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Evaluating Antibacterial Activity of the Iranian Honey Through MIC Method on Some Dermal and Intestinal Pathogenic Bacteria

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Abstract: In this study, the collected multi floral honey from bee colonies in province of East Azerbaijan (Iran) was prepared the doubling dilution and impregnated on paper disks. These disks were used on bacterial cultured media and their antibacterial effects were assessed by disk diffusion test method. The selected bacteria were provided through the standard strains, in addition to that some of the clinical samples were also selected and the taken subsequences were compared with the standard strains. The positive extracts in the disk diffusion method, were selected and used in MIC (Minimum Inhibitory Concentration) method on the specific bacteria. The MBC (Minimum Bactericidal Concentration) method as a complimentary test also was performed on them. In the above mentioned tests, control (untreated) disks were also considered. The results showed that the obtained extracts of Iranian multi floral honey (from the bee colonies of East Azerbaijan Province) have broad spectrum antibacterial activities, although their efficacy showed diversity on different bacterial species.

Key words: Multi floral honey, antibacterial, MIC method, MBC method

INTRODUCTION

Antibacterial activity is present in all higher organisms and some foods to protect against their natural flora of bacteria. Antimicrobial agents include those derived from animals [enzymes such as lysozyme and lactoperoxidase, lactoferrin (protein), histatin (small peptide) and the immune system], those derived from plants (organic acids, essential oils, phenolic compounds, phytoalexins) and those derived from microorganisms (bacteriocins). The effectiveness of antimicrobial agents depends on some factors like the effect of pH or acid dissociation and the specific effect of the antimicrobial agent. There are other factors that can influence antimicrobial activity, including water activity, moisture content, temperature, osmotic pressure and composition of the food, as well as the presence of essential nutrients for growth (Garcia *et al*., 2001).

Honey, whose medicinal uses date from ancient times, has lately been rediscovered as a therapy for wounds (Zumla and Lulat 1989; Molan, 1998). Many publications attest to honey's antimicrobial properties and its use has been successful as a surgical dressing for open wounds, burns and septic infections (Green, 1988). Honey is a supersaturated solution of sugars and presents low pH and low water activity (Garcia *et al*., 2001). It is produced from many sources and its antimicrobial activity varies greatly with origin and processing (Zumla and Lulat, 1989). The antibacterial activity of honey was attributed to the high osmolarity resulting from the sugar of honey applied to the lesion (Green, 1988). But, there are other factors in honey that prevent the growth of microorganisms and show antibacterial activity. Reports vary considerably on the exact nature of the factors responsible for these properties. The 1st report, back in 1937, described the antibacterial effect of honey as “inhibine” (Dold *et al*., 1937). This factor was enzyme-produced hydrogen peroxide and was sensitive to heat and light. It was later shown that the inhibine principle was resultant from the accumulation of hydrogen peroxide produced by the glucose oxidase system. Other authors found antibacterial activity even in heated, sterilized honey and it was reported that antibacterial stability to heat was lowest at pH 7 (Gonnet and Lavie, 1960). Antibacterial activity covers susceptible genera including Gram positive and

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Gram negative bacteria (Ialomiteanu and Daghie, 1973). Antibacterial compounds are introduced by bees during the collection of material and the ripening process. Another nonperoxide activity comes from nectar source (Molan and Russel, 1988).

A study by Willix *et al*. (1992) did specify the type of honey. They determined the sensitivity of wound pathogens to the non-peroxide antibacterial activity of standardized manuka honey and to a standardized honey in which the antibacterial activity was primarily due to hydrogen peroxide.

Essential oils from different plants have been recently assayed for activity against strains of bacteria, fungi, virus and cytotoxic activity (Yang *et al*., 1995; Taylor *et al*., 1996) and it has been observed that methyllin-resistant *Staphylococcus aureus* (MRSA) is inhibited by hydroxyflavanones (Taylor *et al*., 1996) and flavanostilbenes (Sato *et al*., 1995). Not all plants have the same antibacterial components and this could explain the different antibacterial activity for each honey type (Garcia *et al*., 2001). Flavonoids, present in honey and propolis, have an antibacterial effect. These compounds are photoresistant and thermoresistant. It was also an isolated component in honey, showing a chemical behavior similar to lysozyme and with antibacterial properties (Mohrig and Messner, 1968).

The aim of this research was to study the inhibition of some certain strains of bacteria by honey and we have also evaluated honey bactericidal activity on some gram positive and gram negative bacteria that have dermatological and gastrointestinal pathogenic property for human and animals.

MATERIALS AND METHODS

The Province of East Azerbaijan in North West of Iran with 650 floral diversity (medicinal herbs and plants) is appropriate for our studies. So we have tried to study the antibacterial activities of the honeys harvested from this region with its unique floral ecosystem. And also we have investigated the effects of plant diversity in the region on the honey efficacy (the effect of multi floral honeys) on their antibacterial potentials.

A piece of fresh honey comb was sampled from each experimental bee colony and sent to the laboratory of the Islamic Azad University in Shabestar, E. Azerbaijan Provence. The honey were then extracted from these combs and homogenized for further studies. The specimens were stored in the refrigerator. Honey solutions are prepared from honey sample and distillate water in 20, 40, 60 and 80%.

Table 1: Microorganisms used in the experiment

Microorganism	Code
Staphylococcus aureus	(PTCC, 1431)
Bacillus cereus	(PTCC, 1247)
Proteus vulgaris	(PTCC, 1079)
Micrococcus sp.	Clinical sample
Pasturella multicidae	Clinical sample
Bacillus anthracis	Vaccinal strain

Microorganisms: In this study, we evaluated the antibacterial effects of honey on a spectrum of dermatological and gastrointestinal pathogenic bacteria. For this purpose, we purchased some certain strains of bacteria from Organization of Scientific and Industrial Researches of Iran (OSIRI), such as, *Staphylococcus aureus* (PTCC, 1431), *Bacillus cereus* (PTCC, 1247) and *Proteus vulgaris* (PTCC, 1079).

In addition to the above list, we added some isolated bacteria from animal pathological samples such as, *Pasteurella multicida*, *Micrococcus* sp. Additionally we assessed the antibacterial effects of honey on the "Bacillus anthracis Vaccine (Stern strain)", which produced by "Institute of Research and Vaccine Manufacture of Razi". Also, for completion of the test, besides the above bacteria, other pathogenic and nonpathogenic strains were studied (Table 1).

For performing the tests, we prepared a suspension containing 10 powered 6 CFU mL^{-1} from the 24 h culture of each strain. This concentration was according to the test tube number 0.5 of McFarland tubes. The suspension was prepared and used in the sterile saline solution.

Evaluation of antibacterial effects: The antibacterial effect of pure honey was assessed separately in every 4 concentrations of 20, 40, 60 and 80%, on each above mentioned strains of bacteria. At first, the antibacterial effects of each extracts were studied by disc and gel diffusion methods on the available bacteria. The positive cases underwent the MIC test and finally, analyzed by MBC method. We considered one negative control for each dilution. All tests were duplicated.

Disc diffusion method: We prepared the plates containing 20 mL of sterile agar Mueller-Hinton media (from HI Media) and the surface of media were subcultured by swabs from the bacterial suspensions. Then, the different dilutions of honey extracts were transferred onto the discs and the discs prepared by this way were placed on the culture surface. Because of too high viscosity of pure honey and lack of compete desiccation, preparation of appropriate disc of pure honey was impossible. The diameter of the discs was determined as 6 mm. The media (containing discs) were incubated overnight (for 24 h) in

Table 2: The results of Disc Diffusion Test (mm)

	Honey concentration		
Bacteria	80%	60%	40%
Staphylococcus aureus	8	0	0
Bacillus cereus	-	-	-
Bacillus anthracis	8	6	2
Micrococcus sp.	7	0	0
Pasturella multicidae	8	0	0
Proteus vulgaris	-	-	-

Table 3: The results of Gel Diffusion Test (mm)

	Honey concentration			
Bacteria	100%	80%	60%	40%
Bacillus anthracis	20	11	11	5
Proteus vulgaris	25	22	22	8
Bacillus cereus	11	7	0	0
Pasturella multicidae	16	5	0	0
Staphylococcus aureus	21.5	17	11	0

37°C. After this period of 24 h, the clear surrounding zone of growth inhibition was measured by Collis in the 4 concentrations for all strains. The observed results were recorded. A control was considered for every specimen (Table 2).

Gel diffusion method: We prepared the plates containing 20 mL of sterile agar Mueller-Hinton media (from HI Media) and we made a hole with a diameter of 6 mm on the gel surface. The media surface was cultured by swabs from bacterial suspensions and then from the four concentrations of the honey (20, 40, 60 and 80%) was separately, placed into the holes. Again, it was impossible to transferring the pure honey onto the wells. Then these media containing the honey extract were incubated in 37°C for 24 h. After this period of 24 h, the clear surrounding zone of growth inhibition was measured by Collis in the 4 concentrations of all strains. The observed results were recorded. A negative control was considered for every specimen.

Preparation of serial dilutions in test tubes: This method was performed on the bacteria, which had positive results in the both above tests. The serial dilutions were prepared in the test tubes containing Mueller- Hinton broth medium (purchased from HI Media). The concentrations of 0.8-0.025 g mL^{-1} of honey (net) were added to 0.2-0.975 broth medium respectively. After then each tube received 20 μL of bacterial suspension and after post-homogenizing incubated in 37°C for 24 h. The MIC was considered the minimum concentration, in which the turbidity due to bacterial growth was observed and this value was recorded. According to the MIC, the MBC was determined by transferring the clear specimens on the TSA (Trypticase Soy Agar) culture medium and the results were recorded.

RESULTS AND DISCUSSION

The observed antibacterial activities of the honey extracts on dermal and intestinal pathogenic bacteria were interesting and significant. A wide range of bacteria (Table 1) showed positive results in disc diffusion test (Table 2). The antibacterial responses on bacteria in the gel diffusion were satisfactorily and reasonable. These data are summarized in (Table 3). The resulted ranges for honeys of 40, 60 and 80% were considered as 0-2, 0-6 and 7 and 8 mm, respectively. The results of gel diffusion method were also as 40% (0-8 mm), 60% (0-22 mm) and 80% (8.5-22 mm) and for pure honey it differed in the range of 9.5-25 mm (Table 3). The MIC and MBC test were performed for the bacteria having both positive results of gel and disc diffusion tests. In this experiment, honey in different concentration has good antibacterial effect for *Proteus vulgaris*, *Staph. aureus, B. anthracic*, *B. cereus*, *P. multicida* (Table 4 and 5).

In this study, we tried to focus on the efficacy determination of honey extracts on specially dermal and intestinal pathogenic bacteria. The disc diffusion test revealed that the honey extracts were effective on 4 (from 6) bacterial strains. The results of the 2nd method (gel diffusion) were better than the former, because in the latter the sensitivity was in a high degree and the number of sensitive bacteria increased.

In this experiment, honey in different concentration has good antibacterial effect for *Proteus vulgaris*, *Staph. aureus*, *B. anthracic*, *B. cereus*, *P. multicida* with gel diffusion method. Similar result was previously reported by Brady *et al.* (2004) for *Staphylococcus aureus* and by Jason *et al.* (2003) for *B. cereus*.

Surveying the results of MIC test revealed that 5 bacteria were sensitive to the honey and had positive results. This was effective for *Staph. aureus*, *B. anthracis*, *B. anthracis*, *B. cereus*, *P. multicida*. This result was agreement with recent researches about *Proteus vulgaris* and *Staphylococcus aureus*. It is recommended that in the future studies, some more species of bacteria should be studied.

According to the MBC results, it was demonstrated that 4 from 5 above mentioned bacteria were completely sensitive to the honey. Previous research (Subrahmanyam *et al.*, 2001) about *Proteus vulgaris* and *Staphylococcus aureus* showed same effect.

Table 4: The results of MIC Test

	Honey concentration					
Bacteria	0.8 g/ml	0.4 g/ml	0.2 g/ml	0.1 g/ml	0.05g/ml	0.025 g/ml
Proteus vulgaris	+	-	-	-	-	-
Staphylococcus aureus	-	-	+	-	-	-
Bacillus anthracis	-	-	+	-	-	-
Bacillus cereus	-	-	+	-	-	-
Pasturella multicidae	-	-	+	-	-	-

Table 5: The results of MBC Test

	Honey concentration					
Bacteria	0.8 g/ml	0.4 g/ml	0.2 g/ml	0.1 g/ml	0.05 g/ml	0.025 g/ml
Proteus vulgaris	-	-	-	-	-	-
Staphylococcus aureus	-	+	-	-	-	-
Bacillus anthracis	-	+	-	-	-	-
Bacillus cereus	-	+	-	-	-	-
Pasturella multicidae	-	+	-	-	-	-

CONCLUSION

Our results suggest that, using different concentrations of honey has antibacterial activities for 5 bacteria strains (*Proteus vulgaris*, *Staph. aureus*, *Bacillus anthracis*, *Bacillus cereus*, *Pasturella multicidae*) and the best result belong to 80% concentration of honey.

REFERENCES

Andargarchew Mulul, Belay Tessema and Fetene Derbie, 2004. *In vitro* assessment of the antimicrobial potential of honey on common human pathogens Ethiop. J. Health Dev., pp: 18(2).

Brady, N., P. Molan and L. Bang, 2004. A survey of non-manuka New Zealand honeys for antibacterial and antifungal activities. J. Apicult. Res., 43 (2): 47-52.

Dold H., D.H. Du and S.T. Dziao, 1937. Nachweis antibakterieller, hitze-und lichtempfindliche Hemmungsstoffe (Inhibine) in Naturhonig (Blütenhonig). Zeitschrift fuer Hygiene und Infekiontkrankheiten, 120: 155-167.

Garcia M., C. Perez-Arquillue, A. Herrera, T. Juan, M.I. Juan and A. Herrera, 2001. Food Sci. Technol. Int., 7: 155.

Gonnet, M. and P. Lavie, 1960. Influence du chauffage sur le facteur antibiotique present dans les miels. Annales de l'Abeille, 34: 349-364.

Green, A.E., 1988. Wound healing properties of honey. Br. J. Surg., 75: 1278.

Ialomiteanu M. and V. Daghie, 1973. Investigation of the antibiotic qualities of honey. XXIV Congreso Internacional de Apicultura. Buenos Aires.

Jason H. DeMera and Esther R. Angert, 2003. Comparison of the antimicrobial activity of honey produced by *Tetragonisca angustula* (Meliponinae) and *Apis mellifera* from different phytogeographic regions of Costa Rica. Apidologie, 35: 411-417.

Mohrig, W. and B. Messner, 1968. Lysoxyme als antibakterielles Agens im Bienenhonig und Bienengift. Acta Bilogica and Medica Germanica, 21: 55-95.

Molan, P.C., 1998. A brief review of honey as a clinical dressing. Primary Intention, 6: 148-158.

Molan, P.C. and K.M. Russel, 1988. Non-peroxide antibacterial activity of some New Zealand honeys. J. Apicult. Res., 27 (1): 62-67.

Sato N., Y. Kai, G.I. Shemilt and S. Chiku, 1995. Syntesis of 14C-labelled ceflupenam (E1077), a novel parenteral cephalosporin antibiotic. J. Labelled Compounds and Radiopharmaceuticals, 36 (2): 173-178.

Subrahmanyam, M., Archan Hemmady and S.G. Pawar 2001. Antibacterial activity of honey on bacteria isolated from wounds. Ann. Burns Fire Disasters, 14 (11): 22.

Taylor, R.S.L., F. Edel, N.P. Manandhar and G.H.N. Towers, 1996. Antimicrobial activities of Southern Nepalese medicinal plants. J. Ethnopharmacol., 50 (2): 97-102.

Willix, D.J., P.C. Molan and C.J. Harfoot, 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity ofmanuka honey and other honey. J. Applied Bacteriol., 73: 388-394.

Yang, M.S., Y.L. Ha, S.H. Nam, S.U. Choi and D.S. Jang, 1995. Screening of domestic plants with antibacterial activity. Agric. Chem. Biotechnol., 38 (6): 584-589.

Zumla, A. and A. Lulat, 1989. Honey-a remedy rediscovered. J. R. Soc. Med., 82: 384-385.