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Extended Abstract

# The Investigation of the Physicochemical and Antioxidant Properties of Several Types of Honey and Comparing their Antimicrobial Effect on *Acinetobacter baumanii and Enterococcus faecalis*

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## Abstract

Nowadays, many complications from antibiotics have been created for humans, and bacteria have become resistant to a large number of them. Therefore, many researchers have focused on natural substances. The purpose of this study was to investigate the physicochemical and antioxidant characteristics of different honey including Pervoskia abrotanoides, spring product of 2016, Pervoskia abrotanoides, spring product of 2017, Chaste tree, spring product of 2017, cotton and sunflower, fall of 2017, from Neyshabur, On *Acinetobacter baumanii* (PTCC:1797) and *Enterococcus faecalis* (PTCC: 2015). Also, the comparison of the antimicrobial activity of these honeys in different concentrations (10, 20, 50 and 70%) was performed without the use of ampicillin and with ampicillin on the bacteria. The results showed that although all honey had a good quality, the honey of cotton and sunflower, significantly had better physicochemical properties than others. This honey had a higher content of phenolic compounds, antioxidant activity, acidity and proline and a higher specific gravity, and also had lower moisture and overall improved quality. According to the findings, all of the studied honeys had bacteriostatic properties and an increase in the concentration of honey increased their antimicrobial properties. It was also found that with the addition of ampicillin to honey, their antimicrobial effect increased. In terms of antimicrobial properties, the honey taken from cotton and sunflower was the most effective in comparison with the rest of the honey. Finally, the honey and especially fresh honey produced by cotton and sunflower with ampicillin can be effective in preventing the growth of *Acinetobacter baumanii and Enterococcus faecalis* bacteria.

**Keywords**: *Acinetobacter baumanii*, Antimicrobial, *Enterococcus faecalis*, Honey, Physicochemical

## Introduction

Nowadays, humans have faced many complications caused by antibiotics, and bacteria have become resistant to a large number of them. So many researchers are attracted to natural substances. Honey is one of the oldest traditional medicines used to treat microbial infections (Gheysari & Hamidian, 2009; Ray & Ryan, 2004). These characteristics differ from the type of honey that is associated with a group of chemical compounds and physical properties of this foodstuff, which is related to the herbaceous origin, geographic region, and insect studies of honey (Tong *et al*., 2014). *Acinetobacter Baumanii* is the most important member of the

Acinetobacter species in relation to hospital infections worldwide (Lin & Lan, 2014) and *Enterococcus faecalis*, a bacterial in human intestinal tract, and is involved in the development of nosocomial infections (Ray & Ryan, 2004). In the present study, the physicochemical properties and the effects of four different types of honey, alone and in combination with ampicillin, on *Enterococcus faecalis* and *Acinetobacter Baumanii* were investigated. In some countries ampicillin is used in conjunction with sulbactam against *Acinetobacter baumanni* (Smolyakov *et al*., 2003). If proven, the use of honey with ampicillin can inhibit the growth of this bacterium; it can be recommended that this substance be replaced with sulbactam. Also, infections caused by *Enterococcus faecalis* can be treated with ampicillin (Fisher & Phillips, 2009).

## Materials and methods

Different four honeys from Pervoskia abrotanoides, spring product 2015, Pervoskia abrotanoides, spring product 2017, Chaste tree, spring product 2017, cotton and sunflower, fall 2017, purchased from Neyshabur (Khorasan Razavi, Iran) were used. Standard strains of bacteria were obtained from the Iranian Scientific and Industrial Research Organization. Culture media as well as chemical and antibiotic discs were prepared from Merck (Germany).
In order to evaluate the quality of honey, their antioxidant and physicochemical properties were investigated. Moisture, PH, acidity, sugar, Hydroxymethylfurfural (HMF), diastase and proline were tested according to National Iranian Standard Methods (Iranian National Standardization Organization [ISISRI], No. 92, 2013b). Specific gravity, electrical conductivity, ash, antioxidant activity and phenolic compounds were also measured according to standard methods (Bogdanov & Martin, 2002; Ferreira, Aires, Barreira, & Estevinho, 2009; Parvaneh, 2011; Piazza, Accorti, & Persano Oddo, 1991; Zainoldin & Baba, 2009). To investigate the effect of different concentrations of honey on bacteria, Almasaudi *et al*. (2017) methods were performed. Antibiotic test was performed by disc diffusion method. The whole experiment was performed in 3 replications. SPSS software was used to analyze the data and Duncan's test was used at 5% level to compare the means.

## Results and discussion

According to the results (Table 1) and considering the national standard of Iran, moisture content of all samples was within the standard range. Therefore, the studied honey did not have the excess moisture that caused them to ferment and corrode, resulting in low quality and antimicrobial properties of the honey. The lowest moisture content of honeys was observed in the honey coming from cotton and sunflower plants ($P \leq 0.05$). The results (Table 1) confirm the relationship between specific gravity and moisture content. That is, when the moisture content decreases, the specific weight of the honey increases. Considering the desirability of moisture and therefore the desirable condition of the specific weight of honey, it can be concluded that the studied honey of this study was of good quality and natural. Among the honey, the honey of cotton and sunflower seeds had the highest specific weight.

The electrical conductivity of honey is high in content of mineral salts, organic acids and proteins, which is highly variable according to the type of flower (Juszczak, Socha, Rożnowski, Fortuna, & Nalepka, 2009; Terrab, Díez, & Heredia, 2002). According to Table (1), all results were within the standard range (Iranian National Standardization Organization [ISISRI], No. 92, 2013b). The highest electrical conductivity was obtained for honey from cotton and sunflower plants.

**Table 1.** Results of physicochemical and antioxidant tests of honey samples

| Standard | cotton and sunflower | Chaste tree, spring | Pervoskia abrotanoides 1395 | Pervoskia abrotanoides 1395 | Property |
|---|---|---|---|---|---|
| Maximum 20 | $14.59^{b}$±0.040 | $15.14^{a}$±0.00 | $15.35^{a}$±0.00 | $14.75^{b}$±0.00 | Moisture (%) |
| | $1.33^{a}$±0.002 | $1.32^{bc}$±0.00 | $1.33^{b}$±0.00 | $1.33^{c}$±0.00 | Specific gravity($g.ml^{-1}$) |
| Maximum 0.8 | $0.46^{a}$±0.310 | $0.17^{b}$±0.00 | $0.16^{c}$±0.11 | $0.015^{d}$±0.01 | Electrical conductivity ($ms.cm^{-1}$) |
| Maximum 0.6 | $0.18^{a}$±0.001 | $0.06^{b}$±0.01 | $0.01^{c}$±0.00 | $0.00^{c}$±0.001 | Ash (%) |
| Maximum 3.5 | $4.37^{c}$±0.100 | $5.27^{a}$±0.007 | $4.42^{b}$±0.015 | $4.34^{d}$±0.011 | pH |
| Maximum 40 | $19.00^{a}$±0.21 | $17.67^{d}$±0.05 | $17.27^{b}$±0.50 | $15.34^{c}$±0.51 | acidity ($meq.kg^{-1}$) |
| Maximum 40 | $17.98^{a}$±0.23 | $12.57^{c}$±0.31 | $11.189^{d}$±0.11 | $13.80^{b}$±0.15 | )$mg.kg^{-1}$ (HMF |
| at least 65 | $83.25^{a}$±0.23 | $75.15^{c}$±0.47 | $74.85^{d}$±0.90 | $77.85^{b}$±0.11 | Reducing sugars (%) |
| | $1.38^{c}$±0.25 | $4.88^{a}$±0.37 | $2.15^{b}$±0.33 | $1.45^{c}$±0.21 | Sucrose (%) |
| Maximum 5 | $48.83^{a}$±0.99 | $38.27^{d}$±0.31 | $46.83^{b}$±0.30 | $46.38^{c}$±0.47 | Fructose (%) |
| | $35.15^{b}$±1.56 | $36.95^{a}$±1.04 | $27.95^{d}$±0.90 | $31.55^{c}$±0.72 | Glucose (%) |
| at least 0.9 | $1.38^{c}$±0.023 | $1.02^{d}$±0.036 | $1.64^{a}$±0.045 | $1.47^{b}$±0.093 | Fructose-glucose ratio |
| | $12.20^{b}$±0.30 | $8.56^{c}$±0.056 | $19.50^{a}$±0.22 | $5.00^{d}$±0.31 | Diastase (DN) |
| at least 3 | $16.78^{a}$±0.05 | $9.76^{d}$±0.03 | $11.76^{c}$±0.01 | $14.23^{b}$±0.07 | Antioxidant activity (%) |
| | $23.18^{a}$±0.18 | $17.71^{d}$±0.11 | $20.25^{c}$±0.31 | $21.35^{b}$±0.01 | Phenolic compounds ($mgGAE.100g\ honey^{-1}$) |
| at least 180 | $528.75^{a}$±0.40 | $345.13^{b}$±0.03 | $261.23^{d}$±0.11 | $267.81^{c}$±0.1 | Proline (mg.kg-1) |

The results are expressed as the amount of Average ± the standard deviation. Different Latin letters in each row indicate a significant difference at least at ($P$<0.05). Respectively a shows maximum and d shows the least amount of each property.

The amount of ash of honey depends on the type of plant used by the bee (Ramzi, Kashaninejad, Sadeghi Mahoonak, & Razavi, 2015). According to the results presented in Table (1), all honey was pure and well-treated, and within the standard range. As seen in the results, the highest amount of ash and electrical conductivity is related to the honey coming from cotton and sunflower plants.

The pH value was in normal range in all samples. In a study comparing the physicochemical properties of natural honey with counterfeit honey by Ramzi *et al*. (2015), the pH was found to be 3.73, indicating that the honey was natural. Acidity of honey is due to the presence of organic acids, mainly gluconic, pyroic, maleic and citric acids as well as inorganic phosphate, sulfate and chloride ions (Kamkar & Khodabakhshiyan, 2017). According to the results of this study (Table 1), the acidity of honey was in accordance with the standard.

HMF is produced in the heat treatment process by removing the water molecule from acidic hexoses such as fructose and glucose under catalyzed conditions (Terrab *et al*., 2002). According to the results in Table (1), HMF was the highest for honey in cotton and sunflower plants, which was kept in poor condition. However, all values were at the standard level.

The results presented in Table (1) indicated that the amount of reducing sugars in all honey was higher than the amount specified in the National Honey Standard (at least 65). High osmolarity of honey, due to its high levels of sugar, causing an inappropriate environment for bacteria and, as a result, prevents growth. Because honey from cotton and sunflower plants has the highest sugar content, this property can play an important role in its antimicrobial properties. According to the results, the fructose-glucose ratio in all samples is higher than the minimum amount specified in the national honey standard (at least 0.9). Diastasis activity indicates the natural and fresh honey (Cantarelli, Pellerano, Marchevsky, & Camiña, 2008). According to the results presented in Table (1), all honey has a diastolic activity greater than the minimum value (DN 3).

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The antioxidant activity of natural honey is due to the presence of many different substances such as enzymes, organic acids, phenolic compounds, flavonoids, carotenoids, amino acids and ascorbic acid (Hussein, Yusoff, Makpol, & Yusof, 2011). The most antioxidant activity and the highest amount of phenolic compounds related to honey were cotton and sunflower plants (Table 1). (Socha *et al*., 2011) examined the antioxidant activity and phenolic compounds of eight different honey samples and found that with increasing phenolic compounds, the antioxidant activity of honey increased.

Proline is the main amino acid of honey, which is an indicator of the quality of honey and has an acidic effect on the antimicrobial effect of honey (Iranian National Standardization Organization [ISIRI], No. 11145, 2013a). The highest amount of proline was found for honey from cotton and sunflower plants (Table 1), which can be considered as one of the most important antimicrobial agents of this type of honey compared to the rest of the them. In the turbidity method, the results of bacterial growth on Agar culture medium revealed that all of the honey tested had bacteriostatic effects. The most inhibitory effect on both bacteria was honey from cotton and sunflower seeds. Antimicrobial activity was increased by increasing the concentration of honey in the culture medium. The results also showed that the combined use of honey and ampicillin was more effective against *Enterococcus faecalis* and *Acinetobacter baumanii* bacteria. Other studies have shown that honey can destroy the cell wall of bacteria. Honey can, therefore, be used against all bacteria that are resistant to antibiotics that affect the cell wall, such as ampicillin or other lactams, alone or in combination (Brudzynski & Sjaarda, 2014).

## Conclusions

The antimicrobial results of the honey matched with their physicochemical and antioxidant properties The Honey from cotton and sunflower plants had the highest antioxidant activity and its antimicrobial effect was higher than the rest of the honey. This honey, combined with the optimum concentration of ampicillin, showed the highest growth inhibitory effect on *Enterococcus faecalis* and *Acinetobacter Bumanni* bacteria, so they can be used against infections caused by them.

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