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Evaluation of Antioxidant and Antibacterial Activities of *Apis florea* Fabricius (Hymenoptera: Apidae) Honey on *Helicobacter pylori*

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Abstract

Iranian men are at risk of developing gastrointestinal cancer caused by *H. pylori*. It is very imperative to find effective methods to control this bacterium as there are currently no very effective treatments for it. Honey has been shown to have antimicrobial properties against various pathogens. This study analyzed 15 honey samples from *A. florea* bees, collected from different floral and geographical origins, for their antimicrobial efficacy against *H. pylori*. Using atomic absorption measurements, the honey samples were also tested for their phenolic and flavonoid content, protein concentration, and mineral content. Antioxidant activity was determined using the FRAP, DPPH, and ABTS methods. The antibacterial activity of honey samples was investigated both *in-vitro* and *in-vivo* in the gastrointestinal tract of mice. Statistical analysis revealed a significant positive correlation between antioxidant activity and antibacterial activity. All honey samples showed antimicrobial activity *in-vitro*, among which jujube honey from Bushehr exhibiting the highest activity. Differences in antioxidant and antimicrobial activities were likely due to the flora of the plants and the geographic region from which the honey was harvested. Based on these results, *A. florea* honey may be used in the prevention and treatment of *H. pylori*-associated infections and inflammation of the gastrointestinal tract. This feature can be applied to the control of *Helicobacter pylori* along with other available measures.

Keywords: Antioxidant activity, Antimicrobial activities, Honey, *H. pylori*

Introduction

H. pylori is a gram-negative bacterium that is resistant to gastric acid and colonizes the gastrointestinal tract (Khatun *et al.*, 2013). It is a known carcinogen and has been classified as a Class I carcinogen by the World Health Organization. Chronic inflammation caused by *H. pylori* infection increases the risk of gastric and duodenal ulcers, which can lead to gastric cancer (Graham, 2015). It is a known risk factor for gastric cancer development and its pathogenesis is associated with oxidative stress. *H. pylori* infection induces the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the host, leading to oxidative damage in gastric epithelial cells. Host antioxidant systems are activated to counteract this damage, including enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). However, *H.*

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pylori have evolved strategies to evade host immune responses and antioxidant defense systems, resulting in chronic inflammation and increased cancer risk. Despite its prevalence, most infected people (80%) are asymptomatic. However, long-term infection significantly increases the risk of cancer (Saha *et al.*, 2010; Wroblewski *et al.*, 2010). Although many different drugs have been tested to treat *H. pylori*, none have been shown to be effective (Take *et al.*, 2015). Due to the side effects and antibiotic resistance associated with antibiotic treatment, researchers are exploring natural compounds with anti-inflammatory and antimicrobial properties (Bonacorsi *et al.*, 2009). Honey is gaining attention for its antibacterial and antioxidant properties, and its clinical use is increasing (Selahvarzian *et al.*, 2015).

Honey is believed to be the first product discovered by prehistoric people, compared to other products produced by honey bees. Honey is produced by two species of honey bees: the European honey bee (*Apis mellifera*) and the Asian dwarf honey bee (*A. florea*), and is stored in honeycombs after a series of interactions in their digestive tracts. *A. florea* is found in Iran from the South West (Khuzestan Province) to the South (Boushehr Province) and the South East (Sistan and Balouchestan Provinces) (Parichehreh *et al.*, 2020). The high osmolality and antibacterial properties of honey make it a useful substance to promote human health. The effectiveness of honey in this regard is influenced by the species of honey bees, the plants they feed on, and the local climate (Aliyazicioglu and Boukraa, 2015). The antibacterial effect of honey is mainly due to the presence of hydrogen peroxide, which depends on the levels of glucose oxidase and catalase (Weston, 2000). These levels can vary between different types of honey and contribute to differences in their antimicrobial properties. The antibacterial and antioxidant properties of honey are due to the presence of lysozyme, phenolic acids and flavonoids (Snowdon and Cliver, 1995). Honey is a unique wound dressing as it can clear the infection, promote rapid wound healing, inhibit inflammation, minimize scarring, stimulate angiogenesis, and expand epithelial tissue in a short time (Molan, 2002). Numerous studies have investigated the antimicrobial activity of honey from different botanical and geographical origins. For example, the antibacterial activity of honey from *A. mellifera* was studied by Selahvarzian *et al.* (2015), who found that honey from bees fed on licorice extract showed the highest antibacterial activity. Another study by Boyanova *et al.* (2015) found that honey of *A. mellifera* reduced the risk of *H. pylori* infection in 150 Bulgarian patients. Patients who consumed honey more than once a week had a lower rate of *H. pylori* infection than those who did not consume honey. Research conducted by Grego *et al.* (2016) on Italian honey highlighted that the antimicrobial activity of honeydew, polyfloral, and chestnut honey against *S. aureus* was similar to that of manuka honey. A study by Gośliński *et al.* (2020) compared the antioxidant and antimicrobial properties of manuka honey and Polish honey. The results showed that manuka honey had higher antioxidant capacity and stronger antimicrobial activity than Polish honey, suggesting that it may be a more effective natural remedy in the prevention or treatment bacterial infections and oxidative stress-related conditions. Kolayli *et al.* (2020) reported strong antimicrobial activity against *S. aureus* in buckwheat honey (*Fagopyrum eculentum*), heather honey (*Calluna vulgaris*), nettle or urtica honey (*Urtica dioica*), thistle honey (*Silybium marianum*), caltrop honey (*Eryngium campestre*), coriander honey (*Coriandrum sativum*), thyme honey (*Thymus vulgaris*), and honeydew. They also observed moderate antimicrobial activity in heather honey (*Calluna vulgaris*) and honeydew against *E. coli*, and heather honey (*Calluna vulgaris*) against *C. albicans*.

While *A. mellifera* honey has been extensively studied for its antioxidant and antibacterial activity, there is not sufficient research on the case of *A. florea* honey. Therefore, the study aimed at assessing the

antioxidant and antibacterial properties of *A. florea* honey collected from various regions in Iran including Bushehr, Dezful, Iranshahr, Jahrom, and Jiroft, which are characterized by different vegetation types. In this investigation, we tried to clarify the potential health benefits of *A. florea* honey and how it could be used as a natural remedy for various illnesses.

Materials and Methods

Preparation of samples

In August and September 2019, 15 samples of *A. florea* honey (1-2 kg per region) were collected from the southern region of Iran including Bushehr, Dezful, Iranshahr, Jahrom and Jiroft.

Identification of mineral compounds

Mineral content in honey samples was measured using an atomic absorption spectrophotometer (Tosic *et al.*, 2017).

Determination of total phenolic compounds

Total phenolic compounds were determined using the Folin-Ciocalteu colorimetric method using gallic acid as a standard at 760 nm (Singleton *et al*., 1999).

Identification of phenolic and flavonoid compounds

Analysis of phenolic and flavonoid compounds of honey samples was performed by high-performance liquid chromatography (HPLC) (Agilent 1200-Germany) detector of Diode Array at 260 nm. For this purpose, 300 µl of the solution was injected into the instrument. The mobile phase consisted of water/acetic acid (ratio 1.19 v/v) (solvent A) and methanol (solvent B) at a constant flow rate of 1 mL/min. The column temperature was kept constant at 30°C and the chromatograms were processed with Chemstation chromatography software (Mello *et al.*, 2010). A C18 reversed-phase Acquity column (1.7 µm, 150 mm, 4.6 mm) protected by a guard column was used in this study.

Investigation of antioxidant activity of honey samples

The antioxidant activity of honey samples was measured using ABTS, FRAP and DPPH methods.

Trolox equivalent antioxidant activity method (ABTS)

The antioxidant activity of honey was determined according to the method of Re *et al.* (1999) using the Trolox equivalents (TEAC) as a measure of antioxidant activity. ABTS was obtained by reacting the prepared 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate ($K_2S_2O_8$). Samples were stored in the dark at room temperature for 12-16 h. 160 µL of ABTS+ solution was added to 40 µL of the sample at different concentrations. Absorbance at 734 nm was measured after incubation for 10 min at room temperature using a 96-well microplate reader.

FRAP method

The ferric ion regenerative antioxidant activity (FRAP) method was measured following the method of Benzie and Strain (1996) with some modifications. The honey sample was first dissolved in 10 mL of n-hexane-acetone mixture (6:4) and then filtered through the Whatman number 4 filter paper. The honey solution was mixed with 1.8 mL of FRAP reagent, and the absorbance of the reagent mixture was measured spectrophotometrically at 593 nm after incubation for 10 min. The calibration curve was constructed using Trolox, and the results were expressed as mg of Trolox equivalent (TE) per 100 gr of honey.

DPPH method

The antioxidant activity of honey samples was measured by diphenyl picrylhydrazyl (DPPH) at 517 nm according to the method of Von Gadow *et al.* (1997). Honey samples were dissolved in 5 mL of methanol and filtered through Whatman #4. Subsequently, 100 mL of honey, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) in methanol

were mixed with 2.9 mL of a $6 \times 10{-}5$ M solution of DPPH in methanol. The mixture was shaken vigorously and left in the dark at 25°C for 60 min. The absorbance of the solution was measured at 517 nm against a methanol blank using a spectrophotometer (Hitachi U-1900, Japan).

Measuring protein levels

Protein concentration was measured according to the method described by Bradford (1976).

***In-vitro* antibacterial activities of different honey samples**

Testing was performed using the agar well diffusion method (de Queiroz Pimentel *et al*., 2013). After preparing Muller-Hinton agar according to the manufacturer's instruction, 0.5 McFarland bacterial suspension was plated in four wells of each plate using a sterile Pasteur pipette. After the Hinton agar was thawed and sealed, 80 µL of each honey sample was poured into each well. After 37 h of incubation at 37°C, the size of the zone of growth inhibition was measured with a ruler. Each test was repeated three times and recorded.

***In-vivo* antibacterial activities of different honey samples**

Honey samples were tested for antimicrobial activity against *H. pylori* provided by the Iranian National Center for Genetic and Biological Resources.

BALB/c mice (6-7 weeks old) were provided by Razi Vaccine and Serum Research Institute, Tehran and were kept at 25°C and 12:12 L:D under pathogen-free conditions. The mice were fed autoclaved food and water every 2 days. After the bacterial strain was cultured on Nutrient Agar (NA) and placed under anaerobic conditions at 24°C for 24 h, the cells were washed twice with distilled water and a suspension of 10^9 CFU/mL was administered to the mice. In this study, an orogastric tube was inserted into the stomach of mice to observe the occurrence of infection and changes (ulcers, decay, and abnormal tissue growth) (Shamala *et al*., 2002).

Evaluation of gastritis and status of *H. pylori* infection after treatments

A total of sixty healthy female BALB/c mice were prepared and kept at the above-mentioned conditions. There were six groups of 10 mice, and two were considered control groups (one infected with the bacteria but not eating honey, and 1 without the infection). After introducing *H. pylori* into the gastrointestinal tract of mice by gavage, several mice were randomly selected and their stomachs were sampled. After inoculation with *H. pylori*, the mice were fed honey at a concentration of 6:1 (85.7% water: 14.3% honey) every other day, mice stomachs were removed after 75 days and placed in a Styrofoam tray covered with drawing paper. They were then fixed in 10% formaldehyde for 30 days, divided into four equal parts and embedded in paraffin. These four pieces (5 µm thick) were cut horizontally, immersed in albumin solution overnight at 40°C before staining with hematoxylin and eosin (Boldt *et al*., 2015). Stained sections were observed under an optical microscope.

Scoring gastric inflammation

The Sydney system was also used to assess the degree of inflammation in addition to detecting *H. pylori* contamination (Jones *et al*., 2002; Nakamura *et al*., 2002). Grading and scoring are based on endoscopic and histopathological criteria, which are more concerned with topography, morphology, and degree of inflammation (zero = absent, + (mild), ++ (moderate), +++ (severe)). *H. pylori* can also be treated by scoring: zero = absence of *H. pylori*, one = low presence of bacteria up to 5 glands, 2 = moderate presence of bacteria from 6 to 10 glands, 3 = high presence of *H. pylori* from 11 glands upwards.

Statistical analysis

Physicochemical results were reported as mean ± SD (standard deviation) of triplicate samples and statistical differences were tested

using one-way analysis of variance (ANOVA). Differences on the histology score were tested by the Mann-Whitney U test. Both tests considered the results as statistically significant when $p < 0.05$.

Results

Inorganic compounds

The mineral compounds identified in the honey samples are listed in Table 1. The highest and lowest calcium levels were recorded for Bushehr and Jahrom honey samples with 187.70 and 86.08 ppm, respectively. The highest and lowest magnesium levels were measured in Iranshahr (460.09 ppm) and Jiroft (55/43 ppm). In addition, the highest and lowest levels of phosphorus were 7.57 and 0.36 ppm for Iranshahr and Jahrom, respectively. The highest and lowest zinc was recorded for Jiroft (10.03 ppm) and Iranshahr (7.63 ppm), and the highest and lowest percentages of potassium were for Bushehr (846.21 ppm) and Iranshahr (412.15 ppm). Iranshahr honey samples had the highest iron concentration, while Jiroft honey samples had the lowest iron concentration (Table 1).

Table 1- Assessment of mineral compounds in different honey samples

Sample	Ca (ppm)	Mg (ppm)	P (ppm)	Zn (ppm)	K (ppm)	Fe (ppm)
Dezful	187.17	371.07	0.39	9.38	843.82	144.28
Iranshahr	83.80	460.09	5.24	7.63	412.15	219.99
Bushehr	187.70	171.75	0.39	8.51	846.21	117.32
Jahrom	86.08	288.79	0.36	9.50	776.10	114.77
Jiroft	136.32	55.43	7.57	10.03	819.39	30.29

Protein content

The protein content of honey samples was calculated 94.23 ± 0.85, 102.11 ± 0.55, 137.92 ± 0.25, 138.75 ± 0.27, and 133.44 ± 0.12 ppm for Dezful, Iranshahr, Bushehr, Jahrom, and Jiroft respectively (Fig. 1). Statistical analysis of the results showed that the samples differed significantly in protein content (F: 4873.09, df: 14, P: 0.000).

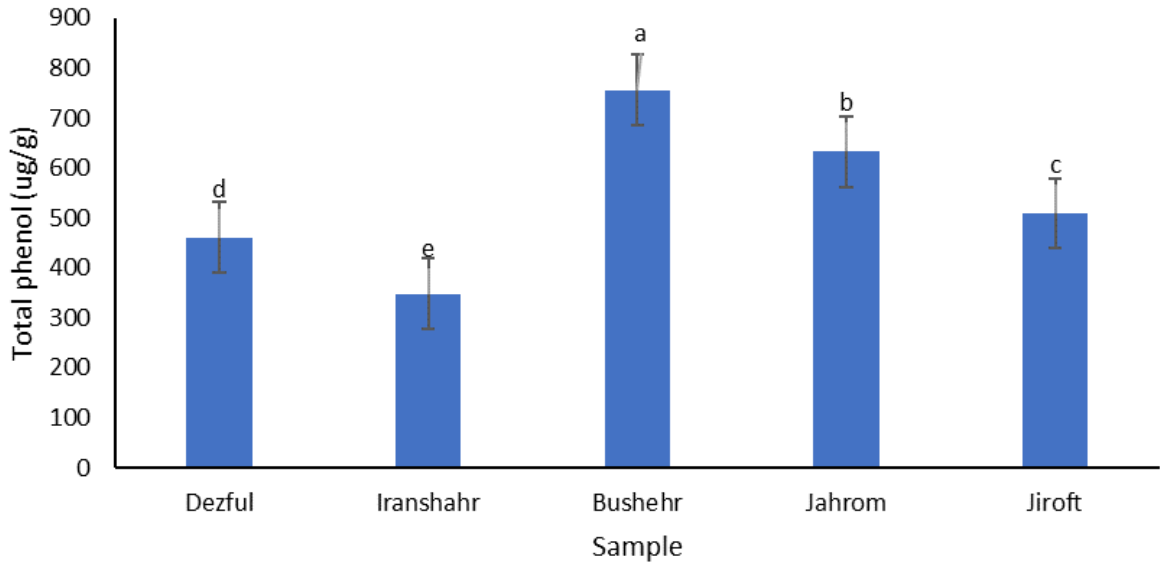

Fig. 1- The amount of total phenol in honey samples
Different letters denote significantly different values from one another (LSD) at $P \leq 0.01$ level.

Phenol and flavonoids

Phenolic and flavonoid compounds of honey samples were identified by HPLC chromatography. Isolated compounds identified in honey samples are shown in Table 2. The compounds included gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, quercetin, kampefrol, hespertin, and apegenin (Table 2). It was found that the most extracted compounds in the extracts of Jiroft, Bushehr, Iranshahr, Jahrom, and Dezful honey were apigenin (54.16%), apigenin (94.51%), kaempferol (66.95%), apigenin (46.90%), and kaempferol (47.15%), respectively.

Table 2- Analysis of phenolic and flavonoid compounds of honey samples by HPLC

Compounds	Dezful	Iranshahr	Bushehr	Jahrom	Jiroft
Gallic acid	0.13*	0.30	0.16	0	0
Chlorogenic acid	0	0	2.64	33.40	0
Caffeic acid	0.69	0.07	0.05	0.10	0.07
p-Coumaric acid	0	0	0	8.04	5.11
Quercetin	0.1	0.14	0.02	0.03	0.05
Kampefrol	2.81	2.35	5.75	11.17	2.78
hespertin	0.31	0.31	0.38	0.62	0.31
Apegenin	1.92	0.34	154.98	47.14	9.83

*Retention time

Total phenol content

The highest and lowest phenol contents were observed in Bushehr (755.83 g/g) and Iranshahr (348.83 g/g), respectively (Fig. 2). Based on statistical analysis, there were significant differences in total phenol content among the collected samples (F: 38.03, df: 14, P: 0.000).

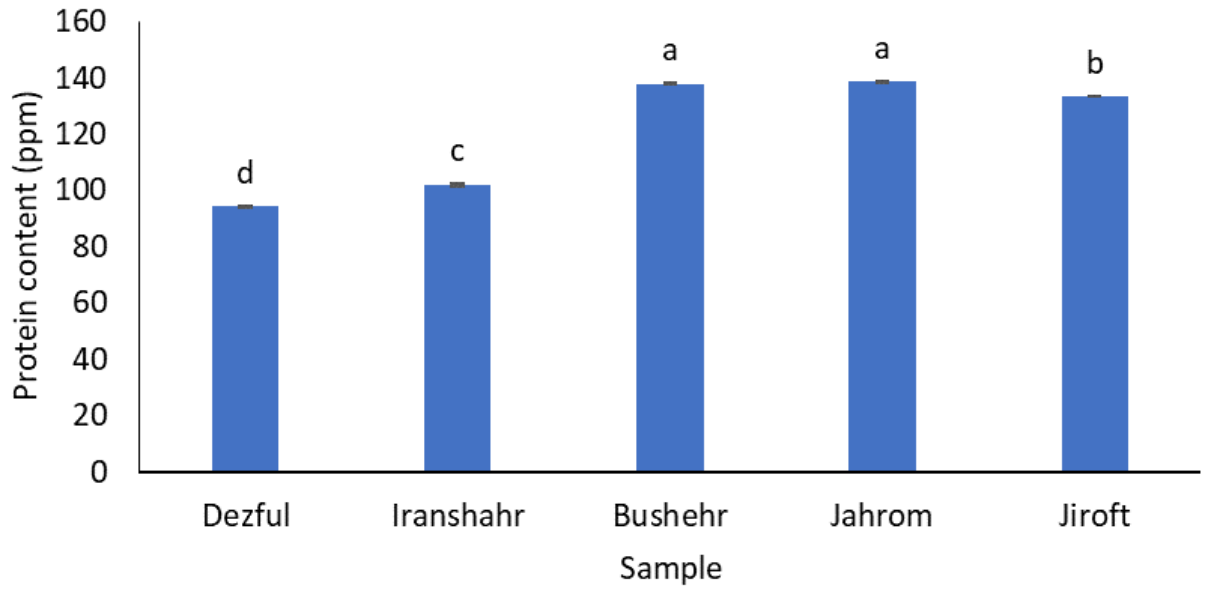

Fig. 2- Measurement of protein content in different honey samples
Different letters denote significantly different values from one another (LSD) at P ≤ 0.01 level.

Antioxidant activity

The antioxidant activity of different honey samples was measured by three methods: ABTS, FRAP, and DPPH. According to ABTS, the highest and lowest levels of antioxidant activity were found in Bushehr (786.40 ± 29.73 µg/g) and Iranshahr (324.90 ± 4.90 µg/g samples (Table 3). Based on statistical analysis,

significant differences were found among honey samples in terms of antioxidant activity (F: 64.40, df: 14, P: 0.000). There were also significant differences in antioxidant activity between different samples according to the FRAP method (F: 14.32, df: 14, P: 0.000). Dezful, Iranshahr, Bushehr Jahrom, and Jiroft honey samples were tested by FRAP method for antioxidant activity, among which Bushehr and Iranshahr samples showing the maximum and minimum antioxidant activity, respectively (Table 3). The DPPH method confirmed the results of the ABTS and FRAP methods for honey antioxidant activity (F: 41.77, df: 14, P: 0.000). According to Table 3, Bushehr honey samples (625.76 ± 26.17 µg/g) and Iranshahr honey samples (393.03 ± 11.98 µg/g) had the maximum and minimum antioxidant activities, respectively (Table 3).

Table 3- Antioxidant power of honey samples collected from different areas
Different letters denote significantly different values from one another (LSD).

Sample	Antioxidant capacity (µgr/gr)		
	ABTS (Mean±SE)	FRAP (Mean±SE)	DPPH (Mean±SE)
Dezful	533.44±19.9 ^{c}	23.28±0.80 ^{ab}	424.64±4.63 ^{d}
Iranshahr	324.90±4.90 ^{d}	14.41±0.95 ^{d}	393.03±11.98 ^{d}
Bushehr	786.40±29.73 ^{a}	26.98±0.92 ^{a}	625.20±26.17 ^{a}
Jahrom	691.96±32.38 ^{b}	23.37±1.81 ^{ab}	570.62±16.58 ^{b}
Jiroft	626.19±6.84 ^{b}	19.97±1.45 ^{c}	472.73±5.59 ^{c}

Different letters indicate significant differences

Antibacterial activities

Table 4 shows the antibacterial activities of *A. florea* honey samples. A 50% decrease in honey concentration *in-vitro* resulted in a decrease in the diameter of the zone of growth inhibition in all samples. This indicates a strong relationship between honey concentration and antimicrobial activity. The antimicrobial activities of honey samples were significantly different based on statistical analysis. Bushehr and Iranshahr honey samples had the highest and lowest antimicrobial activities, respectively, with growth halo diameters of 19.17 ± 0.12, and 13.14 ± 13.09 (mm), respectively.

Based on histopathological observations and the classification scale, inflammation (no = 0, + mild, ++ moderate, +++ severe) was rated (Table 5). A mild inflammatory response and severe mucosal degeneration have been observed in Dezful honey (Fig. 3-a). In the Bushehr honey sample, gastric mucosa showed mild inflammation and mild degeneration, along with severe eosinophilia (Fig. 3-b). Fig. 3c shows gastric mucosa with mild inflammation and moderate degeneration and moderate to severe eosinophilia in the Jahrom honey sample. Furthermore, Jiroft honey samples showed mild inflammatory processes, moderate to severe degeneration, and severe eosinophilia in the gastric mucosa (Fig. 3-e). In the Iranshahr sample, mild inflammatory and moderate to severe degeneration and moderate presence of eosinophils in gastric mucosa were observed (Fig. 3-g). The positive control group showed moderate inflammatory reactions and severe degeneration (Fig. 3-d). According to the results, Bushehr honey and Jahrom honey had less inflammation and degeneration and had higher eosinophil content, so they had higher control against *H. pylori* than other honeys, which is similar to the results of *in-vitro* analysis.

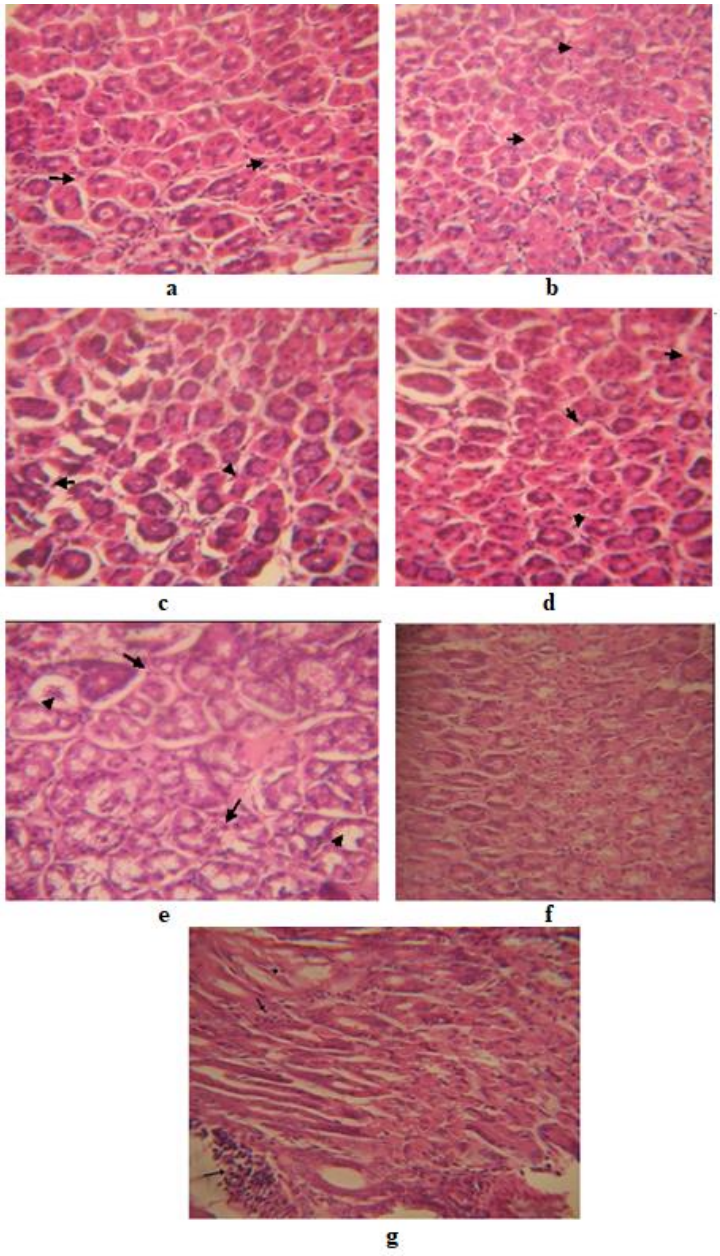

Fig. 3- (a-g). Stomach. Gastric mucosa of mice infected with *Helicobacter pylori* after different honey treatment groups

Stomach. Dezful. Mild inflammatory process and severe degeneration in the gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Bushehr. Mild inflammatory process, mild degeneration and severe presence of eosinophils in the gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Jahrom. Mild inflammatory process, relative degeneration and moderate to severe presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Positive control. Moderate inflammatory process, mild degeneration was observed. Haematoxylin and eosin× 400.

Stomach. Jiroft. Mild inflammatory process, moderate to severe degeneration and severe presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Stomach. Negative control. Haematoxylin and eosin× 400.

Stomach. Iranshahr. Mild inflammatory process, moderate to severe degeneration and moderate presence of eosinophils in gastric mucosa were observed. Haematoxylin and eosin× 400.

Table 4- Diameter of growth inhibition zone of *Heliobacter pylori* in different concentrations of different honey samples

Different letters denote significantly different values from one another (LSD).

Sample	Diameter of growth inhibition zone (mm)	
	100% solution	50% solution
Dezful	14.83±0.15 ^{c}	12.74±0.05 ^{C}
Iranshahr	13.09±0.04 ^{d}	11.32±0.07 ^{D}
Bushehr	19.17±0.12 ^{a}	17.53±0.13 ^{A}
Jahrom	18.83±0.06 ^{ab}	11.65±0.13 ^{D}
Jiroft	18.36±0.15 ^{b}	14.22±0.07 ^{B}

Table 5– Presence of *Helicobacter pylori*, inflammation, degeneration, and eosinophil in the gastrointestinal tract of mice, according to the Sydney System classification scale (0 = Absent, + = Mild, ++ = Moderate, +++ = Severe)

Sample	Inflammation	Degeneration	Eosinophil
Dezful	1	3	1.5
Iranshahr	0.5	2	1.5
Bushehr	0.5	1	3
Jahrom			
Jiroft	1.5	1	1.5
Negative control			
Positive control			

Correlation between antioxidant and antibacterial properties

As shown in Table 6, a Pearson correlation analysis was conducted between the antioxidant and antibacterial activities of honey samples. The results showed that antibacterial activity against *H. pylori* was positively correlated with honey samples' antioxidant content.

Table 6- Correlation between antioxidant and antibacterial activities of honey samples against *Helicobacter pylori*

		ABTS	DPPH	FRAP
Antibacterial peroperties	Pearson correlation	0.929	0.851	0.672
	Sig	0.000	0.000	0.000
	N	15	15	15

Discussion

Honey is a popular natural food that contains both organic and inorganic compounds. Its composition is influenced by natural and anthropogenic factors such as vegetation diversity and geography. Honey contains small amounts of protein and lipids, but its composition is mostly composed of fructose and glucose (65%), water (18%), and trace amounts of protein (Khalil *et al.*, 2001; Silva *et al.*, 2009). The presence of minerals and heavy metals in honey can affect its quality, with light-colored honey containing 0.04% minerals and dark-colored honey containing 0.2% minerals (Bogdanov *et al.*, 2007). Minerals derived from organic or plant sources have been reported to have beneficial effects on human health, whereas minerals from inorganic or heavy metal sources can be toxic (Hernández *et al.*, 2005; Pohl *et al.*, 2009). In this study, we found that 0.04-0.2% of inorganic compounds were present in *A. florea* honey collected from different regions of Iran, which varied in vegetation types, including alfalfa (*Medicago sativa*), *Astragalus* spp., *Citrus*, mesquites (*Prosopis* spp.), and jujube (*Ziziphus spina-christi*).

The phenol and flavonoid profiles of *A. florea* honey have not yet been determined. However, studies of *A. mellifera* honey have shown that the profile of these compounds depends on the dominant flora and

geographical region. Gallic acid has been identified as the most abundant phenolic compound in numerous honey samples (Cheung *et al.*, 2019). HPLC analysis of 40 samples of *A. mellifera* honey revealed 16 phenolic and 14 flavonoid compounds, with gallic acid and chrysin being the most abundant (Cheung *et al.*, 2019). The gallic acid content of honey collected in Australia ranged from 13.9 to 45.2 µg/g, with differences attributed to geographical variations and the types of plants the honeybees were feeding on (Yaoa *et al.*, 2005). In this study, we found that *A. florea* honey samples contained chlorogenic and caffeic acids, which have antibacterial properties (Estevinho *et al.*, 2008). Apigenin was the predominant phenolic compound identified in the Jiroft, Bushehr and Jahrom honey samples, and kampefrol was the predominant compound identified in the Iranshahr and Dezful honey samples. Phenols and flavonoids are naturally occurring compounds found in honey and have been shown to have a positive correlation with the antioxidant effect of honey samples. These compounds also exhibit antimicrobial properties. In this study, the antioxidant activity of honey samples was evaluated using three methods: ABTS, FRAP, and DPPH. The results indicated that the honey samples collected from Bushehr and Iranshahr had the highest and lowest antioxidant activity, respectively, by all three methods. The antioxidant activity of natural honey is attributed to the presence of various compounds such as enzymes, organic acids, phenolic acids, flavonoids, carotenoids, amino acids, and ascorbic acid (Hussein *et al.*, 2011). Different types of honey contain different amounts of antioxidants, especially phenolic compounds, and exhibit different antioxidant activity. These antioxidants are highly dependent on the number of hydroxyl groups attached to the benzene ring of these compounds. . In this study, the total concentrations of phenolic and flavonoid compounds identified in honey samples from Dezful, Iranshahr, Bushehr, Jahrom, and Jiroft honey samples were 5.96, 3.51, 163.98, 100.5, and 18.15 µg/g, respectively. The Iranshahr honey sample had the lowest levels of phenols and flavonoids among the other honey samples, while the Bushehr honey sample had the highest levels. The lower antioxidant activity of Iranshahr honey compared to other honey samples is likely due to the lower concentration of phenolic and flavonoid compounds. . The results of the total phenol content of honey samples also showed that the highest and lowest levels of total phenol were related to Bushehr and Iranshahr honey, respectively. The antioxidant activity of honey was directly related to its phenolic and flavonoid content. Amaral *et al.*, 2017 indicated a positive correlation between phenolic and flavonoid content and antioxidant activity of honey. Some polyphenols have been reported to exhibit antimicrobial activity (Marín *et al.*, 2015). Stagos *et al.* (2018) showed there was a significant moderate positive correlation between the total polyphenolic content and antioxidant activity of honey.

Conclusion

With the rise of antibiotic-resistant strains, there is an urgent need for new effective, low-risk antimicrobial compounds. The search for new antibacterial compounds is therefore one of the interesting topics in the fields of health and medicine (Selahvarzian *et al.*, 2015). Numerous studies have shown that *A. mellifera* honey inhibits the growth of bacteria that are resistant to common antibiotics (Molan, 1992; Al Somal *et al.*, 1994). In laboratory tests, honey was able to effectively control and suppress many pathogens. . Several factors are responsible for the antibacterial activity of honey, including osmotic pressure, acidity, hydrogen peroxide, phenols, flavonoids, and lysozyme (Manyi-Loh *et al.*, 2010). *H. pylori* infection in mice was treated with *A. florea* honey. A positive control group of mice that were treated with *H. pylori* had a higher rate of inflammation than the treatment group. A low rate of inflammation in the stomach of honey-treated mice indicates that honey reduces inflammation severity and has a therapeutic

effect. Degeneration and eosinophils also contribute to this process. In the treatment groups, high levels of eosinophils indicate that honey enhanced mice's immunity against pathogens, including bacteria. There are no studies on the antimicrobial properties of *A. florea* honey. According to this study, *A. florea* honey has been found to have antibacterial effects. Some of the commercial honey samples collected from different regions of Urmia, Iran have been shown to have higher antibacterial activity than other honey samples against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. . This might be attributed to phytochemicals in plants distributed in those areas (Tajik *et al.*, 2007). In Brazil, Amaral *et al*. (2017) investigated the effect of *A. mellifera* honey on *H. pylori* both *in-vivo* and *in-vitro*. Omeprazole, amoxicillin, clarithromycin, and honey were administered to mice after they were infected by *H. pylori*. All treatments were effective in controlling infection, but treatment with honey reduced inflammation and treatment with antibiotics increased eosinophil levels. A study by Rahimifard *et al.* (2019) showed that Thyme honey, royal jelly, and their mixtures exhibited antimicrobial activity against *H. pylori*. They showed that honey due to high osmolality, low acidity, and hydrogen peroxide and non-peroxide content, and royal jelly due to Royalisin protein, fatty acids 10-Hydroxy-2-Decenoic acid (10-HAD), and Jelleins peptides; have a wide spectrum of antibacterial properties. In this study, *A. florea* honey was shown to be effective in treating H. pylori infections. This study found similar results to those obtained by Amaral *et al*. (2017). In particular, jujube honey from Bushehr showed antibacterial and antioxidant properties in this study. This, in combination with other treatments, can reduce infections and inflammation caused by *Helicobacter pylori*.

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Conflicts of interest

The authors have declared that no competing interests exist.

Ethics approval

All experiments with animals were performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the Research Station of Department of Animal Science, Razi Vaccine and Serum Research Institute, Iran**.**

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بررسی خواص آنتی‌اکسیدانی و آنتی‌باکتریالی عسل زنبور عسل کوچک *Apis florea* Fabricius (Hymenoptera: Apidae) علیه *Helicobacter pylori*

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چکیده

هلیکوباکتر پیلوری با آلوده‌سازی دستگاه گوارش فوقانی، منجر به آسیب‌دیدگی در مخاط شده و امکان ابتلا به سرطان لوله گوارش را بویژه در مردان افزایش می‌دهد. با توجه به اینکه امکان درمان آن با روش‌های سنتی همیشه به طور موثری امکان‌پذیر نیست، بنابراین تا زمان تهیه واکسن علیه آن، یافتن روش‌های ایمن تر مبارزه با این باکتری بسیار حائز اهمیت است. عسل یک مکمل غذایی با محتوای کربوهیدرات بالا و فعالیت آنتی‌اکسیدانی و همچنین طیف وسیع ضد میکروبی است که در سال‌های اخیر به عنوان یکی از روش‌های مقابله با طیف گسترده‌ای از عوامل میکروبی از جمله *H. pylori* مطرح بوده است. در این پژوهش برای نخستین بار خواص آنتی‌اکسیدانی و آنتی‌باکتریالی ۱۵ نمونه عسل زنبور عسل کوچک *A. florea* جمع‌آوری شده از بوشهر، دزفول، ایرانشهر، چابهار، رودان، جهرم و جیرفت مورد بررسی قرار گرفت. ترکیبات فنلی، فلاونوئیدی، پروتئین و ترکیبات معدنی عسل‌های جمع‌آوری شده مورد بررسی قرار گرفت. همچنین خواص آنتی اکسیدانی عسل‌ها با استفاده از سه روش DPPH، FRAP و ABTS و خواص ضدباکتریایی در شرایط درون‌تنی و برون‌تنی ارزیابی شد. نتایج نشان داد که همبستگی مثبت و معنی‌داری بین خواص آنتی‌اکسیدانی و آنتی‌باکتریالی عسل‌ها وجود دارد. همچنین براساس نتایج بدست آمده بیشترین خواص آنتی‌باکتریالی علیه هلیکوباکتر پیلوری مربوط به عسل کنار بوشهر بود. طبق نتایج بدست آمده علت تفاوت قدرت آنتی‌اکسیدانی و ضد میکروبی مشاهده شده در بین نمونه‌های عسل به دلیل تفاوت در تنوع فلور گیاهی منطقه و تفاوت جغرافیایی مناطق جمع‌آوری عسل بوده است. بررسی قدرت ضد میکروبی در این پژوهش نشان داد، عسل توانایی بالایی در جلوگیری از آلودگی و نیز درمان آلودگی و التهاب ایجاد شده در دستگاه گوارش به وسیله باکتری هلیکوباکتر پیلوری را داشته است و می‌تواند در کنار سایر روش‌های موجود، در درمان آلودگی به این باکتری مورد استفاده قرار گیرد.

واژه‌های کلیدی: خواص آنتی اکسیدانی، خواص ضد باکتریایی، عسل، *H. pylori*

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