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Comparison of Antibacterial Activities of Natural Urmia Honey and Penicillin Derivatives: An *in vitro* Study

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Abstract: Honey is one of the ancient traditional medicines used for treatment of infected wounds and various illnesses. Medical and therapeutic properties of honey were showed to be related to physicochemical and nature of component of it. The aim of this study was to evaluate antimicrobial efficacy of natural Urmia honey against some potentially pathogen bacteria and in comparison with penicillin family antibiotics. Natural honey which used in this study, was obtained from beehives in Urmia (province of West Azerbaijan; Iran) and no additional procedures were performed. *Staphylococcus aureus*, *Salmonella enteritidis* and *Escherichia coli* were the microorganisms were used in this investigation. Results of the study were showed honey induce inhibition zone against *Staphylococcus aureus* (as the most sensitive control organism to honey) were lesser than ampicillin (as the most potent antibiotic against this microorganism). The inhibition zones from honey against gram negative bacteria (*Salmonella typhimurium*, *Escherichia coli*) were higher than ampicillin and other trial antibiotics. According to these results, honey can be considered as a potent natural anti bacterial agent and its efficacy is comparable with penicillin family antibiotics.

Key words: Antibacterial, Urmia, honey, penicillin, antibiotic

INTRODUCTION

Honey has been used in treatment as long ago as 2000 years (Mathews and Binning, 2002). The ancient Egyptians, Assyrians, Chinese, Greeks and Romans all used honey, in combination with other herbs and on its own, to treat infection and non-infection diseases (Zumla and Honey, 1989). There is an increasing usage of honey as a dressing on infected wounds, burns and ulcers (Efem, 1988; Tovey, 2000; Subrahmanyam *et al*., 2001; Mathews and Binning, 2002). The antibacterial action of honey was reported for the first time in the modern medicine in 1892. The different aspects of the antibacterial properties of honey have been recently extensively reviewed (Molan, 1992). There are 2 sorts of antibacterial agents or so called inhibines. One of them is heat and light sensitive and has its origin in the H_2O_2, produced by honey glucose oxidase (White *et al*., 1963; White and Stubers, 1964; Dustmann, 1972). Some Researchers believe that hydrogen peroxide is the main antibacterial agent (White *et al*., 1963; Dustmann, 1979; Morse, 1986). Other authors Have found that the non-peroxide activity is the more important one (Gonnet and Lavie, 1960; Lavie, 1968; Mohrig and Messner, 1968; Bogdanov, 1984; Radwan *et al*., 1984; Roth *et al*., 1986). The non-peroxide antibacterial activity is insensitive to heat and light (Gonnet and Lavie, 1960; Bogdanov, 1984; Roth *et al*., 1986) and remains intact after storage of honey for longer periods (Lavie, 1968; Bogdanov, 1984). The antibacterial spectrum of natural honey such as other antibacterial agent is not unlimited and some microorganisms may be insensitive to antimicrobial effect of it. For example, there are some reports in related to wound botulism from the Clostridial spores sometimes found in the honey.

The purpose of the present study was to determining antibacterial spectrum of honey and to evaluate antimicrobial activity of natural Urmia honey against some potentially pathogen microorganisms and in comparison with penicillin family antibiotics.

MATERIALS AND METHODS

Natural honey was obtained from beehives in Urmia (province of West Azarbaijan) and no additional procedures were performed. The samples of honey were

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Table 1: Average composition of Urmia honey

Component	Average (%)
Reductant sugars	70.38
Sucrose	2.12
Fructose/Glucose	0.93
Diastase	+
Commercial glucose	-
Mineral components	0.05
Moisture	15.08
Concentration	82.92
Total acid	12.5
pH	3.96

collected and prepared by one investigator while the experiments were performed blindly by the others. Each honey sample was first filtered with a sterile mesh to remove debris and stored at 2-8°C until used. The average composition of the honey is given in Table 1.

Test microorganisms: The microorganisms of this study were include, laboratory control strains: Gram-positive: *Staphylococcus aureus* (ATCC 25923), Gram-negative: *Salmonella typhimurium* (ATCC 1730) and *Escherichia coli* (ATCC 25922) were obtained from Department of Food Hygiene of the Faculty of Veterinary Medicine of Urmia University, Urmia, Iran.

Evaluation of antimicrobial activity: The agar disc diffusion method was employed for the determination of antimicrobial activities of the honey samples (NCCLS, 1999). Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells mL^{-1}) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 50 μL of the and for comparative evaluation of antimicrobial activities, the standard antibiotic discs of penicillin derivatives: Penicillin (10 IU/disc), Ampicillin (10 mcg/disc), Amoxicillin (25 mcg/disc), Carbenicillin (100 mcg/disc) (All standard antibiotic discs were prepared from Padtan Teb, Tehran, Iran), placed on the inoculated plates, they were incubated at 37°C for 24 h for bacteria. The diameters of the inhibition zones were measured in millimeters. The details of this procedure are described in the recent reference. All the tests were performed in duplicate.

RESULTS AND DISCUSSION

The aim of this study was evaluation of antimicrobial efficacy of natural Urmia honey in comparison to penicillin derivative antibiotics. Three control bacteria (*Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*) were used in this investigation. The first microorganism was in gram positive and 2 others were in gram negative bacterial groups. The first point of the bacterial selection was evaluation of antibacterial potency of natural honey against both gram positive and gram negative bacterial groups. The second point of the bacterial selection was the important rule of these bacteria in etiology of many humans and animals diseases. Based on the results of the present study, *Staphylococcus aureus* was the higher sensitive and *Escherichia coli* was the lower sensitive microorganism to antibacterial properties of Urmia honey (Fig. 1).

The issue is in agreement with other reports in this field. *Staphylococcus aureus* is used in many bacteriological studies of honey because high sensitivity of this organism (Cooper, 1998; Molan, 1999).

The reason of the unusual sensitivity of this microorganism is not known. It may be related to the sensitivity of *Staphylococcus aureus* to acidic environment of natural honey (Molan, 1999).

The findings of antibiogram evaluation were revealed that control bacteria were more sensitive to ampicillin than other antibiotics of penicillin family (Fig. 2).

Honey induce inhibition zone against *Staphylococcus aureus* (as the most sensitive control organism to honey) lower than ampicillin (as the most potent antibiotic against this microorganism) (Fig. 1 and 2). Penicillin had the lesser antimicrobial potency than other antibiotics against to the control bacteria (Fig. 2). As an remarkable point, inhibition zones from honey against gram negative bacteria (*Salmonella typhimurium*, *Escherichia coli*) were higher than ampicillin and other trial antibiotics. These variables were similar to inhibition zones from carbenicillin as special antibiotic against gram negative bacteria. The nature of antimicrobial potency of honey is not still clearly known.

The main honey substances are sugars, which by their osmotic effect exert an antibacterial action (Molan, 1992). However, the antimicrobial tests used in different studies are carried out at concentrations where the sugars are not osmotically active. It has been claimed that honey contains lysozyme, a well known antibacterial agent (Mohrig and Messner, 1968). However, in another study no lysozyme activity was found (Bogdanov, 1984). The antibacterial flavonoid pinocembrin is present in honey, but its concentration and contribution to honey's non-peroxide antibacterial activity is small (Bogdanov, 1989). In New Zealand honeys, mainly manuka honey, several aromatic acids with antibacterial activity have been isolated (Russel *et al*., 1988; Molan, 1992). Another investigation claimed, that the low honey pH, besides the high honey osmomolarity was responsible for the antibacterial activity (Yatsunami and Echigo, 1984). Some workers have isolated volatile substances with antibacterial activity (Obaseiki-Ebor *et al*., 1983;

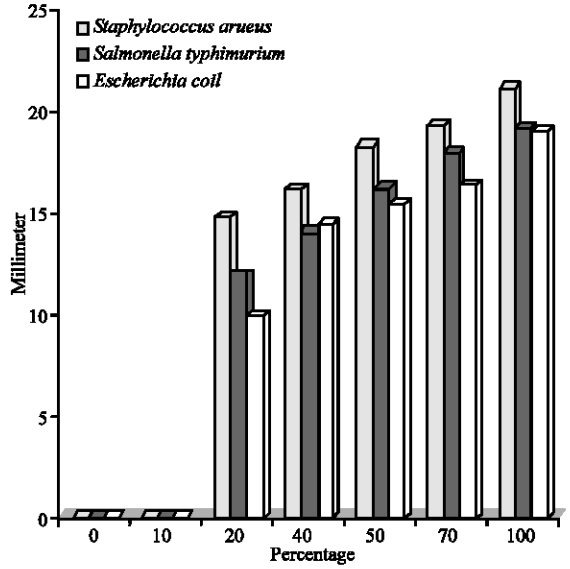

Fig. 1: The comparative diagram of inhibition zones from antibacterial efficacy of different dilutions of natural Urmia honey on control bacteria (*Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*)

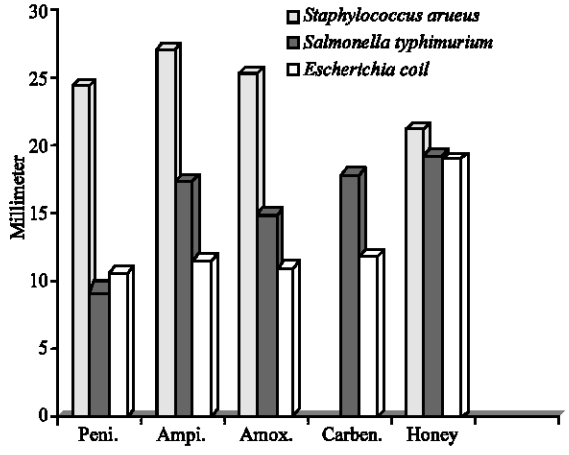

Fig. 2: The comparative diagram of inhibition zones from antibacterial efficacy of natural Urmia honey and antibiotics of Penicilline family on control bacteria.(Peni: Penicillin, Ampi: Ampicillin, Amox: Amoxicillin, Carben: Carbenicillin)

Toth *et al*., 1987), but their quantitative contribution to the antibacterial action of honey was not examined. Other researchers found non-peroxide activity of honey, extractable by organic solvents, but were not able to identify the chemical nature of the substances (Lavie, 1968; Radwan *et al*., 1984).

Despite that there are many studies in related to antimicrobial potency of natural honey (Jeddar *et al*., 1985; Haffejee and Moosa, 1985; Obi *et al*., 1994; Ceyhan *et al*., 2001; Al-Jabri *et al*., 2003) there are few investigation about nature and limitations of antibacterial spectrum of natural honey.

According to results of this study, honey has antimicrobial efficacy against gram positive and gram negative bacterial groups. This broad spectrum and specialty of antimicrobial potency of honey is similar to efficacy of disinfectant agent than to antibiotics.

REFERENCES

Al-Jabri, A.A., B. Nzeako, Z. Al Mahrooqi, A. Al Naqdy and H. Nsanze, 2003. *In vitro* Antibacterial Activity of Omani and African Honey. Br. J. Biomed. Sci., 60 (1): 1-4.

Bogdanov, S., 1984. Characterisation of antibacterial substances in honey. Lebensmittel Wissenschaft and Technologie, 17: 74-76.

Bogdanow, S., 1989. Determination of Pinocembrin in honey using HPLC. J. Apicult. Res., 28: 55-57.

Ceyhan, N. and A. Ugur, 2001. Investigation of *in vitro* Antimicrobial Activity of Honey. Riv. Biol. B. Forum, 94 (2): 363-371.

Cooper, R.A., 1998. The inhibition of bacteria isolated from chronic venous leg ulcers by honey. J. Med. Microbiol., 47: 1140-1146.

Dustmann, J.H., 1972. Ueber den Einfluss des Lichtes auf den Peroxid-Wert des Honigs. Zeitschrift fur Lebensmittel Untersuchung und Forschung, 148: 23-268.

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

Efem, S.E.E., 1988. Clinical observations on the wound healing properties of honey. Br. J. Surg., 75 (7): 679-681.

Haffejee, I.E. and A. Moosa, 1985. Honey in the treatment of infantile gastroenteritis. Br. Med. J., 290: 1866-1867.

Gonnet, M. and P. Lavie, 1960. Influence du chaufage sur le facteur antibiotic du mile. Annales de I'Abeille (Paris), 3: 349-364.

Jeddar, A., A. Khasany, V.G. Ramsaroop, A. Bhamjei and I.E. Haffejee *et al*., 1985.The antibacterial action of honey: An *in vitro* study. S. Afr. Med. J., 67: 257-8.

Lavie, P., 1968. Proprie'te's Antibacte'riennes et Action Physiologique des Produits de la Ruche et des Abeilles. Traite' de Biologie de l'Abeille. In: Chauvin, R. (Ed.). Masson and Cie, pp: 2-115.

Mathews, K.A. and A.G. Binning, 2002. wound management using honey. Compend. Con. Edu., 24 (1): 53-59.

Mohrig, W. and R. Messner, 1968. Lysozym als antibacterielles Agens im Honig und Bienengift. Acta Biol.Med. Germanica, 21: 85-95.

Molan, P., 1992. The antimicrobial activity of honey. Bee World, 73: 59-76.

Molan, P. C., 1999. Why honey is effective as a medicine. Its use in modern medicine. Bee World, 80 (2) : 80-92.

Morse, R.A., 1986. The antibiotic properties of honey. Pan-Pacific Entomol., 62: 337-370.

National Committee for Clinical Laboratory Standards, 1999. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: Approved standards. Document M31-A, 19 (11): 9-10.

Obaseiki-Ebor, E.E., T.C.A. Afonya and A.O. Onyekweli, 1983. Preliminary report on the antimicrobial activity of honey distillate. J. Pharm. Pharmacol., 35: 748-749.

Obi, L., E.O. Ugoji, S.A. Edun, S.F. Lawal and C.E. Anyiwo, 1994. The antibacterial effect of honey on diarrhea causing bacterial agent isolated in Lagos, Nigeria. African Journal of Medical Sciences.

Radwan, S.S, A.A. El-Essawy, M.M. Sarhan, 1984. Experimental evidence for the occurrence in honey of specific substances active against micro-organisms. Zentralblatt fur Mikrobiologie, 139: 249-255.

Roth, L.A., S. Kwan and P. Sporns, 1986. Use of a disc assay to detect oxytetracycline residues of honey. J. Food Protect., 49: 436-444.

Russel, K.M., P. Molan, A.L. Wilkin and P.T. Holland, 1988. Identification of some antibacterial constituents of NewZealand manuka honey. J. Agric. Food Chemist., 38: 10-13.

Subrahmanyam, M., A.G. Sahapur, N.S. Nagan, V.R. Bhagwat and J.V. Ganu, 2001. Effect of topical application of honey on burn wound healing. Annals of Burns and Fire Disasters, XIV (3): 157-161.

Toth, G., E. Lemberkovics and Kutasi-Szabo, 1987. The volatile components of some Hungarian honeys and their antimicrobial effects. Am. Bee J., 127: 496-497.

Tovey, F., 2000. Honey and suger as dressing for wounds and ulcers. Trop. Doctor, 30: 1.

White, J.W., M.H. Stubers and A.I. Schepart, 1963. The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochem. Biophys. Acta, 73: 57-70.

White, J.W. and M.H. Stubers, 1964. Studies of honey inhibine. The effect of heat. J. Apicul. Res., 3: 454-450.

Yatsunami, K. and T. Echigo, 1984. Antibacterial activity of honey and royal jelly. Honey Bee Sci., 5: 125-130.

Zumla, A. and L.A. Honey, 1989. A remedy discovered. J. R. Soc. Med., 82: 384-385.