilage bacteria. This suggests that these tested honeys could be used for treatment of local bacterial infections and for preservation of food from spoilage.

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REFERENCES

Allen, K.L., P.C. Molan and G.M. Reid, 1991. A survey of the antibacterial activity of some New Zealand honeys. J. Pharm Pharmacol. 43: 817-22.

Adams, C.J., C.H. Boult, B.J. Deadman, J.M. Farr, M.N. Grainger, M. Manley-Harris and M.J. Snow, 2008. Isolation by HPLC and characterization of the bioactive fraction of New Zealand Manuka *(Leptospermum scoparium*) honey. *Carbohydrate Res*., 343: 651–659.

Atrott, J. and T. Henle, 2009. Methylglyoxal in Manuka Honey: Correlation with Antibacterial Properties, *Czech J Food Sci*., 27: S163-S165.

Al-Somai N., K.E. Coley, P.C. Molan and B.M. Hancock, 1994. Susceptibility of Helicobacter pylori to the Antibacterial Activity of Manuka Honey. *J Royal Soc Med*., 87: 9-12.

Ali, A.T., M.N. Chowdhury and M.S. Al-Humayyd, 1991. Inhibitory effect of natural honey on *Helicobacter pylori. Tropical Gastroenterol*. 12: 139-143.

Allen, K.L., G. Hutchinson and P.C. Molan, 2000. The potential for using honey to treat wounds infected with MRSA and VRE. First World World Healing Congress, Melbourne, Australia.

Bunting, C.M., 2001. The production of hydrogen peroxide by honey and its relevance to wound healing. MSc thesis. University of Waikato.

Cooper R.A.., P.C. Molan, and K.G. Harding, 2002. The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol 93: 857-863.

Chute, R.K., N.G. Deogade and M. Kawale, 2010. Antimicrobial activity of Indian honey against clinical Isolates. Asiatic J. Biotech. Res. 1: 35-38.

Cooper, R., 2008. Using honey to inhibit wound pathogens. Nurs Times. 104(3): 46-49.

Dustmann, J.H., 1979. Antibacterial Effect of Honey. *Apiacta* 14: 7-11.

Effem, S.E., 1988. Clinical Observations on the Wound Healing Properties of Honey. *Br J Surg* . 75**:** 679-681.

Frankel, S., G.E. Robinson, and M.R. Berenbaum, 1998. Antioxidant capacity and correlated characteristics of 14 unifloral honeys. J Apic Res. 37: 27-31.

Fakoor, M. and M.H. Pipelzadeh, 2007. A study on the healing effect of honey on infected open fracture wounds. *Pak. J. Med. Sci*. 23: 327-329.
Pathogenic Bacteria. *Arch Med Res*. 36: 464-467.

George, N.M. and K.F. Cutting, 2007. Antibacterial Honey (Medihoney): in-vitro Activity Against Clinical Isolates of MRSA, VRE, and Other Multiresistant Gram-negative Organisms Including Pseudomonas aeruginosa. Wounds 19:231-236.

Giamarellou, H. and A. Antoniadou, 2001. Antipseudomonal antibiotics. *Med. Clinics North America*. 85: 19-41.

Haffejee, I.E. and A. Moosa, 1985. Honey in the Treatment of Infantile Gastroenteritis. *Br. Med. J.* 290**:** 1866-1867.

Halawani, E.M.A. and M.M. Shohayeb, 2011. Survey of the antibacterial activity of Saudi and some international honeys(under publication).

Harris, A., C. Torres-Viera, L. Venkataraman, P. DeGirolami, M. Samore and Y. Carmeli, 1999. Epidemiology and clinical outcomes of patients with multiresistant *P. aeruginosa*. *Clinl Infect Dis*. 28: 1128-33.

Hsu, D.I., M.P. Okamoto, R. Murthy and A. Wong-Beringe, 2005. Fluoroquinolone-resistant *P. aeruginosa*: risk factors for acquisition and impact on outcomes *J Antimicrob Chemother*. 55: 535-541.

Iurlina, M.O. and R. Fritz, 2005.Characterization of microorganisms in Argentinean honeys from different sources. *International J Food Microbiol*. 15: 297-304.

Jeddar, A., A. Kharsany, U.G. Ramsaroop, A. Bhamjee, I.E. Haffejee and A. Moosa, 1985. The antibacterial action of honey; an in vitro study. *South Afr Med J*., 67: 257-258.

Jeffrey, A.E. and C.M. Echazarreta, 1996. Medical uses of honey. *Rev Biomed*. 7: 43-39.

Krell, R., 1996. Value-added products from beekeeping. FAO Agric. Sev. Bull., No 124.Retrived from: htt/www.fao.org.

Kwakman, P., te A. Velde, L. de Boer, D. Speijer, C. Vandenbroucke-Grauls and S. Zaat, 2010. How honey kills bacteria FASEB J. 24: 2576-2582.

Kandil, A., S. El-Banby, G.K. Abdel-Wahed, M. Abdel-Gawwad and Fayez M., 1987. Curative properties of true floral and false non-floral honeys on induced gastric ulcers. *J Drug Res* . 17**:** 103-106.

Lusby, P.E., A.L. Coombes and J.M. Wilkinson, 2005. Bactericidal Activity of Different Honeys against

Molan, P.C., 1992. The Antibacterial Activity of Honey. 1. The Nature of the Antibacterial Activity. *Bee World* 73: 5-28.

Molan, P.C., 1992. The Antibacterial Activity of Honey. 2. Variation in the Potency of the Antibacterial Activity. *Bee World*, 73: 59-76.

Majno, G., 1975. The Healing Hand. Man and Wound in the Ancient World. Harvard University Press Cambridge, Massachusetts.

McInerney R. J. 1990. Honey - a Remedy Rediscovered. *J Royal Soc Med*. 83: 127-130.

Mundo, M.A., O.I. Padillla-Zakour and R.W. Worobo, 2004. Growth inhibition of foodbone pathogens and food spoilage organisms by select raw honeys. *International J Food Microbiol*. 97: 1-8.

Molan, P.C., 2001. Potential of honey in the treatment of wounds and burns. American J Clin Dermatol. 2: 13-16.

Natarajan, S., D.J. Williamson,Grey, K.G. Harding and R.A. Cooper, 2001. Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. *J Dermatol Treatment*. 12: 33-36.

Smith, T., K. Legel and J.R. Hanft, 2009. Topical leptospermum Honey (Medihoney) in recalcitrant venous leg wounds: A preliminary case series. Advances Skin Wound Care. 22: 68-71.

Subrahmanyam M., Hemmady A. and Pawar SG .2001. Antibacterial activity of honey on bacteria isolated from wounds. Ann Burns Fire Disasters. 14: 198-201.

Tumin, N., N.A. Halim, M. Shahjahan, N.J. NoorIzani, M.A. Sattar, A.H. Khan and S.S.J. Mohsin, 2005. Antibacterial activity of local Malaysian honey, Malay J Pharmaceutical Sci, 3:1-10.

Turner, F.J., 1983. Hydrogen Peroxide and Other Oxidant Disinfectants , 3rd ed. Philadelphia: Lea and Febiger Publishers.

Wahdan, H., 1998. Causes of the antimicrobial activity of honey. Infection 26: 30-35.

Wilkinson, J.M. and M.A. Cavanagh, 2005. Antibacterial activity of 13 honeys against Escherichia coli and Pseudomonas aeruginosa. J Med Food. 8: 100-103.

Yoirish, N., 1977. Curative properties of honey and bee venom,1th edn. San Francisco :New Glide Publication.

Zumla, A. and A. Lulat, 1989. Honey: Remedy Rediscovered. J Royal Soc Med., 82: 384-385.