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Physicochemical Properties, Antioxidative and Antibacterial Activities of the Persian Medicine-Based Maolasal Honey: An Experimental Study

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ABSTRACT

Background: Maolasal is a honey-induced product which may lead to several beneficial effects from the Persian Medicine (PM) point of view. Heating process as a major step of Maolasal preparation may decrease the honey quality and deteriorate thermal treatment indicators of honey. **Methods:** This study was conducted on four honey samples (from different floral sources). Maolasal preparation process was done according to one of the most important PM references "Gharabadin Kabir". Thermal treatment indices, antibacterial and antioxidative activities of products were measured. **Results:** The 5-Hydroxymethylfurfural (HMF) concentrations of PM-based Maolasal samples of polyfloral, monofloral, and sugar honey were higher than 40 mg/kg; while PM-based Maolasal sample of monofloral honey had acceptable HMF concentration (38.9 mg/kg). Proline content of all samples decreased thorough Maolasal preparation and simple heating processes in all samples and these reductions were greater in monofloral honey and sugar honey Maolasal samples. Maolasal preparation processes decreased diastase activity of all samples. Although Maolasal preparation process led to sucrose content reduction in the three Maolasal samples, sucrose content of Maolasal sample of monofloral honey was higher than its raw sample. Maolasal preparation process increased total phenolic content of all samples. Finally, Maolasal preparation process led to an increase in antibacterial and antioxidant activities of all the samples. **Conclusion:** Maolasal samples reduced diastasis activity and proline content and increased HMF concentration. Maolasal preparation process may lead to an increase in antibacterial and antioxidative activities of honey which may be helpful for special preventive/therapeutic medicinal objectives.

Keywords: *Honey; Differential thermal analysis; Traditional medicine; Heating*

Introduction

Honey is known as an important macro- and micro-nutrients source including mono-, di-, and tri-saccharides, enzymatic proteins, free amino acids (especially proline), and variable amounts of minerals such as calcium, copper, potassium, iron, manganese, phosphorus, magnesium, selenium,

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sodium, and zinc (Ajibola *et al.*, 2012, Sadeghi *et al.*, 2019, Samarghandian *et al.*, 2017) . Although it has been used as a natural sweetener, clinicians administered the honey for different medicinal and nutritional aims since the ancient times (Ajibola *et al.*, 2012, Samarghandian *et al.*, 2017). As it has been reported in previous studies, a major portion of honey protein content (derived from bees, pollen, and nectar) include glucose oxidase, invertase, and amylolytic enzymes such as α-amylases (Ajibola *et al.*, 2012, Samarghandian *et al.*, 2017). Moreover, presence of organic acids such as gluconic acid and aspartic acid lead to acidity of honey which contribute to honey antimicrobial activity, flavor, and the stability of its matrix (Ajibola *et al.*, 2012). Finally, flavonoids and non-flavonoids polyphenols of honey which are dependent on the floral origin of the honey are considered as a major bioactive component of different types of honey (Ajibola *et al.*, 2012, Cianciosi *et al.*, 2018, Mandal and Mandal, 2011). These components make honey having beneficial health effects including antidiabetic, antimicrobial, and anti-carcinogenic effects, cardiovascular and respiratory systems protection, tissue regeneration, wound healing, and symptoms revealing of gastrointestinal disorders (Ajibola *et al.*, 2012, Eteraf-Oskouei and Najafi, 2013, Mandal and Mandal, 2011, Sadeghi *et al.*, 2019, Samarghandian *et al.*, 2017).

In Persian Medicine (PM), as a school of complementary medicine, paying attention to nutrition and its modification has been superior to prescribing medication in disease prevention and treatment.

Maolasal is considered as a honey-induced product which has different health benefits of raw honey from the PM point of view. Therapeutic effects on respiratory diseases, decreasing risk of gastrointestinal, renal and rheumatologic disorders, as well as increasing appetite and improving gastric function are some major beneficial health benefits of Maolasal according to PM references (Aghili, 2008). On the other hand, Maolasal preparing process contains heating process which may lead to decreased enzymatic activity, increased hydroxymethylfurfural (HMF) concentration, decreased proline content, and subsequently, reduced antioxidative and antibacterial activities and quality of honey (Karabournioti and Zervalaki, 2001, Molaveisi *et al.*, 2019, Shapla *et al.*, 2018, Zarei *et al.*, 2019).

There has been no study investigating the Maolasal preparation process effects on honey thermal treatment indices, anti-oxidative and antibacterial activities. Therefore, the present study was carried out to compare heating process-related physicochemical properties of raw, simple heated and PM-based Maolasal honey samples.

Materials and Methods

This experimental study was carried out in January 2020, Mashhad, Iran. The parameters for which the samples were tested include diastase activity, HMF content, proline level, total phenolic content (TPC), total antibacterial and antioxidative activity.

Honey samples: Four samples of honey from different regions were collected in December 2019; and stored in -20 ± 2 ºC until preparation and analysis date to prevent physicochemical changes due to the laboratory conditions. All samples were provided by Koohdasht Honey Company, Mashhad, Iran.

Maolasal preparation and simple heating processes: Preparation of samples including simple heating with water (honey/water: 2, for 90 min in 95 ºC), and Maolasal preparation (according to one of the most important PM references "Gharabadin Kabir" (Aghili, 2008, 2009)(Aghili, 2008, 2009)(Aghili, 2008, 2009), honey/water: 2, heating for 90 min in 95 ºC, excluding the covering foam and bubbles of the product) were done in one round, at the laboratory of the School of Persian and Complementary Medicine, Mashhad, Iran.

HMF concentration and diastase activity: According to the harmonized methods of the international honey commission (IHC) (Bogdanov *et al.*, 2002), HMF concentration was assessed thorough its Ultraviolet (UV) absorbance at 284 nm using the White method (spectrophotometer,

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Hanon i3) and diastase activity was measured according to the Schade method (Bogdanov *et al.*, 2002).

Proline content: Proline content was determined by spectrophotometric comparison with a standard sample of proline (following complex formation with ninhydrin) (Bogdanov *et al.*, 2002).

Total phenolic content: For TPC measurement, 5 ml of 10% water diluted of each sample was added to 2.5 ml of Folin-Ciocalteu 0.2 N solution. Folin-Ciocalteu reagent was used to determine the TPC of the samples. Two ml of sodium carbonate (7.5%) were added to the tubes following 5 min standing and the test tubes were agitated. The tubes were allowed to stand at room temperature for 120 min and then the absorbance of samples were measured at 760 nm (Meda *et al.*, 2005). Gallic acid solutions with 10–1000 mg/l concentrations were used as standard for the calibration curve production.

Free radical scavenging activity: Free radical scavenging activity of honey samples was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (with minor modification of the method described by Isla (Isla *et al.*, 2011). By dissolving 2 mg of DPPH in 100 ml methanol, DPPH solution was prepared. Different concentrations of methanolic honey solution (20-40 mg/ml) were added to DPPH solution (0.75 ml methanolic honey solution to 1.5 ml of DPPH solution). Ascorbic acid was used as the positive control and after 15 min of incubation, the absorbance was measured at 517 nm.

Sucrose content: Determination of the apparent sucrose was done by titration method (the Fehling's test/ Lane and Eyon modified method (Folin and Peck, 1919) .

Antibacterial activity measurement: The antimicrobial activities of all honey samples were determined using agar well diffusion method. Phenol equivalent assays were performed using phenol (as reference in solutions of 1–10 % (w/v) in distilled water). Antimicrobial activity was evaluated by comparing the square of the mean diameter of the clear zone from E. coli ATCC 25922 in nutrient agar of the honey sample with standard curve (Allen *et al.*, 1991, Mahmoodi-Khaledi *et al.*, 2015).

All physicochemical tests were performed regarding the Iranian national standardization organization (INSO) 92, 6th revision, 2008 (Institute of Standards and Industrial Research of Iran, 2008). All measurements were triplicated and the mean values of all 3 times were reported as the final value. The amounts of the studied indicators were expressed as mean ± SD.

Ethical consideration: This study was approved by the Ethics Committee of Mashhad University of Medical Sciences (MUMS) with ethical code of IR.MUMS.REC.1398.185 (28/9/2019).

Results

General characteristics of 4 honey samples are shown in **Table 1**. According to the results, HMF concentrations of sugar, monofloral, polyfloral raw honey samples were in acceptable range (< 40 mg/kg). Heated and PM-based Maolasal samples of polyfloral, monofloral (thyme), and sugar honey had HMF concentrations higher than 40 mg/kg, while only heated and PM-based Maolasal sample of monofloral honey had acceptable HMF concentrations (38.9, and 4.4 mg/kg, respectively). The raw thyme monofloral honey sample had a HMF concentration of 57.6 mg/kg. The monofloral raw honey sample had the lowest HMF concentration, while the maximum HMF content was recorded for heated monofloral honey sample (0.3 and 164.25 mg/kg, for monofloral raw and heated monofloral (thyme) honey samples, respectively).

As depicted in **Table 2**, sucrose content of heated and Maolasal samples of monofloral honey and also heated sample of the monofloral thyme honey were higher than their raw samples. However, heated and Maolasal preparation processes led to sucrose content reduction in other heated and Maolasal samples. Proline content of raw sugar honey sample was higher than other 3 raw samples (581.3 vs. 266.2, 137.4, and 262.3 mg/kg for sugar, monofloral, polyfloral, and

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monofloral (thyme), respectively). Proline content of all samples decreased thorough heating and Maolasal preparation processes in all samples. The reduction in proline content was greater through heating process in sugar, monofloral samples in comparison to Maolasal preparation process. However, Maolasal preparation process led to more proline reduction in polyfloral and monofloral (thyme) honey samples in comparison to simple heating process. As it is shown in **Table 2**, heating and Maolasal preparation processes decreased diastase activity of all samples and these reductions were greater in heating process except for monofloral (thyme) honey.

According to analysis results of the study (**Table 3**), antibacterial activity of heated sample of sugar honey was lower than its raw sample (1.5 vs. 2% for heated and raw samples, respectively). However, heating and Maolasal preparation processes led to an increase in antibacterial activity of all other samples and this increase was greater in Maolasal sample of monfloral thyme honey rather than its heated sample; 3.5 vs. 2.5% for Maolasal and heated monofloral thyme samples, respectively.

The results of TPC measurement showed that heating and Maolasal preparation processes increased TPC of all samples except for Monofloral honey Maolasal sample, and this increase was greater in heating process in all the samples. Furthermore, heating and Maolasal preparation processes led to an increase in antioxidant activity of all samples, while this increase was higher in heated samples of sugar and monofloral thyme honey samples compared to the Maolasal samples.

Table 1. Floral and geographical sources of the studied honey samples.

Samples	Origin
Sugar honey	Khorasan Razavi province, Iran
Monofloral honey (Rhamnus sp. /Nabk-Tree)	Khoozestan province, Iran
Polyfloral honey	Khorasan Razavi province, Iran
Monofloral honey (Thyme)	Khorasan Razavi province, Iran

Table 2. Thermal treatment indicators of different honey samples.

Honey samples	Sucrose (g/100 g)	Proline (mg/kg)	HMF (mg/kg)	Diastasis activity (Schade units)	TPC (mg/100 g GAE)
Sugar honey					
Raw sample	2.1	581.3	5.7	32.9	0.04
Simple heated sample	1.6	395.1	115.8	0	0.05
Maolasal product sample	1.5	375.8	75.9	0	0.04
Monofloral honey (Rhamnus sp.)					
Raw sample	3.2	266.2	0.3	20.0	0.08
Simple heated sample	5.4	199.7	38.9	11.9	0.18
Maolasal product sample	4.4	183.1	4.4	11.3	0.01
Polyfloral honey					
Raw sample	8.4	137.4	3.4	5.3	0.02
Simple heated sample	6.5	67.0	105.4	0.8	0.07
Maolasal product sample	5.9	106.4	64.5	0	0.06
Monofloral honey (thyme)					
Raw sample	3.3	262.3	57.6	5.5	0.03
Simple heated sample	4.5	168.3	164.2	0	0.07
Maolasal product sample	2.4	184.3	127.5	0.4	0.04

HMF**:** hydroxymethylfurfural, TPC: total phenolic content, GAE: Gallic acid equivalent.

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Table 3. Antibacterial and antioxidant activities of different honey samples.

Honey samples	Antibacterial activity (%)	Antioxidant activity (mg/ml)
Sugar honey		
Raw sample	2.0	555.5
Simple heated sample	1.5	580.5
Maolasal product sample	2.5	552.5
Monofloral honey (Rhamnus sp. /Nabk-Tree)		
Raw sample	3.0	684.3
Simple heated sample	4.0	762.4
Maolasal product sample	4.0	765.9
Polyfloral honey		
Raw sample	4.0	509.5
Simple heated sample	4.0	590.0
Maolasal product sample	4.0	643.5
Monofloral honey (Thyme)		
Raw sample	3.0	551.7
Simple heated sample	2.5	583.7
Maolasal product sample	3.5	554.2

Discussion

To the best of the authors' knowledge, this is the first study investigating Maolasal preparation process effects on honey thermal treatment indices. The results showed that Maolasal preparation with honey/rain water ratio of 2, excluding the covering foam and bubbles of the product, and heating for 90 min in 95 ºC led to a decrease in quality and deterioration of nutritional thermal treatment indicators, such as sucrose, proline content, HMF concentration, and diastasis activity. However, our study results showed improvement of antioxidant and antimicrobial activities of honey following Maolasal preparation process in comparison to raw honey samples.

According to the results of the present study, diastase activity decreased through simple heating and Maolasal preparation processes as heated and Maolasal samples of polyfloral, thyme monofloral, and sugar honey had diastase number (DN) values lower than 8 and did not meet Iranian honey standards (Institute of Standards and Industrial Research of Iran, 2008). Only monofloral honey heated and Maolasal samples had DN values greater than 8 Schade units (11.6 and 11.36 Schade units for heated and Maolasal samples, respectively). Given that the storage time and temperature conditions of all samples in the study were similar, this result may indicate the adverse effect of Maolasal preparation process on the quality of this honey sample. The naturally enzyme content of floral source of the honey samples should be considered in the interpretation of the results, as two monofloral (thyme) and polyfloral raw honey samples from Khorasan Razavi Province of Iran had DN values lower than 8 Schade units (Moloudian *et al.*, 2018, Wang and Li, 2011).

Table 2 shows that despite three raw honey samples HMF contents were in acceptable range, only monofloral honey Maolasal sample had an acceptable HMF content and all of the 7 heated and Maolasal samples had HMF contents greater than 40 mg/kg. However, this parameter can be blow 80 mg/kg in tropical and blended honey (Moloudian *et al.*, 2018, Shapla *et al.*, 2018). According to previous studies, fresh raw honey should not contain HMF and based on the present study results, raw monofloral thyme honey sample was not qualified in this parameter. Although heating and Maolasal processes lead to HMF content increase in almost all samples (except for monofloral Rhamnus sp. honey), this increase was greater in heated samples compared to Maolasal honey samples. However, we cannot use the HMF content as the only quality assessment index for honey because it is correlated to pH, diastase activity, the floral source and blending processes, so it should be interpreted in combination with all of these characteristics of

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samples (Moloudian *et al.*, 2018, Shapla *et al.*, 2018).

Although heating and Maolasal preparing processes led to sucrose content decrease in sugar and polyfloral honey samples, the sucrose content of monofloral heated and Maolasal honey samples increased through thermal treatments. All samples of the polyfloral honey including the raw sample and simple heated Rhamnus sp. Monofloral honey sample had sucrose levels higher than the allowed maximum levels in accordance to Iran standard (> 5 g/100 g) (Institute of Standards and Industrial Research of Iran, 2008).

Proline was considered as the most important amino acid containing approximately half of the total amounts of amino acids of honey. Proline content of honeydew was reported to be higher in comparison to nectar honey (Moloudian *et al.*, 2018). Proline content of different honey samples varies in accordance to their different floral sources (Gulfraz *et al.*, 2010, Moloudian *et al.*, 2018, Saxena *et al.*, 2010). Despite the fact that proline is not considered as a common quality assessment indicator, according to the parameters of animal science research institute (ASRI) of Iran, honey samples with proline content < 183 mg/kg are considered as adulterated honey samples (Gulfraz *et al.*, 2010, Moloudian *et al.*, 2018, Saxena *et al.*, 2010). According to the results of the present study, all raw samples were adulterated honey samples except for sugar honey sample. Moreover, heating and Maolasal preparation processes led to a decrease in proline content of all samples and these reductions were greater in heated samples compared to Maolasal samples with an exception for monofloral honey (Rhamnus sp.).

The results of the present study revealed that Maolasal preparation and simple heating lead to increased antioxidant activity of honey samples compared to similar raw samples. However, polyphenol profile of heated samples was higher than Maolasal samples. As the floral origin of honey can affect the polyphenol profile of the samples, the heated monofloral honey (Rhamnus sp.) sample had the highest polyphenol profile among all processed samples (Gulfraz *et al.*, 2010, Meda *et al.*, 2005, Moloudian *et al.*, 2018, Ramzi *et al.*, 2015, Saxena *et al.*, 2010). Furthermore, Maolasal preparation process lead to increase in antibacterial activity of all honey samples.

There are different objectives for honey consumption including honey intake for nutritional aims as well as administration of the honey for different medicinal objectives (Kaveh *et al.*, 2015, Nikzadeh Kigal *et al.*, 2020). According to PM, the honey product recommendation should be performed in accordance to the different nutritional/medicinal objectives. For example, raw honey is recommended for vascular disorders, metabolic diseases, nephrolithiasis, and cholelithiasis, while Maolasal product should be administered for gastrointestinal reflux disease, steatohepatitis, and respiratory system disorders (Aghili, 2008, Bogdanov *et al.*, 2002)(Aghili, 2008, Bogdanov *et al.*, 2002)(Aghili, 2008, Bogdanov *et al.*, 2002)(Aghili, 2008, Bogdanov *et al.*, 2002)(Aghili, 2008, Bogdanov *et al.*, 2002). Therefore, greater antioxidant and antimicrobial activity of Maolasal may be more important in comparison to nutritional effects of honey including sucrose and proline content in such diseases in PM school.

There are several different recipes for Maolasal preparation process according to PM references. There are different honey/water ratios as well as different types of recommended water, including distilled water, or ordinary drinking water. Moreover, different references suggested different goal volumes as reaching to 1/2-1/3 of the raw water + honey volume. Finally, there are different methods of heating including heating on the fire or being under natural sunlight to reach to the target volume in PM (Aghili, 2008, Bogdanov *et al.*, 2002, Isra'ili, 1992, Kaveh *et al.*, 2015).

Deterioration of thermal treatment indicators may be due to the heating process which was performed thorough Maolasal preparation process as well as inappropriate provided raw honey samples. On the other hand, not selecting an appropriate recipe for Maolasal preparation process may be another reason for such results.

This experimental study was carried out on four

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honey samples and only thermal treatment indices were evaluated following simple heating and Maolasal preparing processes and this was a major limitation of the study. Investigation of all physicochemical characteristics of several honey samples from different regions following different Maolasal preparation processes may help have better judgment on the best Maolasal formulation and determination of exact indications of Maolasal administration for different medicinal (preventive/therapeutic) objectives. The authors did not have several samples to do statically analysis and compare the significance of the differences**.** Therefore, further studies are required to find whether there is any safe, high quality Maolasal product to have different health beneficial effects. Moreover, clinical trials especially randomized clinical trials are needed to investigate the possible effects of Maolasal on organ functions and disease prevention and treatment in comparison to raw honey.

Conclusion

Maolasal preparation process may lead to increased HMF concentration and reduced proline content and diastasis activity. Therefore, Maolasal preparing process may lead to thermal treatment indicators deterioration in honey samples. On the other hand, Maolasal samples had higher antibacterial activity in comparison to raw honey samples. Additionally, all heated and Maolasal honey samples except for sugar honey Maolasal sample had higher antioxidant activity in comparison to their raw samples. The aforementioned parameters may be helpful for special preventive/therapeutic objectives. Further experimental and clinical trials are necessary to investigate the best Maolasal formulation and exact indications for prescribing the Maolasal product.

Conflict of interests

The authors report that there is no conflict of interest.

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Authors' contributions

Zakerian M, Roudi F, Salari R, and Hosseini SR designed the study. Refahi B, Zakerian M, and Hosseini SR were involved in the sample preparation. Ramezani M and Ramezani M were involved in the laboratory analysis of the samples. Zakerian M, Roudi F, and Salari R prepared the manuscript. Salari R and Hosseini SR supervised the research project. All the authors read and approved the manuscript.

References

Aghili S 2008. Makhzan- Al' Advieh. Tehran University publication: Tehran.

Aghili S 2009. Gharabadin Kabir. 1st edition. Tehran: Iran University of Medical Sciences, Institute of history of Medicine studies and Islamic medicine: Tehran.

Ajibola A, Chamunorwa JP & Erlwanger KH 2012. Nutraceutical values of natural honey and its contribution to human health and wealth. *Nutr Metab (Lond).* **9 (1)**: 1-12.

Allen K, Molan P & Reid G 1991. A survey of the antibacterial activity of some New Zealand honeys. *Journal of pharmacy and pharmacology.* **43 (12)**: 817-822.

Bogdanov S, Martin P & Lullmann C 2002. Harmonised methods of the international honey commission. *Swiss Bee Research Centre, FAM, Liebefeld.* **5**: 1-62.

Cianciosi D, et al. 2018. Phenolic Compounds in Honey and Their Associated Health Benefits: A Review. *Molecules.* **23 (9)**: 2322.

Eteraf-Oskouei T & Najafi M 2013. Traditional and modern uses of natural honey in human diseases: a review. *Iran J Basic Med Sci.* **16 (6)**: 731-742.

[DOR: 20.1001.1.24767417.2023.8.1.11.7]

[DOI: 10.18502/jnfs.v8i1.11773]

Folin O & Peck EC 1919. A Revision of the Copper Phosphate Method for the Titration of Sugar. *Journal of biological chemistry.* **38 (2)**: 287-291.

Gulfraz M, et al. 2010. Quality assessment and antimicrobial activity of various honey types of Pakistan. *African journal of biotechnology.* **9 (41)**: c-6906.

Institute of Standards and Industrial Research of Iran 2008. Honey specifications and test methods, 6th Revision.

Isla MI, et al. 2011. Physico chemical and bioactive properties of honeys from Northwestern Argentina. *LWT-food science and technology.* **44 (9)**: 1922-1930.

Isra'ili IS 1992. Al-Aqziah va al-adviah. Izzuddin Publications: Beirut.

Karabournioti S & Zervalaki P 2001. The effect of heating on honey HMF and invertase. *Apiacta.* **36 (4)**: 177-181.

Kaveh S, et al. 2015. The role of honewater (Maul asL) in treatment of respiratory diseases from Iranian traditional medicine point of wiew. *Msdical history.* **7 (23)**: 29-50.

Mahmoodi-Khaledi E, Kashef N, Habibi-Rezaei M & Moosavi-Movahedi AA 2015. In vitro characterization of antibacterial potential of Iranian honey samples against wound bacteria. *European food research and technology.* **241 (3)**: 329-339.

Mandal M & Mandal S 2011. Honey: its medicinal property and antibacterial activity. *Asian Pacific journal of tropical biomedicine.* **1 (2)**: 154-160.

Meda A, Lamien CE, Romito M, Millogo J & Nacoulma OG 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food chemistry.* **91 (3)**: 571-577.

Molaveisi M, Beigbabaei A, Akbari E, Noghabi MS & Mohamadi M 2019. Kinetics of temperature effect on antioxidant activity, phenolic compounds and color of Iranian jujube honey. *Heliyon.* **5 (1)**: e01129-e01129.

Moloudian H, et al. 2018. Characterization and Classification of Iranian Honey Based on Physicochemical Properties and Antioxidant Activities, with Chemometrics Approach. *Iranian journal of pharmaceutical research.* **17 (2)**: 708-725.

Nikzadeh Kigal D, Habibzadeh S, Ebrahimi HA, Mohammadi Monfared M & Miran M 2020. Clinical Evaluation of Honey Throat Spray for Auxiliary Treatment of Pharyngitis. *Journal of Ardabil University of medical sciences.* **20 (1)**: 79-93.

Ramzi M, Kashaninejad M, Mahoonak ARS & Razavi SMA 2015. Comparison of physico-chemical and rheological characteristics of natural honeys with adulterated and sugar honeys. *Iranian Food Science & Technology Research Journal.* **11 (4)**: Pe392-Pe407.

Sadeghi F, Salehi S, Kohanmoo A & Akhlaghi M 2019. Effect of Natural Honey on Glycemic Control and Anthropometric Measures of Patients with Type 2 Diabetes: A Randomized Controlled Crossover Trial. *Int J Prev Med.* **10 (3)**: 1-7.

Samarghandian S, Farkhondeh T & Samini F 2017. Honey and Health: A Review of Recent Clinical Research. *Pharmacognosy Res.* **9 (2)**: 121-127.

Saxena S, Gautam S & Sharma A 2010. Physical, biochemical and antioxidant properties of some Indian honeys. *Food chemistry.* **118 (2)**: 391-397.

Shapla UM, Solayman M, Alam N, Khalil MI & Gan SH 2018. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. *Chemistry central journal.* **12 (1)**: 1-18.

Wang J & Li QX 2011. Chapter 3 - Chemical Composition, Characterization, and Differentiation of Honey Botanical and Geographical Origins. In *Advances in Food and Nutrition Research* (ed. S. L. Taylor), pp. 89-137. Academic Press.

Zarei M, Fazlara A & Tulabifard N 2019. Effect of thermal treatment on physicochemical and antioxidant properties of honey. *Heliyon.* **5 (6)**: e01894-e01894.

[DOR: 20.1001.1.24767417.2023.8.1.11.7]
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