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Antibacterial Activity of Honey Bee Products Collected from Three Different Climate in Golestan Province in Northern Iran

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ABSTRACT

The biological activities of bee products vary according to plant origin, geographical region and climatic characteristics. This study aimed to investigate the antibacterial activity of honey and propolis samples from different geographical regions with different climate. Samples included three honey samples and three propolis samples were collected from three areas of the Plain, Mountain and Forest of Golestan province in north of Iran. Antibacterial activity was evaluated by agar well diffusion method and MIC and MBC were determined by broth macrodilution tube method. The results of the present study revealed that antibacterial activity of the honey collected from forest areas is higher than other honey samples so that the MIC of this honey sample was in the range of 12.5 to 25%. The propolis collected from plain areas showed the highest antibacterial activity with MBC in the range of 12.5 to 50 mg/ml. The gram-positive bacteria in comparison of gram-negative bacteria and standard strains in comparison of native isolates were showed more sensitive to these bee products. The standard strain of *S. aureus* and the native isolate of *P. aeroginosa* were the most sensitive and the most resistant of the bacteria respectively. Difference and variation of antibacterial activity of bee products can be due to the difference in the various plants that bees have fed. Due to the complications associated with antimicrobial chemical compounds, identifying of effective compounds of these products can hope for us to introduce a natural drug combination or a natural food additive.

1. Introduction

One of the problems of new medicine, despite the appearances benefits in comparison of traditional medicine, is the increasing consumption of chemical drugs and the growing proliferation of antibiotic resistance which is a major concern of the World Health Organization. For this reason, research on the introduction of new natural-antimicrobial agents with a natural origin is necessary in order to reduce both antibiotic resistance and the unwanted side effects of chemical agents

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(Khairy et al., 2013; Ventola, 2015; Chanda et al., 2010).

The honey bee is one of the most useful insects on the one hand with pollination of crops, gardens and pasture plants, increases the yield of the products and residues of the vegetation of the rangelands, and on the other hand produce honey, which is the main production of colonies, and other products such as propolis, royal jelly, Pollen, wax ... brings a lot of benefits to humans.

Honey and propolis are of the most important products of honey bee, which many biological activities attributes to them. These biological activities vary according to herbal origin, geographical region and other climatic characteristics (Kumazawa et al., 2004; Bankova, 2005).

Honey is a very useful food with high potential for antimicrobial and other biological properties such as anti-tumor, anti-inflammatory, anti-oxidant and antiviral properties. These attributes relate to a group of intrinsic compounds of this nutrient that relates to the herbaceous origin and the geographical area and the honey entomology (Molan, 2002).

Antimicrobial effects of honey are due to acidity and high osmolarity, as well as hydrogen peroxide and other non-peroxide factors. Amounts of diastase, invertase, glucose oxidase, protease, catalase and phosphatase enzymes play an active role in antimicrobial activity of honey. Also amylase hydrolyzes starch in honey and, by producing dextrose and maltose, increases the effect of honey's osmotic effect and, as a result, increases its antibacterial activity. The presence of chemical compounds such as methyl glyoxal also influences antimicrobial activity of honey (Paulus et al., 2012; Moussa et al., 2011; Oddo et al., 1999; Boukraa and Amara, 2008; Mavric et al., 2008).

Propolis is a resinous, brownish, rigid material made from bees by gum from various trees and plants, collected in pollen baskets, and after combining it with wax and saliva as a sealant and peeling agent in the hive, Disinfection of wax cells after the emergence of infants and before the laying of the queen in them and mummification of the carcasses of the injured in the hive is used. This material is a by-product of bee honey that has been used by humans in traditional medicine for centuries (Bankova, 2009).

Propolis contains about 50% gum or plant resin, 30% wax, 10% essential fatty acids, 5% pollen and 5% other organic compounds, vitamins and minerals. Using biochemical analyzes, various compounds including flavonoids, alcohols, alpha acids, amino acids, aromatic acids, aromatic esters, terpenoids, sugars and steroids, and hundreds of other substances were identified in the propolis (Bankova, 2009).

Chemical compounds that are responsible for beneficial biological activity of propolis, especially antimicrobial and antioxidant activities, include flavonoids (flavones, flavonols, flavans, dihydroflavonols) and other phenolic compounds, the main ones are cinemic acids and other esters. Studies on the chemical composition of propolis have shown that various samples of propolis are very varied in this regard, and various types of chemical compounds varies depending on the herbal origion, the geographical region and the Meteorological features of the area where the bee has been used (Bankova, 2005; Marcucci, 1995; Banskota et al., 2004; Kumazawa et al., 2004).

Gastrointestinal infections are responsible for high morbidity and mortality worldwide. Due to the increasing in the prevalence of gastrointestinal diseases in the world that are the most common digestive diseases after respiratory infections and the importance of *E. coli*, *S. aureus*, *B. cereus* and *P. aeroginosa* in causing these diseases This study aimed to investigate the antibacterial activity of three types of honey and propolis collected from bee hives from three geographical regions with different climate from Golestan province in northern Iran against these bacteria.

2. Materials and Methods

2.1. Honey and propolis samples

In March 2017, samples included three honey samples and three propolis samples from bee hives were collected in three geographical regions with different climate from Golestan province in north of Iran and until the experiments were kept at the refrigerator of the Microbiology Laboratory at Islamic Azad University of Azadshahr branch.

Bee products (honey and propolis) from bee hives in forest areas of the *Jahan Nama* in *Kord Kuy* township (Forest), the plains of the *Luve* in Galikesh township (Plain) and Mountain areas of the *Ghale Maran* in *Ramian* township (Mountain) were collected.

2.2. Preparation of ethanolic extract of propolis samples

To extraction of the ethanolic extract, the propolis was divided into small pieces and placed in a 80% ethanol (1-10 w/v) on a shaker at room temperature for 48 hours. Then, the solution of ethanolic extract was filtered with Whatman No. 4 filter paper using by the vacuum pump, and in order to condensation and solvent removal (ethanol) was placed in a rotary evaporator under vacuum conditions at 50°C. After evaporation of the solvent, the crude extract was obtained with a brownish viscous. This pure extract (1000 mg/ml) was again dissolved in ethanol at %80 to obtain a concentration of 100 mg/ml (working solution) and, using serial dilutions, different concentrations ethanolic extract of propolis samples (50 to 0.78 mg/ml) were prepared in Nutrient Broth (Merck) (Dziedzic et al., 2013).

2.3. Preparation of serial dilution of honey samples

Dilution of honey samples were carried out in sterilized distilled water and serial dilutions of 1/2 (50%), 1/4 (25%), 1/8 (12.5%), 1/16 (6.25%) and 1/32 (12.3%) of each of the honey samples were perpared.

2.4. Bacterial Strains

In this study, antibacterial activity of honey and propolis samples against 4 bacterial native isolates and 4 bacterial standard strains were evaluated. Native isolates include *Staphylococcus aureus* isolated from the nasal cavity of the carrier, *Escherichia coli* isolated from the stool, *Pseudomonas aeruginosa* isolated from water, and *Bacillus cereus* were isolated from the soil. These isolates were identified using routine microbial laboratory tests for each (Selective media culture, gram stain, catalase and oxidase tests, biochemical tests, …). The standard strains used in this study two types of gram-negative bacteria included *E. coli* (PTCC 1338) and *P. aeruginosa* (PTCC 1811) and two types of gram-positive bacteria included *S. aureus* (PTCC 1112) and *B. cereus* (PTCC 1154) which were provided from the Iranian Research Organization for Science and Technology (IROST) in a lyophilized form. Then, they recovered in BHI medium (Merck) for 24 h at 37^0C. The 24-hour culture of each of bacteria were inoculated into Nutrient Broth culture medium (Merck) and it was incubated at 37°C for to obtain turbidity equal to 0.5 McFarland = 1.5×10^8 CFU/ml. When turbidity of the tube was equal to 0.5 McFarland standard, the absorbance value was between 0.08 to 0.1 using 625 nm wavelength (Cockerill et al., 2012; Bagheri et al., 2016; Jafarzadeh Kashi et al., 2011).

2.5. Evaluation of antibacterial activity of honey and propolis samples

Antibacterial activity of honey and propolis samples was carried out on the basis of agar well diffusion method. Also, Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each specimen were tested by broth macrodilution tube method.

2.5.1. Agar well diffusion method

In well-method, from bacterial suspensions equal of 0.5 McFarland (equal to 1.5×10^8 CFU/ml) each of the bacteria including standard strains and native isolates the uniform spread culture was prepared using sterile cotton swabs on Muller Hinton Agar medium. Then, using by sterilized cork borer wells at a diameter of 6 mm on the medium were created and 100µl each of serial dilutions of honey samples and different concentrations of ethanolic extract of propolis samples were poured into wells. Then the plates were incubated at 37°C for 24 hours. After incubation, using a millimeter ruler the diameter of inhibition zone was measured and recorded (Cockerill et al., 2012; Jafarzadeh Kashi et al., 2011; Bagheri et al., 2017)

2.5.2. Determination of MIC and MBC

Determination of MIC of honey and propolis samples were carried out based on turbidimetric assay and by using macrodilution tube method. For this purpose, serial dilutions of honey samples and different concentrations of ethanolic extract of propolis samples were prepared in Nutrient Broth (Merck) then to each of these tubes from was added, suspensions equivalent to 5×10^5 CFU/ml from each of the bacteria and incubated for 24 h at 37°C. There were also control tubes containing of serial dilutions of honey samples and different concentrations ethanolic extract of propolis samples were prepared in Nutrient broth (without bacterial suspension) as negative controls and bacterial suspension of 5×10^5 CFU/ml (without honey and propolis samples) as positive controls. The results after 24 h of incubation for microbial turbidity of visible were recorded. The last dilution (lowest concentration) in which microbial turbidity was not observed, as the MIC was considered. For the determination of MBC, from the tube that contained honey and propolis concentrations higher than the MIC were cultured onto the Nutrient agar medium. The MBC was defined as the lowest concentration that allowed no visible growth on the agar (Cockerill et al., 2012; Pimentel et al., 2013; El Soheimy and Masrey, 2014).

2.6. Statistical Analysis

All data were expressed as the mean ± standard deviation (n = 3). Data analysis was performed through one-way analysis of variance (SPSS 18) software, and for means comparison, the Duncan test was used at 5% significance level.

3. RESULTS

The results of the well method for the evaluation of antibacterial activity of honey and propolis samples collected from bee hives from three different geographic regions with different climates are presented in Tables 1 and 2.

The results obtained from the well method showed that the antibacterial effects of honey and propolis samples were concentration-dependent and, with increasing sample concentration, the mean diameter of the inhibition zone (antibacterial activity) increased. Also, there was a significant difference between the mean diameter of the inhibition zone (antibacterial activity) of honey and propolis samples in different climates ($P < 0.05$).

As seen in table 1, honey samples collected from bee hives in the areas of the Forest of the *Jahan Nama* in *Kord Kuy* township (Forest) have shown significant antibacterial activity ($P < 0.05$). This sample of honey in the pure concentration were affected against all tested bacteria, so that the mean diameter of the inhibition zone of standard strains of *S. aureus*, *E. coli*, *B. cereus* and *P. aeroginosa* was 22.5, 16.5, 15.5 and 14 mm respectively. The mean diameter of the inhibition zone of native isolates for this honey was 20.5, 16.5, 12 and 12 mm, respectively. This honey sample, even at 12.5% concentration, has been able to prevent the growth of standard strain and native isolate of *S. aureus* with a mean diameter of the inhibition zone 13.0 and 13.5 mm, respectively (Table 1).

The propolis sample collected from bee hives of the plains of the Luve in Galikesh township (Plain) showed more antibacterial activity than the other samples ($P < 0.05$). So that at

concentration of 6.25 mg/ml of ethanolic extract this propolis, the standard strains of *S. aureus*, *E. coli*, *B. cereus* and *P. aeroginosa* with mean diameter of the inhibition zone of 14, 14.5, 10.5, and 13.5 mm respectively, were affected, and except for *B. cereus*, even at concentration of 3.12 mg/ml of ethanolic extract this propolis, inhibitory activity against other tested bacteria was observed (Table 2). Of course, like other honey and propolis samples, native isolates tested showed more resistance to this propolis sample. So that the native isolates of *E. coli* and *P. aeroginosa* were inhibited only in the concentration of 100 mg/ml of ethanolic extract this propolis sample with mean diameter of inhibition zone 16 and 12 mm respectively (Table 2).

Generally, in comparing gram positive and gram negative bacteria to different concentrations of honey and propolis samples, the results showed that gram-positive bacteria were more sensitive to the products of the honeybee compared to the gram-negative bacteria. Standard strain of *S. aureus* was recognized as the most susceptible bacteria tested ($P < 0.05$), so that this gram positive bacterium was sensitive to all three honey samples even at a concentration of 25%. Also ethanolic extract all three samples of propolis at concentrations 3.12-6.25 mg/ml were inhibited the standard strain of *S. aureus* and at concentrations of 3.12-12.5 mg/ml for native isolates of this bacterium (Table 1,2). The most resistant bacteria studied to the different concentrations of honey and propolis was native isolate of the gram-negative bacteria of *P. aeruginosa*. So that this bacterium as a gram-negative bacterium were resistant to most of the concentrations of the propolis, and only concentrations of 100 mg/ml ethanolic extract of propolis collected from bee hives of *Ghale Maran* in *Ramian* township (Mountain) and *Luve* in *Galikesh* township (Plain) with mean diameter of inhibition zone of 13.5 and 12 mm, respectively were able to inhibit the growth of this bacterium (Table 2).

Statistical analysis showed that overall, native isolates compared with the standard strains showed higher resistance to honey and propolis samples ($P < 0.05$).

The results of the MIC and MBC of honey and propolis samples are shown in Tables 3 and 4. MIC and MBC are honey samples ranging from 12.5- to 50% V/V. In this method, like the well method, the honey samples collected from Forest areas of the *Jahan Nama* in *Kord Kuy* Township (Forest) in comparison with the other two regions showed the most antibacterial activity against the tested bacteria. So that the MIC of this honey sample for standard strains of *S. aureus*, *B. cereus*, *P. aeroginosa* and *E. coli* was in the range of 12.5 to 25%, but the MIC of honey samples collected from bee hives of plain areas of Luve in Galikesh township (Plain) for these standard strains was obtained the 25%, and MIC of honey sample of montain areas of Ghale Maran in Ramian township (Montain) ranged from 25% to 50% (Table 3).

The MBC of honey samples collected from bee hives of the forest areas of Jahan Nama in Kord Kuy township (Forests) for standard strains and native isolates was in the range of 12.5-50% and 25-50%, respectively, which indicate more resistance of isolates native (Table 3).

The more sensitivity of gram-positive bacteria in comparison with gram-negative bacteria to different concentrations of honey samples collected from forest areas of *Jahan Nama* in *Kord Kuy* township (Forest) and plain areas of *Luve* in *Galikesh* township (Plain), in Table 3 are clearly evident. So that the MBC of these honey samples were for standard strains and native isolates of gram-positive bacteria of *S. aureus* and *B. cereus*, of 25%, but MBC of honey sample collected from bee hives of plain areas of *Luve* in *Galikesh* township (Plain) for gram-negative bacteria of *E. coli* and *P. aeroginosa*, including strains standard strains and native isolates were obtained at 50%. Also MBC of honey sample of collected from bee hives of forest areas of Jahan Nama in Kord Kuy township (Forest) for standard strain and native isolate of *P. aeroginosa* was a 50%, and for standard strains and native isolate of *E. coli* it was 12.5% and 25% respectively (Table 3).

able 1. The mean diameter of inhibition zone of bacteria in the presence of different concentrations of honey samples in well method (mm)

Honey sample	Concentration (%)	Standard Strains				Native Isolates			
		S. aureus	*E. coli*	*B. cereus*	*P. aeroginosa*	*S. aureus*	*E. coli*	*B. cereus*	*P. aeroginosa*
Jahan Nama (Forest)	100%	22.5 ± 0.5^{aA}	16.5 ± 1.5^{bA}	15.5 ± 0.5^{bA}	14 ± 1^{bcA}	20.5 ± 1.5^{aA}	16.5 ± 0.5^{bA}	12 ± 0^{cC}	12 ± 1^{cB}
	50%	16 ± 1^{aCD}	$11\pm1b^{bB}$	-	-	16 ± 0^{aB}	11.5 ± 0.5^{bB}	-	-
	25%	13 ± 1^{aE}	-	-	-	13.5 ± 0.5^{aC}	-	-	-
	12.5%	-	-	-	-	-	-	-	-
	6.25%	-	-	-	-	-	-	-	-
	3.12%	-	-	-	-	-	-	-	-
	Negative Control	-	-	-	-	-	-	-	-
Luve (Plain)	100%	16.5 ± 0.5^{bC}	9 ± 1^{dBC}	14 ± 1^{cA}	9 ± 1^{dB}	19 ± 1^{aA}	-	10 ± 2^{dC}	7.5 ± 0.5^{dC}
	50%	14.5 ± 0.5^{bDE}	8.5 ± 0.5^{cC}	8.5 ± 0.5^{cB}	6.5 ± 0.5^{dC}	16 ± 0^{aB}	-	7.5 ± 0.5^{cdD}	6.5 ± 0.5^{dC}
	25%	9 ± 0^{aF}	-	-	-	7 ± 1^{bD}	-	-	-
	12.5%	-	-	-	-	-	-	-	-
	6.25%	-	-	-	-	-	-	-	-
	3.12%	-	-	-	-	-	-	-	-
	Negative Control	-	-	-	-	-	-	-	-
Ghale Maran (Montain)	100%	18.5 ± 0.5^{abB}	18.5 ± 1.5^{abA}	-	12.5 ± 1.5^{cA}	21 ± 1^{aA}	8 ± 0^{dC}	18.5 ± 0.5^{abA}	16.5 ± 1.5^{bA}
	50%	16 ± 1^{aCD}	16 ± 1^{aA}	-	-	16 ± 0^{aB}	-	16 ± 0^{aB}	13 ± 0^{bB}
	25%	8 ± 1^{aF}	-	-	-	8 ± 0^{aD}	-	-	-
	12.5%	-	-	-	-	-	-	-	-
	6.25%	-	-	-	-	-	-	-	-
	3.12%	-	-	-	-	-	-	-	-
	Negative Control	-	-	-	-	-	-	-	-

Different lowercase letters in the columns (a, b, ...) show a significant difference between different treatments.
Different capital letters in rows (A, B, ...) show a significant difference between different treatments.

Table 2. The mean diameter of inhibition zone of bacteria in the presence of different concentrations of ethanolic extract of propolis samples in well method (mm)

Propolis samples	Concentration (mg/ml)	Standard Strains				Native Isolates			
		S. aureus	*E. coli*	*B. cereus*	*P. aeroginosa*	*S. aureus*	*E. coli*	*B. cereus*	*P. aeroginosa*
Jahan Nama (Forest)	100	25.5 ± 0.5^{aA}	15 ± 1^{cBC}	22.5 ± 0.5^{bA}	10.5 ± 1^{dC}	24.5 ± 0.5^{aA}	14.5 ± 0.5^{cB}	18.5 ± 0.5^{bA}	-
	50	20 ± 1.5^{aCD}	-	17.5 ± 0.5^{bC}	-	18.5 ± 0.5^{abBC}	-	14.5 ± 0.5^{bC}	-
	25	17 ± 1^{aEF}	-	16.5 ± 0.5^{aCD}	-	17.5 ± 0.5^{aC}	-	12.5 ± 0.5^{aCD}	-
	12.5	16 ± 0^{aF}	-	14.5 ± 1.5^{aDE}	-	16 ± 0^{aD}	-	10.5 ± 1.5^{aDE}	-
	6.25	16 ± 0^{aEF}	-	13.5 ± 0.5^{bE}	-	15.5 ± 0.5^{aDE}	-	-	-
	3.12	14 ± 0^{aG}	-	10 ± 0^{bG}	-	15 ± 1^{aDE}	-	-	-
	Negative Control	-	-	-	-	-	-	-	-
Luve (Plain)	100	24 ± 0^{aB}	21 ± 1^{bA}	21 ± 1^{bAB}	20.5 ± 0.5^{bA}	20 ± 1^{bB}	16 ± 1^{cB}	20 ± 0^{bB}	12 ± 0^{dB}
	50	18.5 ± 0.5^{aDE}	20 ± 1^{aA}	16.5 ± 0.5^{bCD}	20 ± 1^{aA}	20 ± 0^{aB}	-	16.5 ± 0.5^{bC}	-
	25	17 ± 0^{bEF}	16.5 ± 0.5^{bB}	14 ± 0^{cE}	19.5 ± 0.5^{aA}	19.5 ± 0.5^{aB}	-	14 ± 1^{cD}	-
	12.5	16 ± 0^{aF}	15 ± 1^{bBC}	12 ± 0^{cF}	14.5 ± 0.5^{bB}	14.5 ± 0.5^{bE}	-	12.5 ± 0.5^{cDE}	-
	6.25	14 ± 1^{aG}	14.5 ± 0.5^{aC}	10.5 ± 0.5^{bG}	13.5 ± 0.5^{aB}	14 ± 0^{aE}	-	10.5 ± 1.5^{bE}	-
	3.12	13.5 ± 0.5^{aG}	12.5 ± 0.5^{aD}	-	12.5 ± 1.5^{aBC}	12 ± 1^{aF}	-	-	-
	Negative Control	-	-	-	-	-	-	-	-
Ghale Maran (Montain)	100	23.5 ± 0.5^{aB}	20.5 ± 0.5^{bA}	20 ± 0^{bB}	13 ± 0^{cB}	20 ± 0^{bB}	20 ± 0^{bA}	22.5 ± 0.5^{aA}	13.5 ± 0.5^{cA}
	50	21.5 ± 0.5^{aC}	15 ± 1^{cBC}	-	10.5 ± 0.5^{dC}	18 ± 1^{bBC}	15 ± 0^{cB}	18.5 ± 1.5^{bBC}	-
	25	18.5 ± 0.5^{aDE}	-	-	-	15 ± 0^{bDE}	-	14 ± 1^{bD}	-
	12.5	18 ± 1^{DE}	-	-	-	-	-	-	-
	6.25	18 ± 0.5^{DE}	-	-	-	-	-	-	-
	3.12	-	-	-	-	-	-	-	-
	Negative Control	-	-	-	-	-	-	-	-

Different lowercase letters in the columns (a, b, ...) show a significant difference between different treatments.

Different capital letters in rows (A, B, ...) show a significant difference between different treatments.

As can be seen in Table 4, propolis samples collected from beehive hives in plains areas of *Luve* in *Galikesh* township (Plain) and mountain areas of *Ghale Maran* of *Ramian* township (Mountain) were showed the highest and lowest antibacterial activity against the tested bacteria, respectively. So that the MIC of propolis sample of collected from beehive hives in plains areas of *Luve* in *Galikesh* township (Plain) was in the range of 1.56-25 mg/ml, MIC of proplos samples collected from forest areas of the *Jahan Nama* in *Kord Kuy* township (Forest) was in the range of 1.56 to 50 mg/ml, and the MIC of propolis sample collected from beehive hives in mountain areas of *Ghale Maran* of *Ramian* township (Mountain) was in the range of 6.25-50 mg/ml (Table 4). These results confirm the results of the well method.

Also, more sensitivity of the gram-positive bacteria to the ethanolic extract of propolis samples was observed. So that MBC of propolis sample collected from bee hives in plains areas of *Luve* in *Galikesh* township (Plain) for gram-positive bacteria was in the range of 1.56-6.25 mg/ml, while the MBC of this propolis sample for gram-negative bacteria was 25mg/ml. This sensitivity in other samples of propolis is also evident in Table 4.

MIC of propolis sample collected from bee hives in plains areas of *Luve* in *Galikesh* township (Plain) for standard strains in the range of 1.56-6.25 mg/ml and for native isolates in the range of 3.12 to 25 mg/ml was obtained. MIC of propolis sample collected from bee hives in forest areas of *Jahan Nama* in *Kord Kuy* township (Forest) for standard strains in the range of 1.56-50 mg/ml and for native isolates in the range of 3.12 to 50 mg/ml was obtained. Finally that amounts for propolis sample collected from bee hives in mountain areas of *Ghale Maran* in *Ramian* township (Mountain) against standard strains in the range of 6.25-25 mg/ml and for native isolates in the range of 12.5 to 50 mg/ml was obtained (Table 4).

These results indicate the resistance of native isolates compared to standard strains to different concentrations of ethanolic extract of propolis samples, which were also observed in well method.

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of honey samples (%)

Honey samples		***Jahan Nama* (Forest)**		***Ghale Maran* (Montain)**		***Luve* (Plain)**	
Tested Bacteria		MIC	MBC	MIC	MBC	MIC	MBC
	Standard	12.5	25	25	25	25	25
S. aureus	Native	25	25	25	50	25	25
	Standard	12.5	25	50	50	25	25
B. cereus	Native	12.5	25	25	50	25	25
	Standard	25	50	25	25	25	50
P. aeroginosa	Native	50	50	25	25	50	50
	Standard	12.5	12.5	50	50	25	50
E. coli	Native	25	25	25	50	50	50

Table 4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Propolis samples (mg/ml)

Propolis samples / Tested Bacteria		*Jahan Nama* (Forest)		*Ghale Maran* (Montain)		*Luve* (Plain)	
		MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	Standard	1.56	3.12	6.25	6.25	1.56	1.56
	Native	3.12	3.12	12.5	25	3.12	3.12
B. cereus	Standard	3.12	6.25	6.25	6.25	1.56	3.12
	Native	6.25	12.5	12.5	25	3.12	6.25
P. aeroginosa	Standard	25	25	25	50	6.25	25
	Native	25	50	50	50	25	25
E. coli	Standard	50	50	12.5	25	6.25	25
	Native	50	50	25	25	12.5	25

4. Discussion

The results of the present study indicated the antibacterial activity of the bee products tested. Also the products collected from different climates had different antibacterial activity. In between, the honey samples of collected from forested areas and propolis samples collected from the bee hives of the plains showed the most antibacterial activity.

The difference and variation of antibacterial activity of bee products studied in this study can be due to the difference in the various plants that the bees have fed. In other words, different species of a plant in different regions contain different compounds, and the resulting honey and propolis will not be the same, and therefore their biological effects will be different.

Generally, different species of plants and pollen from the flower that the bees use for nectar, due to climatic conditions, geographical origions, season conditions, rainfall and soil compositions are different and the honey and propolis will not be the same. Therefore, the biological effects They will also be affected by these conditions (Bankova et al., 2012; Kumazawa et al., 2004; Miguel et al., 2014; Tumin *et al*., 2005).

The results of the well method showed a significant correlation between the concentrations and the diameter of inhibition zone and showed that the antibacterial activity of honey and propolis samples were concentration-dependent and with increasing sample concentration, the mean diameter of the inhibition zone (antibacterial activity) increased which this issue has been seen in similar studies (Khairy et al., 2013; Sherlock et al., 2012).

Also geographically, there was a significant difference between the mean diameter of the inhibition zone (antibacterial activity) of honey and propolis samples in different climates ($P < 0.05$).

Alzahrani et al. (2012) also found that the differences in antibacterial and antioxidant activity of various honey was associated with natural changes in the origin of flora and geographical origions of honey (Alzahrani et al., 2012).

The results of this study showed that gram-positive bacteria were more susceptible than gram-negative bacteria to the products of the honey bees. The more sensitivity gram-positive bacteria in comparison with gram-negative bacteria to propolis and honey samples has also been reported in other studies (Marcucci, 1995; Kujumgiev et al., 1999; Nieva et al., 1999; Yaghobi et al., 2007; Sherlock et al., 2011; Fidaleo et al., 2011).

Uzel et al. (2005) also in the study of chemical compounds and antimicrobial activity of four samples of propolis in Turkey showed more susceptibility of gram-positive bacteria (Uzel et al., 2005).

In a study by Sherlock et al. (2010), strains of *S. aureus* compared to *E. coli* and *P. aeroginosa* were more sensitive to honey samples, which was consistent with the present study (Sherlock et al., 2011).

The results of this study showed more antibacterial activity of the honey collected from Forest areas of the *Jahan Nama* in *Kord Kuy* township (Forest) in comparison to honey samples of other regions. Filipic et al. too reported a higher sensitivity of gram-positive bacteria compared to gram negative bacteria to honey samples collected from forest areas Italy and Spain (Filipic et al., 2016).

The standard strain of gram-positive bacteria of *S. aureus* and the native isolate of gram-negative bacteria of *P. aeroginosa* were the most sensitive and the most resistant of the present study bacteria respectively ($P < 0.05$). In the study of Sillici and Kutluca in order to the evaluation of antibacterial activity, three samples of propolis, too gram positive bacteria of *S. aureus* were the most susceptible bacteria and gram negative bacteria of *P. aeroginosa* and *E. coli* the most resistant bacteria to propolis samples (Sillici and Kutluca, 2005).

Seidel et al., in a study to evaluate the antibacterial activity of ethanolic extract of 40 samples of propolis from different geographical regions with different climates, antibacterial activity of ethanolic extract of propolis samples against gram positive bacteria were reported. They were reported the most antibacterial activity associated with samples of propolis collected from the climates of rainy forests (Seidel et al., 2008).

Studies have shown that the cell wall of the gram-positive bacteria compared to gram-negative bacteria is highly permeable and susceptible to many antibiotics, antimicrobial chemical compounds and even many herbal medicines. The more sensitivity of most gram-positive bacteria is related to their cell wall structure. The existence of a layer of lipopoly saccharide of cell wall and periplasmic space is one of the important reasons for relative resistance to gram negative bacteria (Nikaido, 2003).

Conclusion

Different species of a plant in different regions contain different compounds, and the resulting honey and propolis will not be the same, and therefore their biological effects will be different. Finally due to the increasing drug resistance and unwanted side effects of chemical agents and the prevalence of gastrointestinal diseases in the world and due to the antibacterial activity of honey bee products studied in this study, identifying the chemical and effective compounds of these products can hope for us to introduce a natural drug combination or a natural food additive with unique characteristics. More comprehensive research and studies on extraction and purification of honey and propolis components should be undertaken. It is also necessary to evaluate their effects on pathogenic bacteria in vitro and in vivo in animal models to make a more accurate judgment of the antibacterial activity of these products.

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