

Effect of Iranian Ziziphus honey on growth of some foodborne pathogens

Maryam Ekhtelat,
Karim Ravaji¹,
Masood Parvari¹

Medicinal Plants and Natural Products Research Center, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, ¹Agricultural Jihad of Applied Science and Technology University of Ahvaz, Ahvaz, Iran

Address for correspondence:

Dr. Maryam Ekhtelat, Medicinal Plants and Natural Products Research Center, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. E-mail: maryam_ekhtelat@yahoo.com

Abstract

Background: Honey has previously been shown to have wound healing and antimicrobial properties, but this is dependent on the type of honey, geographical location, and flower from which the final product is derived. We tested the antimicrobial activity of a natural honey originating from the *Ziziphus spina-christi* tree, against selected strains of bacteria. Ziziphus honey among more than a 100 varieties of honey is known to have the greatest value of energy and minerals in it. **Materials and Methods:** The aim of this study was to determine the antibacterial activity of Ziziphus honey in 10%, 20%, 30%, and 40% dilutions (v/v) against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus*. Viable count enumeration of the sample was investigated after 0, 24, 72, and 120 h postinoculation with any of the bacteria using pour-plate method. **Results:** The findings indicate that Ziziphus honey was effective against these pathogenic bacteria. In a comparative trial, antibacterial activity of Ziziphus honey was higher after 120 h incubation for each four bacteria in most dilutions. The microbial count showed 3-7.5 log reduction comparing with control after 120 h. **Conclusions:** Therefore, it is recommended using Ziziphus honey as a natural preservative and antibacterial agent. Also, it could potentially be used as therapeutic agents against bacterial infection particularly to the tested microorganisms.

Key words: Antibacterial activity, foodborne pathogens, Ziziphus honey

INTRODUCTION

Honey has been reported to show a significant antibacterial activity against a wide range of bacteria.^[1] It has been demonstrated in many studies that the antibacterial effects of honey are attributed to its high osmolarity, low pH, hydrogen peroxide content, and other non-peroxide factors such as lysozyme, phenolic acids, and flavonoids.^[2,3] The quality of honey is mainly determined by its sensorial, chemical, physical, and microbiological characteristics. Honey is a sweet and flavorful natural product which has been consumed for its high nutritive value and its

contribution to human health that depend largely on the floral source. These properties could be associated to honey's high osmolarity, antibacterial properties, and antioxidant capacity.^[3,4] The antioxidant and antibacterial activity of honey, however, varies greatly depending on the honey floral source.^[5] Ziziphus is a multipurpose tree or shrub, belonging to *Rhamnaceae* family. There are about 50 species in the tropical Asia, Africa, America, and the temperate regions. In Flora Iranica, five species were listed for Iranian Plateau, namely *Zizyphus spina-christi*, *Zizyphus oxyphylla nummularia*, *Zizyphus oxyphylla jujuba*, *Zizyphus*

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Ekhtelat M, Ravaji K, Parvari M. Effect of Iranian Ziziphus honey on growth of some foodborne pathogens. J Nat Sc Biol Med 2016;7:54-7.

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.175069

oxyphylla mauritiana, and *Zizyphus oxyphylla*.^[6] *Z. spina-christi* L. known by the Persian name “Konar” or “Sidr” and widely grow in the southern of Iran. Biological and pharmacological tests have shown antibacterial, antiviral, and antidiabetic effects of the extracts or fractions of the leaves of this plant.^[7] Its flowers are important source for honey bee. The honey collected from the flowers of the Sidr is in high demand by citizens for its medicinal qualities in addition to its excellent taste and fragrant smell. The composition of active components in plants depends on various factors, particularly plant bio- and chemo type and climatic conditions. Consequently, it can be reasonably expected that honey properties from different locations are different.^[3] Newly identified honeys may have advantages over or similarities with known honey due to enhanced antimicrobial activity, local production (thus availability), and/or greater selectivity against medically important organisms.^[8] On this basis, other honeys need to be assessed and evaluated.^[9] Therefore, the aim of the present study was an *in vitro* evaluation of antibacterial effectiveness of *Zizyphus* honey originating from the *Z. spina-christi* L. tree (Tangestan, Bushehr) against *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus* as foodborne pathogens.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation

S. aureus (ATCC 6538) and *L. monocytogenes* (ATCC 19118) as Gram-positive and *S. typhimurium* (ATCC 14028) and *E. coli* (ATCC 25922) as Gram-negative bacteria were used. For the antimicrobial activity measurement, first of all active cultures were generated by inoculating single colony of each bacterium into 5 ml sterile nutrient broth (Merck) and incubated at 37°C for 24 h. Freshly synchronized cultures of bacterial strains from initial inoculums were prepared after 2 times overnight cultures (24 h) of each bacterium by successively transferring 100 µl of the vegetative cells into tryptic soy broth (TSB, Merck, NJ, USA). Then the optical densities of the active freshly synchronized cultures were adjusted at 600 nm. The broth cultures prepared in this way had a final culture density of approximately 10⁶ colony forming unit (CFU)/ml.^[10] This had been used as adjusted inoculum for all the further studies.

Honey sample and preparation of concentrations

Natural *Zizyphus* honey was collected from Tangestan apiaries in Bushehr province, Iran and stored in the dark at room temperature. Different concentrations of honey constituting 10%, 20%, 30%, and 40% (v/v) were made using sterile nutrient broth. Before, honey was placed in a 37°C water bath to aid dissolving.

Measurement of antibacterial activity

The natural *Zizyphus* honey in 10%, 20%, 30%, and 40% dilutions (v/v) were provided in nutrient broth medium as mentioned above. Then, 1 ml (about 10⁶ CFU/ml) of each of bacteria separately added to 1 ml of each dilution of honey and incubated at 35°C for 120 h. Concurrently, a positive control by inoculating 1 ml of the microbial suspension into 1 ml of the nutrient broth and a negative control by adding 1 ml of the nutrient broth (without bacteria) to 1 ml of each dilution of honey were made. Total viable count enumeration of each sample was investigated after 0, 24, 72, and 120 h postinoculation. Total viable count was carried out using the pour-plate method as described by Harrigan.^[11] The count was expressed as CFU per ml. All tests were performed in triplicate and were repeated 3 times to obtain reliable results.

Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) and *post-hoc* tests (Scheffe and Duncan) by SPSS 16 software from IBM Company. Differences were considered significant when $P \leq 0.05$.

RESULTS

Viable count enumeration of different concentrations of *Zizyphus* honey (10%, 20%, 30%, and 40%) was investigated after 0, 24, 72, and 120 h postinoculation with any of the bacteria. Then, analysis of data was carried out by SPSS 16 software. Based on our result and statistical analysis using one-way ANOVA, significant difference at different times between different concentrations of honey was observed about all the researched bacteria. The results were expressed as means \pm standard deviation log-transformed bacterial concentration (CFU/ml). Figure 1 indicates antibacterial activity of *Zizyphus* honey against *S. aureus* at different concentrations and times. The results showed 3-7 log reduction of *S. aureus* in 30% and 40% concentration of honey, respectively, at different times. Also in *post-hoc* tests (Scheffe and Duncan) in 24 h of incubation was observed significant difference between the concentrations of 0% with the other concentrations of honey ($P < 0.05$). On the other hand, between the concentrations

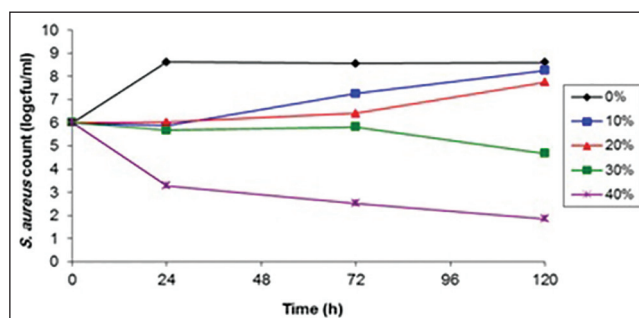


Figure 1: Growth curve of *Staphylococcus aureus* in different dilutions (10%, 20%, 30%, and 40%) in 0, 24, 72, and 120 h

of 10%, 20%, and 30% showed no any significant difference ($P > 0.05$). In 120 h of incubation, the concentrations of 30% and 40% were observed significant difference compared to other concentrations ($P < 0.05$). The statistical analysis showed no significant difference between 24, 72, and 120 h incubation on *S. aureus* in 40% dilution. Antibacterial activity of Ziziphus honey against *E. coli* indicates 3-7 log reduction in different concentrations of honey, after 120 h of incubation [Figure 2]. Statistical analysis observed a significant difference between concentrations of 30% and 40% with other concentrations at 24 and 72 h of incubation ($P < 0.05$). Based on the results, *L. monocytogenes* showed 2-5 log reduction in different concentrations of honey, after 72 and 120 h of incubation [Figure 3]. Also, a significant difference between different concentrations of honey with 0% concentration was observed at 72 and 120 h of incubation ($P < 0.05$). The effect of Ziziphus honey against *S. typhimurium* showed 1.5-6 log reduction in different concentrations of honey, after 72 and 120 h of incubation [Figure 4]; also, a significant difference between different concentrations of honey with 0% concentration was observed at 72 and 120 h of incubation ($P < 0.05$). Statistical analysis between different concentrations indicated a significant difference between 30% and 40% concentrations of honey with other concentrations after 24 h of incubation ($P < 0.05$). Also, it showed no significant difference between 30% and 40% dilution on salmonella ($P > 0.05$). The total results showed that Ziziphus honey has a good antibacterial activity against these bacteria. In a comparative trial, antibacterial activity of Ziziphus honey was higher after 120 h incubation, especially in 30% and 40% concentrations of honey. *L. monocytogenes* showed a lower sensitivity at concentrations of 30% and 40%.

DISCUSSION AND CONCLUSION

These days, abundant use of antibiotics has resulted in widespread resistance; with the development of novel antibiotics, alternative antimicrobial strategies are urgently needed.^[12] Honey has an extensive history to traditional human medicine use and also is an attractive ingredient for healthy foods.^[13,14] It has been proposed that the healing effect of honey could be due to various physical and chemical properties.^[15] The high osmolarity and acidity of honey are among the physical characteristics that contribute to its antibacterial activity. Hydrogen peroxide, volatiles, organic acids, flavonoids, beeswax, nectar, pollen, and propolis are important chemical factors that provide antibacterial properties to honey. In a study, Shin and Ustunol related the sugar composition of honeys from different floral sources to the growth inhibition of various intestinal bacteria.^[16] Thus, the floral source of honey plays an important role on its biological properties. For example, Manuka honey from New Zealand is recognized

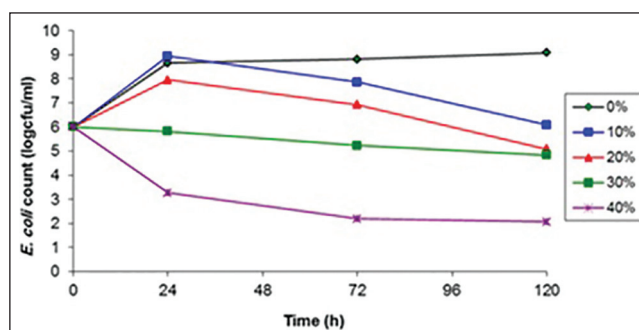


Figure 2: Growth curve of *Escherichia coli* in different dilutions (10%, 20%, 30%, and 40%) in 0, 24, 72, and 120 h

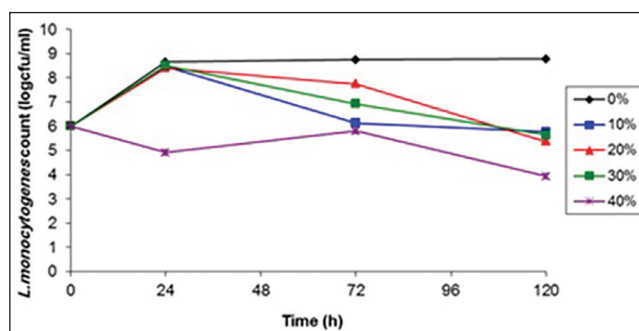


Figure 3: Growth curve of *Listeria monocytogenes* in different dilutions (10%, 20%, 30%, and 40%) in 0, 24, 72, and 120 h

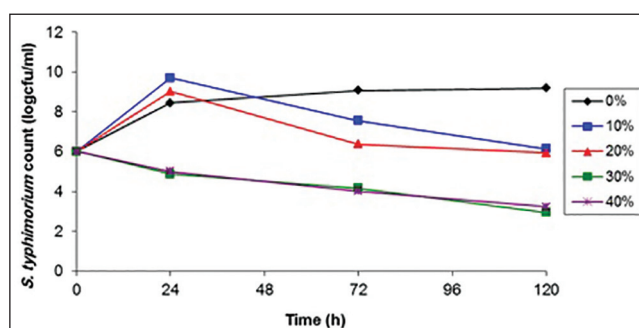


Figure 4: Growth curve of *Salmonella typhimurium* in different dilutions (10%, 20%, 30%, and 40%) in 0, 24, 72, and 120 h

for its therapeutic properties. The composition of honey has been shown to depend largely on its floral source. In consequence, it would not be surprising that the provenance of honey could determine its antibacterial properties.^[17] *Z. spina-chirsti* plant has versed medicinal and nutritional values. Some useful phytochemicals that include flavonoids, tannins, lipids, terpenes, alkaloids, steroids, free sugar, and mucilage have been isolated from the plant.^[18] This research is the first study to provide direct evidence for the efficacy of Iranian Ziziphus honey against these foodborne pathogens. Our data reveal that the foodborne pathogens tested were susceptible to Ziziphus honey, although this honey was not able to produce complete inhibition of bacterial growth during 120 h incubation, but antibacterial activity was increased with additional concentration of honey and the

microbial count showed about 3–7.5 log reduction after 120 h in most cases. It can be related to increase antibacterial factors and duration effect of the honey. Nzeako and Hamdi^[19] in their study of six commercial honeys found that inhibition of *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* did not occur at honey concentrations <40%. In a research in Saudi Arabia, antimicrobial effects of different honey samples including Sidr honey against multiresistant pathogens showed that the most sensitive pathogens were *Aspergillus nidulans*, *S. typhimurium*, and *Staphylococcus epidermidis*.^[20] Alandejani *et al.* showed effectiveness of Sidr (Yemen) and Manuka (New Zealand) honey on *S. aureus* and *P. aeruginosa* biofilms, respectively. The bactericidal rates for the Sidr and Manuka honeys against methicillin-susceptible *S. aureus*, methicillin-resistant *S. aureus*, and *P. aeruginosa* biofilms were 63–82%, 73–63%, and 91–91%, respectively. These rates were significantly higher than those seen with single antibiotics commonly used.^[21] In a research, Hegazi investigated antibacterial activity of seven Egyptian honeys and one Saudi honey (Sidr) against some bacteria. He reported that the Sidr honey was more effective against *S. aureus* and *P. aeruginosa*.^[22] Also, antimicrobial activity of three types of honey (Sidr, Sunflower, and Sunut) was examined in Sudan. The Sidr honey showed antimicrobial activity against *S. aureus*, *P. aeruginosa*, and *Klebsiella aerogenes*, while *E. coli* showed high resistance.^[23] Other researchers have also found differences in susceptibility of microorganisms to Ziziphus (Sidr) honey produced in different countries. There is a great interest in controlling the growth or eliminating foodborne pathogens using natural antimicrobials. If honey can slow or stop the growth of spoilage organisms or food pathogens, then its incorporation into foods as a preservative can be explored. In light of the enormous potential for application of honey, it is important that research continues not only into those honeys recognized as antibacterial, but also into other locally produced, as yet untested honeys.^[24] Therefore, it is recommended using Ziziphus honey as a natural preservative and antibacterial agent and it may play an important role as antibacterial natural product. However, further research could indicate whether Ziziphus honey has potential as a preservative in foods and oral medications.

Acknowledgments

This study was financially supported by Agricultural Jihad of Applied Science and Technology University of Ahvaz, Iran. Also we acknowledge the deserve support of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Molan PC. Honey: Antimicrobial actions and role in disease management. In: Ahmad I, Aqil F, editors. *New Strategies Combating Bacterial Infection*. Weinheim: Wiley VCH; 2009. p. 229–53.
- Molan PC. The antibacterial properties of honey. *Chem NZ* 1995;59:10–4.
- Alvarez-Suarez JM, Tulipani S, Díaz D, Estevez Y, Romandini S, Giampieri F, *et al.* Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food Chem Toxicol* 2010;48:2490–9.
- Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M. Contribution of honey in nutrition and human health: A review. *Med J Nutrition Metab* 2010;3:15–23.
- Gheldof N, Engeseth NJ. Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of *in vitro* lipoprotein oxidation in human serum samples. *J Agric Food Chem* 2002;50:3050–5.
- Dinarvand M, Zarinkamar F. Anatomy-taxonomy of the genus *Ziziphus* in Iran. *Iran J Bot* 2006;12:36–41.
- Jafari A, Zolfaghari B, Shirani K. Cytotoxicity of different extracts of arial parts of *Ziziphus spina-christi* on hela and MDA-MB-468 tumor cells. *Adv Biomed Res* 2014;3:38.
- Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, *et al.* Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement Altern Med* 2010;10:47.
- Basuny AM, Arafat SM, Farag HA. Utilization from fruits and leaves of Napek (*Ziziphus spina-christi* L.) as a source of bioactive components. *Nat Sci* 2013;1:29–36.
- Basti AA, Razavilar V. Growth response and modeling of the effects of selected factors on the time-to-detection and probability of growth initiation of *Salmonella typhimurium*. *Food Microbiol* 2004;21:431–8.
- Harrigan WF. *Laboratory Methods in Food Microbiology*. 3rd ed. Academic Press, London, UK: Gulf Professional Publishing; 1998.
- Nathan C. Antibiotics at the crossroads. *Nature* 2004;431:899–902.
- Williams E, Jeffrey J, Barminas J, Toma I. Studies on the effects of the honey of two floral types (*Ziziphus* spp. and *Acelia* spp.) on organism associated with burn wound infections. *Afr J Pure Appl Chem* 2009;3:98–101.
- Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. Functional properties of honey, propolis, and royal jelly. *J Food Sci* 2008;73:R117–24.
- Snow M, Manley-Harris M. On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. *Food Chem* 2004;84:145–7.
- Shin HS, Ustunol Z. Carbohydrate composition of honey from different floral sources and their influence on growth of selected intestinal bacteria: An *in vitro* comparison. *Food Res Int* 2005;38:721–8.
- Basualdo C, Sgroy V, Finola MS, Marioli JM. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet Microbiol* 2007;124:375–81.
- Adzu B, Haruna AK. Studies on the use of *Ziziphus spina-christi* against pain in rats and mice. *Afr J Biotechnol* 2007;6:1317–24.
- Nzeako B, Hamdi J. Antimicrobial potential of honey on some microbial isolates. *Med Sci* 2000;2:75–9.
- AL-Waili N, Al Ghamdi A, Ansari MJ, Al-Attal Y, Al-Mubarak A, Salom K. Differences in composition of honey samples and their impact on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi. *Arch Med Res* 2013;44:307–16.
- Alandejani T, Marsan J, Ferris W, Slinger R, Chan F. Effectiveness of honey on *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. *Otolaryngol Head Neck Surg* 2009;141:114–8.
- Hegazi AG. Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. *Res J Microbiol* 2011;6:488–95.
- El-Toum SK, Yagoub SO. Compression study of anti-microbial activity of honey-bees. *Res J Microbiol* 2007;2:776–81.
- Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. *Arch Med Res* 2005;36:464–7.