

Full Length Research Paper

Evaluation of *in-vitro* inhibitory effect of honey on some microbial isolate

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The aim of this study was to investigate the antimicrobial activity of honey sample from Basrah region against certain microbial isolate. Different concentrations (25.0, 50.0, 75.0 and 100.0%) of honey sample were checked for their antimicrobial activities, using some medically important micro-organisms including *Escherichia coli*, *Pseudomonas* spp. and *Staphylococcus aureus*. The minimum inhibitory concentrations (MIC) of the honey sample were determined on the selected micro-organisms by using broth dilution technique. The sample of honey show inhibitory effect *in vitro* at 50, 75 and 100% concentration on the various investigated micro-organism except at 50% concentration where no inhibition zone on *S. aureus*. However, no effect was observed at 25% concentration. The MIC for *E. coli*, *Pseudomonas* spp. and *S. aureus* were 6.25, 1.5 and 12.5 mg/ml respectively. The study shows that honey, like antibiotics, has certain organisms sensitive to it, and provides alternative therapy against certain bacteria and is also shown to have antimicrobial action against a broad spectrum of bacteria (both gram- positive and -negative bacteria).

Key words: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, honey, antibiotics, sensitivity, antimicrobial.

INTRODUCTION

The antibacterial activity of honey was first recognized in 1892, by van Ketel (Dustmann, 1979). Honey is produced from many sources and its antimicrobial activity varies greatly with origin and processing (Molan, 1992). Honey has been used as a medicine in many cultures for a long time (Quinn et al., 1994). It has been rediscovered by the medical profession and it is gaining acceptance as an antibacterial treatment of topical infections resulting from burns and wounds (Abuharfeil et al., 1999). Numerous studies demonstrate that honey possesses antimicrobial activity (Dustmann, 1979; Molan, 1992). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positives and gram-negatives (Molan, 1992), it destroys and/or inhibits the growth of some pathogenic vegetative micro-organisms (Chick and Shin, 2001). An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium* (Quinn et al., 1994), as well as all the common dermatophytes (Brady et al., 1997).

Honey possesses inherent antimicrobial properties, some of which are due to high osmotic pressure/low

water activity, in which the low water activity of honey is inhibitory to the growth of the majority of bacteria and to many yeasts and moulds. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth. Even when diluted with water absorbed from wounds, honeys would be likely to retain a water activity sufficiently low to inhibit growth of most bacteria. Honey is mildly acidic, with a pH between 3.2 and 4.5, gluconic acid is formed in honey when bees secrete the enzyme glucose oxidase, which catalyses the oxidation of glucose to gluconic acid, the low pH alone is inhibitory to many pathogenic bacteria and, in topical applications at least, could be sufficient to exert an inhibitory effect (Molan, 1995).

Hydrogen peroxide, the end product of the glucose oxidase system and tetracycline derivatives has the antibacterial properties against pathogens (Snowdon and Cliver, 1996). Low concentrations of this known antiseptic are effective against infectious bacteria and can play a role in the wound healing mechanism (Molan, 2001) and in stimulation and proliferation of peripheral blood

lymphocytic and phagocytic activity (Tonks et al., 2001). Other factors, such as low protein content, high carbon to nitrogen ratio, low redox potential due to the high content of reducing sugars, viscosity/anaerobic environment and other chemical agents/phytochemicals are also likely to play some role in defining antibacterial activity of honey (Honey, 2002). Furthermore, honey has been employed to shorten the duration of diarrhea in patients with bactericidal gastro-enteritis due to bacterial infection (Haffejee and Moosa, 1985). However, honey has other important beneficial characteristics that are less influenced by storage conditions (Cooper et al., 2002).

MATERIAL AND METHODS

Honey samples

The honey sample used in this study was collected from Basrah province / Iraq, (Almuttia region); it was collected in sterile container and checked for purity on blood agar plate by streaked on blood agar plate, and incubated overnight. The honey sample was diluted by physiological saline to 25.0, 50.0, 75% and the non diluted honey (100.0%) referred to as neat. The study done in Al Sader teaching hospital/College of medicine, it was carried out during the period from January, 2009 to April, 2009.

Microorganisms

S. aureus, *E. coli* and *Pseudomonas* spp. were obtained from the Al Sader teaching hospital laboratory as clinical isolates and maintained in blood and Macconkey media and sub cultured in Müller Hinton media.

Antimicrobial susceptibility testing

The disc diffusion technique was used as previously described by Dustmann (Dustmann, 1979) using different types of antimicrobials. All isolates were inoculated into Müller-Hinton broth (in 10 ml) and incubated for 18 - 24 h; the density was then adjusted to 10^5 CFU/ml with sterile saline solution.

Microbiological tests

Preparation of honey suspensions for the disc diffusion test

The disc diffusion test was carried out as described by Mirsa, Wamota and Helms et al. (2002). Eight millimetre diameter-filter paper was saturated with 0.1 ml of each of the honey suspensions. The density of the isolates was the same as that used in the antimicrobial susceptibility testing of the various chemotherapeutic agents. All the tests were performed in triplicate.

Minimum inhibitory concentration (Broth dilution method) against the isolated organisms

The broth dilution technique was used to ascertain the minimum inhibitory concentration (MIC) of the honey samples. The test was carried out as described by Heuvelink et al. (1998) A suspension of the organism was adjusted to 1.5×10^5 organism/ml and further diluted to 1:200 in Müller Hinton broth. Five millilitres each of Müller

Hinton broth was pipetted into ten sterile screws capped test tubes. A weight of 100 mg/ml of the honey was dissolved completely in the first tube. A serial dilution of honey, with a dilution factor of half was established. Tube number 10 served as a positive growth control containing Müller Hinton broth and bacterial inoculum only, and an additional tube containing broth only was used as a negative control. A volume of 0.1 ml of the bacterial suspension (7.5×10^5 organism/ml) was added to each tube. The tubes were incubated at 37°C for 18 h and visually examined for evidence of turbidity. The lowest concentration of honey in the series that inhibited the growth of the organism was taken to be the MIC, expressed in mg/ml.

RESULT

Honey sample showed marked inhibition of growth on *Pseudomonas* spp., the maximum inhibition zone was shown at concentration of 100% as 23 mm, which reduce to 10 mm at 75% and 8 mm at 50% concentration (Table 1). Also the table showed that *E. coli* grow with inhibition zone at concentration of 100% as 22 mm, and the inhibition zone reduce to 12 mm at 75% and 8 mm at 50% concentration. *S. aureus* showed a little less inhibition zone with honey sample. These were 20 mm at 100% and 11 mm at 75% concentration, however, no effect was observed at 25% concentration (Table 1).

Table 2 shows the zone of inhibition on the net concentration of honey that produced a greater inhibition than tetracycline and gentamicin on *Pseudomonas* spp. (23, 0 and 16 mm respectively), and on *E. coli* (22, 18, 20 mm respectively), Except for *S. aureus*, where the tetracycline produced similar inhibition of honey 20 and 18 mm on gentamicin.

Studies on the minimum inhibitory concentration (MIC) of the honey on the test organisms showed that the low MIC were demonstrated against *Pseudomonas* spp. (1.5 mg/ml) and the low MIC was exhibited against *S. aureus* (12.5 mg/ml), while the MIC for *E. coli* was equal to 6.25 mg/ml (Table 3).

DISCUSSION

This study was undertaken to investigate *in vitro* antimicrobial activity of honey against certain microbial isolates. In the study, honey sample showed the antimicrobial activity and our result were in agreement with Wi11ix et al. (1992) who found that honey inhibited the growth of *S. aureus*, *E. coli* and *Pseudomonas* sp. and also in agreement with Bilal et al. (1998) who found honey exhibited a fairly good antimicrobial activity against both Gram-negative and -positive bacteria and a remarkable activity was observed with *P. aeruginosa* and *S. aureus*.

The study show antimicrobial activity against *S. aureus*, and this result is in agreement with Molan (1992a) who found the *S. aureus*, as one of the bacterial species most susceptible to the antibacterial activity of honey. These might be due to the osmotic effect, the effect of pH and the sensitivity of these organisms to hydrogen peroxide,

Table 1. Antibacterial activities of different concentrations of honey against microbial isolate.

Inhibition zone (diameter in mm)			
Concentrations % (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>P. spp.</i>
100	20	22	23
75	11	12	10
5	0	8	8
25	0	0	0

$\chi^2 = 1.8$; $df = 2$; $P > 0.05$.

Table 2. Antibacterial activities of net honey against certain microbial isolate compared with Gentamicin and Tetracycline.

Inhibition zone (diameter in mm)			
Organisms	Honey 100%	Gentamicin	Tetracycline
<i>S. aureus</i>	20	18	20
<i>E. coli</i>	22	20	18
<i>P. spp.</i>	23	16	0

$\chi^2 = 7.24$; $df = 2$; $P < 0.01$.

Table 3. The minimum inhibitory concentration (MIC) of honey on the test organisms.

Organisms	Minimum inhibitory concentration (MIC) (mg/ml)
<i>S. aureus</i>	12.5
<i>E. coli</i>	6.25
<i>P. spp.</i>	1.5

which represented an 'inhibine', factor in honey (Postmes et al., 1993).

The potency of neat honey (100% concentration) was found to be superior against all bacteria tested, and the best antimicrobial activity of honey occurs with *Pseudomonas* sp. followed by *E. coli*, these result of the study is in agreement with Adeleke et al. (2005) where it shows an evident in the percentage levels of bacterial sensitivity; as high as 100% for *P. aeruginosa* and 96.4% for *E. coli*. Also of interest is the finding that the activity of gentamicin, both 4.0 and 8.0 $\mu\text{g/ml}$, was found to be virtually lower than that of undiluted honey or any of its aq. dilutions.

And these result is in agreement with Abd-el et al. (2007) who showed that honey have a greater inhibitory effect on isolated gram-negative bacteria (*P. aeruginosa*, *Enterobacter* spp. and *Klebsiella*). Also El-Sukhon et al. (1994) showed gram negative bacteria to be more sensitive to action of honey than Gram-positive bacteria. Moundoi et al. (2001) discovered that the antimicrobial activity of honey was more with *Pseudomonas* and *Acinetobacter* spp, both with resistance to some

antibiotics like gentamicin, Ceftriazone, Amikacin and Tobramicin than other bacteria tested.

Also in agreement with Subrahmanyam et al. (2001) who showed that strains of *P. aeruginosa* resistant to routinely used and higher antibiotics were sensitive to the antibacterial action of honey.

Taormina et al. (2001) studied the antimicrobial effect of honey on gram negative bacteria and attributed it to the presence of factors as high content of tetracycline derivatives, hydrogen peroxide and powerful antioxidants, as also to a naturally low pH, which is unsuitable for bacterial growth and to the presence of phenolic acids, lysozyme and flavanoids.

The demonstration of MIC shows that the most susceptible micro-organisms to the honey are *Pseudomonas* spp. Cooper (1999) has reported that manuka honey had MIC of less than 10% against 17 strains of *P. aeruginosa* from infected wounds, and honeys which have a MIC of 10 - 20%, can be expected to be effective in preventing growth of *Pseudomonas*, followed by *E. coli* and *S. aureus* and these result is in agreement with Willix et al. (1992) who found the MIC

(minimum inhibitory concentration) of the honeys to ranged from 1.8 - 10.8% (v/v), indicating that the honeys had sufficient antibacterial potency to stop bacterial growth if diluted at least nine times and up to 56 times in the presence of *S. aureus*.

The high antibacterial effect of honey sample in the disc diffusion test and the low MIC may be attributable to the presence of glucose oxidase, which is activated by dilution in water resulting in the production of hydrogen peroxide which is toxic to bacteria (Stinson et al., 1960).

REFERENCES

- Abd-El Aal AM, El-Hadidy MR, El-Mashad NB, El-Sebaie AH (2007). Antimicrobial effect of bee honey in comparison to antibiotic on organisms isolated from infected burns. *Annals of Burns and Fire Disasters* 2.
- Abuharfeil N, Al-Oran R, Abo-Dhehada M (1999). The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytosis. *Food Agric. Immunol.* 11: 169-177.
- Adeleke OE, Olaitan JO, Okpeke EI (2005). Comparative antibacterial activity of honey and gentamicin against *E. coli* and *Pseudomonas aeruginosa*. *Annals of Burns and Fire Disasters*: (ISSN 1592-9566).
- Bilal AN, Molan PC, Sallal AK (1998). Antimicrobial activity of honey on selected microorganisms: A preliminary study. *Biomed. Res. (India)*. 9: 51-54.
- Brady NF, Molan PC, Harfoot CG (1997). The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Pharm. Sci.* 2: 1-3.
- Chick HHS, Shin Z (2001). Ustunol, Growth and acid production by lactic acid bacteria and bifidobacteria grown in skim milk containing honey., *J. Food Sci.* 66: 478-481.
- Cooper R (1999). The use of honey as an antiseptic in managing *Pseudomonas* infections. *J. Wound Care.* 8: 161-164.
- Cooper RA, Molan PC, Harding KG (2002). The sensitivity to honey of gram-positive cocci of clinical significance isolated from wounds. *J. appl. Microbiol.* 93(5): 857-863.
- Dustmann JH (1979). Antibacterial effect of honey. *Apiacta.* 14(1): 7-11.
- El-Sukhon SN, Abu-Harfeil N, Sallal AK (1994). Effects of honey on Bacterial Growth and Spore Germination. *J. Food Prot.* 57(10): 918-920.
- Haffejee IE, Moosa A (1985). Honey in the treatment of infantile gastroenteritis. *Br. Med. J.* 290: 1866-1867.
- Helms M, Vastrup P, Gerner-Smidt P, Molbak K (2002). Excess mortality associated with antimicrobial drug resistant *S. typhimurium*. *Emerg. infect. Dis.* 8(15), 490-495.
- Heunvelink AE, van den Biggelaar FL, Zwartkruis-Nahuis JTM, Herbes RG, Huyben R, Nagelkerke N, Melchers WJ, Monnens LA, de Boer E (1998). Occurrence of verocytotoxin-producing *E. coli* O157 on Dutch dairy farms. *J. Clin. Microbiol.* 36(12): 3480-3487.
- Honey – Health and Therapeutic Qualities, The National Honey Board, Longmont, CO, USA (2002) (<http://www.nhb.org>)
- Molan P (1992a). The antibacterial activity of honey 1. The nature of the antibacterial activity. *Bee World.* 1: 5-28.
- Molan PC (1992). The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee World* 73(1): 5-28.
- Molan PC (1992). The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World* 73:59-76.
- Molan PC (1995). The antibacterial properties of honey. *Chem in NZ*, pp 10 – 14.
- Molan PC (2001). Why honey is effective as a medicine. 2. The scientific explanation of its effects. *Bee World* 82(1): 22-40.
- Moundoi MA, Padila-Zakour OI, Worobo RW (2001). Antimicrobial activity of honey against food pathogens and food spoilage microorganisms. *New York State Agricultural Experiment Station.* 1: 61-71.
- Postmes T, Van den Bogaard AE, Hazen M (1993). Honey for wounds, ulcers and skin graft preservation. *Lancet.* 341: 756-757.
- Quinn PJ, Carter ME, Markey BK, Carter GR (1994). Enterobacteriaceae. In *Clinical veterinary microbiology*. Wolfe Publishing, an imprint of Mosby-Year Book Europe Ltd. London. pp. 109-135.
- Snowdon JA, Cliver DO (1996). Microorganisms in honey. *International J. Food Microbiol.* 31: 1-26.
- Stinson EE, Subers MH, Petty J, White JW (1960). The composition of honey. V. Separation and identification of the organic acids. *Arch. Biochem. Biophys.* 89: 6-12.
- Subrahmanyam M, Hemmady AR, Pawar SG (2001). Antibacterial activity of honey on bacteria isolated from wounds. *Ann. Burns Fire Disasters.* 14(1):198-201.
- Taormina PJ, Niemira BA, Beuchat LR (2001). Inhibitory activity of honey against food-borne pathogens as influenced by the presence of hydrogen peroxide (H₂O₂) and level of antioxidant power. *Int. J. Food Microbiol.* 69: 217-25.
- Tonks A, Cooper RA, Price AJ, Molan PC, Jones KP (2001). Stimulation of TNF-alpha release in monocytes by honey. *Cytokine.* 14: 240-242.
- Willix DJ, Molan PC, Harfoot CG (1992). A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. *J. Appl. Bacteriol.* 73(5): 388-394.
- Willix DJ, Molan PC, Harfoot CG (1992). A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *J. Appl. Bacteriol.* 73(5): 388-94.

