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In vitro Evaluation of Antimicrobial Efficacy of Natural Honey in Comparison with Sulfonamide Derivatives

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Abstract: Honey produced by honeybees (*Apis mellifera*) is one of the ancient traditional medicines used for treatment various infection and diseases. This study was an assessment the antimicrobial activity of natural honey against control bacteria and its properties was compared to some antibiotics from sulfonamide family. Honey samples were obtained from beekeepers in Urmia (West Azerbaijan; Iran). Control microorganisms were used in this examination include: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*. According to findings of this investigation, the most sensitive organism to natural honey was *Staphylococcus aureus* ($p<0.05$) and the most effective sulfonamide against this organism was sulfadimidine ($p<0.05$). The inhibition zones were induced by honey against the most sensitive control organism (*Staphylococcus aureus)* was lesser than the most potent antibiotic against this microorganism (sulfadimidine). With attention to the results and other related reports in this field, despite the honey samples produced lesser inhibition zones than antibiotics of sulfonamide family but it can be still considered as a powerful natural antibacterial agent.

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

INTRODUCTION

With introduction potent antibiotics such as penicillin, this up until this past decade seemed like panaceas for every microbe under the sun. But with the over-use and abuse of such antibiotics and the growing number of diseases, which seem to evolve to become more virulent with each generation, investigations into potent yet more natural antimicrobials seemed the sagacious step to take. The invasion of pathological organisms is on the rise. To combat this unseen enemy, medical scientists have created ever-stronger antibiotics. But these organisms have brought new meaning to the word 'mutant'. They mutate faster than science can create new antibiotics. Each new generation seems more aggressive in its invasion and less responsive to antibiotics. To add to the dilemma, the immune system has undergone its own 'mutation' process, failing to respond to older, redundant antibiotics and quickly becoming 'immune' to the effects of newer ones. The immune system becomes weakened over extended use of antibiotics, making it more susceptible to the invasion of pathological organisms. In short, a paradox has been created. Stronger organisms require stronger antibiotics, which create stronger organisms. The therapies have drawn the interest of both public and medical communities. Current research has mainly focused on herbal and aromatherapy products. However, a number of other products such as honey have shown therapeutic promise. Honey has been used in treatment different diseases as long ago as 2000 years (Mathews and Binning, 2002). The use of honey as a medicine has continued into present-day medicine. Laboratory studies have shown that pure honey has significant antibacterial activities against the major wound-infecting species (Postmes *et al*., 1993; Subrahmanyam, 1994) specially those with the potential to develop antibiotic resistance such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), β-haemolytic streptococci, Vancomycin-Resistant Enterococci (VRE) and *Pseudomonas* (Molan, 2002; Cooper *et al*., 1999). Hence, a continuing need exists for potent natural antimicrobial agent, which is cheaper, easily available and effective in preventing and treatment infection. This study was designed to evaluate antimicrobial activity of natural honey against some microorganisms (control and wound pathogen bacteria) and in comparison with sulfonamide family antibiotics.

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Table 1: Average composition of Urmia honey

Component	Average (%)
Reductant sugars	80.38
Sucrose	2.24
Fructose/glucose	0.95
Diastase	+
Commercial glucose	-
Mineral components	0.07
Moisture	13.02
Concentration	84.50
Total acid	12.60
pH	3.52

MATERIALS AND METHODS

Natural honey was obtained from beehives in Urmia area (North-West of Iran) and no additional procedures were performed. The samples of honey were 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. In the present study, 3 control microorganisms were used for evaluation of antimicrobial efficacy of honey (*Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeroginosa* (ATCC 25922) and *Escherichia coli* (ATCC 25922)), which were obtained from Department of Food Hygiene of the faculty of veterinary medicine of Urmia University, Urmia, Iran. Antimicrobial activities of the honey were assessed by the agar disc diffusion method. A suspension of the tested microorganism (0.1 mL of 10^8 cells mL^{-1}) was spread on the solid media plates. For comparative evaluation of antimicrobial activities, honey discs were prepared by impregnation of honey samples in 6 mm diameter filter paper discs and the standard antibiotic discs of sulfonamide derivatives: Sulfadimidine, Sulfacetamide, Sulfadiazine and Sulfamethoxazole were used. They were 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 mm.

RESULTS AND DISCUSSION

The purpose of this present study was assessment of antibacterial effectiveness of natural honey in comparison to sulfonamide derivative antibiotics. There are many reports about antibacterial properties of natural honey, but these properties of honey are not unlimited such as other antibacterial agents. Antimicrobial activity of honey is thought to be due to physicochemical properties (high content of reducing sugars, high viscosity, high osmotic pressure, low pH, low water activity (AW), low protein content)[28] and hydrogen peroxide (Hyslop *et al*., 1995; Molan and Cooper, 2000). Also, Radwan *et al*. (1984), attributed the antibacterial activity to specific chemicals in honey. The nature of these chemicals and the mechanisms of their action are not fully understood even though Thin Layer Chromatography (TLC), polyacrylamide gel electrophoresis (PAGE) or High Performance Liquid Chromatography (HPLC) have shown that honey contains fatty acids, lipids, amylases and ascorbic acids (Bergman *et al*., 1983; Oka *et al*., 1987). 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). Results of this investigation revealed, *Staphylococcus aureus* (19.25±0.52 mm) was the higher sensitive and *Pseudomonas aeroginosa* (6.40±0.44 mm) was the lower sensitive microorganism to antimicrobial properties of natural honey (Fig. 1). The findings of this study are in agreement with other reports. Because high sensitivity of *Staphylococcus aureus*, it is used in many microbiological evaluations of honey (Cooper, 1999; Molan, 2002). 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, 2002). The results of the evaluation were showed that sulfadimidine were the more potent antibiotic against to control bacteria than other antibiotics of sulfonamide family (Fig. 2). Honey induce inhibition zone against *Staphylococcus aureus* (as the most sensitive control organism to honey) (19.25±0.52 mm) lesser than the most potent antibiotic of sulfonamide family (Sulfadimidine: 28.14±20 mm) (Fig. 2). Sulfacetamide had the lower antimicrobial potency than other antibiotics of this family against to the control bacteria (Fig. 2). Honey induce inhibition zone against control organisms greater than Sulfacetamide's inhibition zone (Fig. 2). With attention to this fact, antibiotics of sulfonamide family prevent to synthesis folic acid in pathogenic microorganisms thus they have more bacteriostatic than bactericide efficacy. The antimicrobial property of honey is thought to be due to non-specific mechanisms (physicochemical and peroxidial properties), this value is more similar to disinfectants than to antibiotics. Thus can be expected to honey should be possess broad-spectrum antimicrobial potency and very low microbial resistant to it. Researches relating honey show that pure honey is bactericidal for many pathogenic organisms, including various gram-negative and gram-positive bacteria (Haffejee and Moosa, 1985; Ceyhan and Ugur, 2001; Al-Jabri *et al*., 2003). On basis of these results, natural both gram positive and negative bacteria. Despite honey produce lesser inhibition zone than tested antibiotics in honey has broad spectrum antibacterial efficacy against

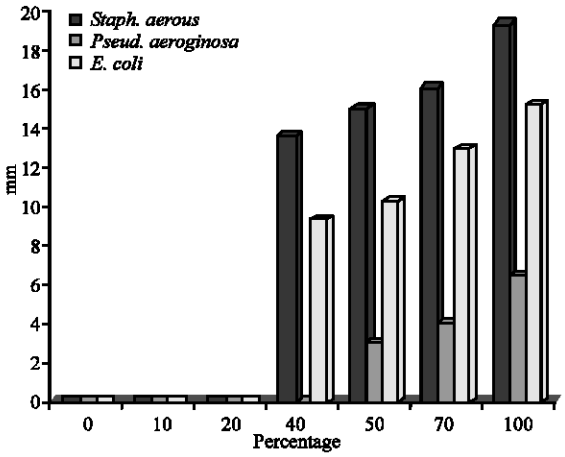

Fig. 1: The comparative diagram of inhibition zones from antibacterial efficacy of different dilutions of natural Urmia honey on control bacteria

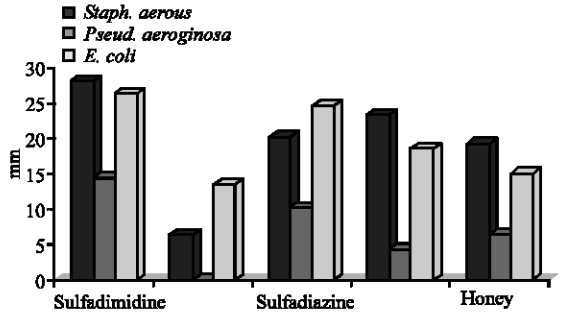

Fig. 2: The comparative diagram of inhibition zones from antibacterial efficacy of natural Urmia honey and antibiotics of Sulfonamide's family on control bacteria

control bacterial culture but it can be considered as an acceptable antimicrobial agent in comparison with sulfonamide family antibiotics. It need to the further microbiological and pharmacological investigations to clarify all unobvious viewpoint of this field.

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