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Antimicrobial activity of different honey samples against *Pseudomonas aeruginosa in vitro*

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Abstract. Honey is a natural product from honeybees *Apis mellifera* that was found to possess antimicrobial activity. *Pseudomonas aeruginosa* is opportunistic, nosocomial pathogen that infects urinary and pulmonary tracts, burns, wounds and also causes the other blood infections. The concentration of reducing sugar (any sugar with aldehyde or a ketone group) and sucrose and diastase enzyme were assessed. Because the antimicrobial activity of honeys is related to their geographical regions or from where they are collected, we compared the antibacterial activity of 15 different samples of honeys against *Pseudomonas aeruginosa*. *P. aeruginosa* was not inhibited in low concentration of honey while higher concentration of honey affected its growth. The samples of honey without diastase enzyme exhibited antimicrobial activity in high concentrations.

KeyWords: Honey, Antibacterial activity, diastase activity, *Pseudomonas aeruginosa.*

Introduction

Pseudomonas aeruginosa, an opportunistic human, plant pathogen is a gram negative bacterium that is the most common agent of infectious burn injuries, otitis externa, community acquired and ventilator associated pneumonias being one of the most important bacteria for research (Driscoll et al. 2007, Yetkin et al. 2006). *P. aeruginosa* is naturally resistant to many commonly used antibiotics and it acquired resistance to effective antibiotics during the treatment. These reasons result in severe adverse outcomes (Carmeli et al. 1999). So the efforts directed towards preventing the emergence of antibiotic resistance are very important.

Natural products such as jelly royal, propolis, pollen and honey from honeybee have gained interest as antimicrobial agents. There are many reports on antimicrobial activity of bee products (Noori 2004, Fontana et al. 2004, Kartal et al, 2003).

Honey has long been known as an antimicrobial agent and its activity was first recognized by Van Ketel in 1892 (Dustmann 1979). The antimicrobial activity of honey is depended on its chemical components and remarkably varies to its geographical sources or collected regions.

These differences are related to the amount of hydrogen peroxidases that is enzymatically present in different types of honey or other antimicrobial constituents with origin of nectar with which the bee is nourished (Molan 1992). There are many reports about antipseudomonal activity of honey from different parts of the world (Mullai & Menon 2005, Cooper et al. 2002, Subrahmanyam 1991) but there are two reports about Iranian honey against *P. aeruginosa* (Hazrati et al. 2010, Tajik et al. 2009). The first study has evaluated the effect of honey on pseudomal burn injuries in rats and others; *in vitro* has used the Urmia honey (North West of Iran) against *P. aeruginosa*.

The aim of this study was to evaluate the antimicrobial activity of 15 samples of honey against *P. aeruginosa* ATCC 27853 *in vitro* condition; also we determined the diastase enzyme, sucrose and reducing sugars in different samples.

Materials and methods

Sample collection and analysis

Fifteen honey samples were randomly collected from different sources. The botanical origin of these honey samples was unknown and concentrations of reducing sugars and sucrose were analyzed. All samples were saved in glass flasks and cool places and analyzed within two months from sampling. Reduction of sugar was carried out using the Layne-Enyon method. 2.6 g of honey were weighed and then transferred to a 500 mL volumetric flask. Five milliliters of standardized Fehling A and B solutions were transferred to a 250 ml Erlenmeyer, with 7 mL of water and 15 mL of honey solution. The Erlenmeyer was heated and 1 mL methylene blue 0.2 % was added. Titration was carried out adding the diluted honey solution until the indicator was decolorized. Determining sucrose content was carried out by inversion, adding 10 mL of diluted HCl, 50 mL diluted honey solution and water to a 100 mL volumetric flask, heating in water bath, then cooling and diluting to mark. Finally the Lane-Enyon method was applied and sucrose content was obtained by difference (Rajakylä & Paloposki 1983). The diastase activity of the honey samples were assessed for demonstration of diastase enzyme. The diastase or amylase catalyses the starch and it produces maltose. We used the starch as substrate and determined the diastase activity (Schade et al. 1958).

Microbial evaluations

Pseudomonas aeruginosa ATCC 27853 was purchased from Institute of Standard and Industrial Research of Iran (ISIRI). The antimicrobial activity of honey samples against *P. aeruginosa* was determined by agar dilution assay (CLSI/NCCLS 2009). Briefly, Muller Hinton agar (MHA) containing different concentrations of honey (5-30%) were used as plates. *P. aeruginosa* were grown in BHI broth at 37°C for 12 hours before the analysis, bacterial suspensions were adjusted to a 0.5 McFarland standard by Spectrophotometer Equipment (OD_{600}). The bacterial suspension was diluted to a concentration of culture of 10^5-10^6 CFU/ml. 1 ml of bacterial suspension that serial is diluted was inoculated on the surface of the medium containing the honey samples. The plates were incubated at 35°C for 24 h. MHA containing sugar (equivalent to sugars of each honey sample) and without it is used as control plates. Then, bacterial growth was observed and set in special checklists as 0 (no growth), +1 (< 50000 colonies/plate), +2 (50000-100000 colonies/plate), and +3 (> 100000 colonies/plate). For heat sensitivity evaluation of honey samples against *P. aeruginosa*, the honey samples are heated at 80 °C for 1 hour and their antibacterial activity were compared before heating.

Results

Evaluation of reducing sugar and sucrose in 15 honey samples exhibited that six samples had enough scale for acceptance. Two honey samples were atypical samples. The diastase activity showed that two samples had diastase activity and others did not have any diastase activity. Therefore, 2 honey samples with positive diastase activity, 4 samples without diastase activity and 2 atypical samples with high concentration of sucrose were used for further evaluation. As results are shown in Table 1, two samples with diastase activity had 59, 60% and 63, 64% sugar before and after hydrolysis, respectively. Other honey samples had sugar ranging between 45.5%-51% and 55%-58%, respectively.

Table 1. Diastase activity and sugar concentration of honey samples.

Honey samples	Reducing sugar (%)		Sucrose (%)	Diastase enzyme
	Before heating	After heating		
1	59	63	3.8	+
2	60	64	3.9	+
3	51	55	3.8	-
4	51.5	54.5	2.8	-
5	51	54	3	-
6	55	60	4.7	-
7	50	60	9.6	-
8	45.5	58	11.8	-

The antimicrobial activity of honey samples are summarized in Table 2. The MIC values of honey samples against *P. aeruginosa* were in the ranges of 200->300 mg/ml. The most antipseudomonal activity of honey samples was related to sample 2 with 60% reducing sugar and 3.9% sucrose. The other effective honey was sample 1 with 59% and 3.8% reducing sugar and sucrose, respectively. The honey sample 7 showed less activity against *P. aeruginosa* with high concentration of sucrose (9.8%) and 50% reducing sugar. The hydrolysis of honey increased the concentration of reducing sugar. The heating of honey decreased the antimicrobial activity of honey samples. The heating increased the reducing sugar in honey and decreased the antipseudomonal activity of honey (Table 2, 3).

Table 2. Antibacterial activity of honey samples against *P. aeruginosa.*

Honey samples	Honey (% W/V)						Control
	5	10	15	20	25	30	Sugar
1	+3	+1	+1	+1	0	0	+3
2	+3	+3	+2	0	0	0	+3
3	+3	+3	+3	+3	+2	0	+3
4	+3	+3	+3	+3	+2	+2	+3
5	+3	+3	+3	+3	+2	+1	+3
6	+3	+3	+3	+3	+2	+2	+3
7	+3	+3	+3	+3	+3	+3	+3
8	+3	+3	+3	+3	+3	+2	+3

0: no growth, +1: < 50000, +2: 50000-100000, +3: > 100000; CFU/plate

Table 3. The effect of heating on antibacterial activity of natural honey 1.

Honey samples	Honey (% W/V)						Control
	5	10	15	20	25	30	Sugar
Before	+3	+1	+1	+1	0	0	+3
After	+3	+3	+2	+1	+1	0	+3

0: no growth, +1: < 50000, +2: 50000-100000, +3: > 100000; CFU/plate

Discussion

Honey is clinically used as an antiseptic and there is little or no information on the honey type. Different honey samples from different sources had varied antibacterial activity. Our investigation exhibited that different types of honey did not have any standard range of reducing sugars (68%). The concentration of sugars in some samples was in the upper range of 65% and it can relate to high sucrose concentration in honey. Antibacterial activity of honey samples against *P. aeruginosa* exhibited that *P. aeruginosa* grows rapidly in low concentrations of honey, whereas in higher concentrations, the bacterial growth was different. A direct relation was between antibacterial activity and honey concentration. The antibacterial activities of natural honeys without diastase enzymes were seen in higher concentrations and diastase enzymes decreased the dose effective on *P. aeruginosa*. So, the type of honey is a very important criterion for therapy purposes. Other literature showed that 25% honey have antibacterial activity against *P. aeruginosa* (Al-Jabri et al. 2003), but in another study the minimal inhibitory concentration of honey against *P. aeruginosa* was lower than 10% honey (Al-Waili 2004). Our study is in agreement with Al-Jabri et al. The high concentration of sugar had no antipseudomonal activity and could be used as positive culture in experiments. Heating the honey to 80 °C for 1 hour could decrease the antimicrobial activity of honey (Cooper et al, 2002). Honey is a complex mixture of components which affect its antimicrobial activity. It is not been yet cleared which component of honey has antimicrobial activity. Sixteen sulfonamide (Al-Waili 2004), Chloramphenicol (Pang et al. 2005), Acaricides (Al-Waili 2004), and other non-organic compounds such as oxalic acid, formic acid, lactic acid (Ishii et al. 2006), phenolic compounds (Arraez-Roman et al. 2006), and tylosin A-D (Nozal Nalda et al. 2006) were found in the honeys, which all components have antimicrobial activity. The antimicrobial activity of honey is related to its components or synergistic/antagonistic effect. Also, the kind of components and their amount is related to their sources of honeys.

Conclusion: Our results of this study and other similar research exhibited that high concentrations of natural types of honey have strong antimicrobial activity against resistant organisms such as *P. aeruginosa* which has different kind of resistant mechanisms to different antibiotics. So, therapeutic uses of honey for antiseptic purposes is needed to identify the natural type from its spurious honeys and because they are different in antimicrobial activity, we should evaluate each honey for its antimicrobial activity and then use its antiseptic agents.

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