Examination the antioxidant potentials and antidiabetic properties of phenolic extracts of some Iranian honeys

Mahmoodreza Moein[1,2], Soheila Moein[1,3*] , Fatemeh Farmani[1] , Sahere Rozbehan[3] , Zahra Sabahi[1]

[1]Medicinal Plants Processing Research Center, Shiraz University of Medical Sciences, Shiraz, Fars, Iran
[2]Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Fars, Iran
[3]Department of Biochemistry, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Hormozgan, Iran

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ABSTRACT

Introduction: In most ancient cultures, honey has been used for both nutritional and medical purposes.
Objectives: In this research, phenolic extracts of four Iranian honeys were evaluated to determine the antioxidant potentials using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, nitric oxide (NO) radical scavenging and reducing power by ferric reducing activity of plasma (FRAP) method. Additionally, anti-diabetic properties of honey and phenolic extracts were evaluated by determination of α-amylase and α-glucosidase inhibition.
Patients and Methods: Besides, reducing potential was evaluated by ferric-reducing antioxidant power method. Moreover, determination of phenolic and flavonoid contents was performed. Moreover, inhibition of α-amylase and α-glucosidase of honey and phenolic extracts were evaluated.
Results: With considering to antioxidant potentials, Gavan (Astragalus) sample showed the greatest phenolic (3817 ± 1.52 mg GAE/100 g), flavonoid contents (3.1 ± 0.005 mg QE/100 g), and DPPH radical scavenging ($IC_{50} = 2 \pm 0.003$ mg/mL). Bahareh honey had the highest NO radical scavenging ($IC50 = 0.0403 \pm 0.0009$ mg/mL) and Meymand honey possessed the highest reducing potential by FRAP method ($IC_{50} = 0.0018 \pm 0.000003$ mg/mL). The maximum inhibition of α-glucosidase was shown in Meymand honey extract ($46 \pm 0.1\%$). After sugar isolation, Zataria honey had the highest inhibition of α-glucosidase ($54 \pm 0.6\%$) and the mode of α-amylase inhibition was noncompetitive by this honey. Whole extract ($23 \pm 0.1\%$) and phenolic extract of Gavan honey presented the maximum inhibition of α-amylase ($31.2 \pm 0.1\%$).
Conclusion: Honey samples showed antioxidant potentials and anti-diabetic properties by retardation of α-amylase and α-glucosidase.

Introduction

Honey is a natural compound synthesized by bees that have been consumed by people from ancient times. *Apis mellifera* is a bee, which produces honey from the sweet liquid in flower or from secretions of trees and plants (1). Honey prevents lipid oxidation in meat (2) and light, heat, and metal-induced oxidation reactions in food (2).

The other research immunologically confirmed the presence of α-glucosidase in stomach, α-glucosidase II in stomach, blood and lymph, and α-glucosidase III beneath the hypopharyngeal apparatus that the enzyme may be released into nectar and collected by honeybees

(3). However, α-amylase inhibitory capacities of phenolic extracts of honey reveal its anti-diabetic potential. Diabetes occurred when oxidative stress is more than antioxidants production (4). The most important management for treatment of diabetes is to decrease blood glucose after meal, which is performed through inhibition of glucose uptake by means of retardation α-amylase and α-glucosidase known as carbohydrate hydrolyzing enzymes (5).

Moreover, flavonoids (such as kaempferol, catechin, and quercetin) and phenolic acids (such as caffeic acid and gallic acid) are the most significant components of honey

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Corresponding author: Soheila Moein, Email: soheila_9@yahoo.com, Smoein@hums.ac.ir

which have anti-cancer properties (6).

Objectives

In this study, phenolic extracts of four Iranian honey were evaluated to determine the antioxidant potentials using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, nitric oxide (NO) radical scavenging, and reducing potential was evaluated by FRAP (ferric reducing ability of plasma) method. Besides, determination of phenolic and flavonoid contents was performed. Moreover, anti-diabetic potentials of four kinds of Iranian honey and their phenolic extracts were evaluated by determination of α-amylase and α-glucosidase inhibition.

Materials and Methods

Materials

 Butylated hydroxyl toluene (BHT), α-amylase, and bovine serum albumin (BSA) were obtained from Sigma-Aldrich. The other materials were bought from Merck Chemical Co.

Collection of samples

The honey samples were purchased from local stores in Shiraz and Meymand, Iran.

Preparation of phenolic extracts of honey

In order to prepare phenolic extracts of honey, 30 g of Amberlite XAD-2 resin was immersed in methanol for 24 hours. Afterwards, most of the methanol was discharged and substituted with distilled water. After 5–10 minutes, the glass column (25 cm × 2 cm) was filled with this mixture. Then, honey (50–100 g) was dissolved in 70 mL of distilled water. The mixture was passed gradually through the column of Amberlite XAD-2 resin. The column was washed with 350 mL of mixture of water and hydrochloric acid (pH=2) and afterward washed with 400 mL of distilled water to separate carbohydrates as polar compounds from honey. By using 400 mL of methanol, the phenolic components were eluted from the sorbent. The methanol extracts were concentrated under vacuum by using a rotary evaporator at 40°C (7).

Evaluation of DPPH radical scavenging

The DPPH scavenging was performed according to the method of Bruits et al with some modifications (8). Before transferring to a 96-well microplate, different concentrations (12.5-3200 µg/mL) of honey samples (3.2 mg) were mixed with 200 µL of DPPH (100 mM). Negative controls contained 20 µL of methanol and 200 µL DPPH in triplicate. The microplate was held for 30 minutes at 25°C and the absorbance was detected by using a microplate reader (Biotek, ELX 800) at 495 nm (8). Quercetin was used as the antioxidant standard. The percent inhibition was plotted against the concentrations of the honey and the IC$_{50}$ was estimated by the fitted linear curve. The results were exhibited at the mean ± SD of three replicates.

Evaluation of NO radical scavenging

For evaluation of NO radical scavenging, 50 µL of nitroprusside (10 mM nitroprusside dissolved in phosphate buffer, 20 mM, pH 7.4) was blended with 50 µL of honey extract (200 µg/mL) and the mixture was put for 150 minutes at 27°C (9). After that, 100 µL Griess solution was added and the absorbance was determined at 542 nm. The blank contained honey extract without any reagent. The control contained 50-µL methanol, 100-µL Griess solution, and 50 µL of nitroprusside. Inhibition of NO activity was determined as follows (9):

Inhibition: A0 – A / A0 × 100
A0: is the absorbance of control
A: is the absorbance of test - absorbance of blank (9)

Evaluation of ferric-reducing antioxidant power

In this method, 20 mmol/L of FeCl$_3$, 0.3 mol/L of acetate buffer (pH = 3.6), and 10 mmol/L of TPTZ (2, 4, 6-tripyridyl-S-triazine) solution were mixed in HCl (40 mmol/L). In a 96-well microplate, 20 µL of each sample and 180 µL of FRAP reagent were mixed and put for 10 minutes at 37°C. The absorbance of the mixture was determined at 593 nm (10).

Determination of phenolic compound

Phenolic compounds levels were dedicated as stated by Miliauskas et al (8). Around 0.15 mL of the sample (10 mg/mL) was blended with 0.75 mL of Folin-Ciocalteu (diluting 1:10 with distilled water) and after 3 minutes, 0.6 mL of 7.5% w/v of sodium carbonate was added to the mixture. Then, the combination was held for 1 hour in the dark and the absorbance was read at 765 nm (8). Various concentrations of Gallic acid (0.0003- 0.0024 mg/mL) were used for plotting the standard curve. Phenolic compounds of the samples were estimated by the subsequent Equation

$$C = c \cdot v/m'$$

 While C is the phenolic contents (mg/g), c is the concentration of GA obtained from the standard curve (mg/mL), v is the extract volume (mL), and m' is the extract weight (g).

Determination of flavonoid compounds

Flavonoid compounds of the samples were determined through the colorimetric method (8). In this method, 0.5 mL solution of the sample was blended with 2 mL of distilled water and with NaNO$_2$ 15% solution. After 6 minutes, 2 mL of 4% NaOH solution was added to the combination. Then, water was added and the final volume became 5 mL. The combination was blended and permitted to stand for extra 15 minutes. At 510 nm, the absorbance of the combination was detected (8). Quercetin was used as a standard and all values were stated as milligram of quercetin equivalent per 1 g of

extract. Data were presented as mean± SD.

Evaluation of α-amylase inhibition

Alpha-amylase inhibition was performed by Ademiluyi et al method (11). Solution of enzyme (4 unit/mL) was made by blending 0.001 g of α-amylase (EC 3.2.1.1) in sodium phosphate buffer (pH = 6.9, 20 mM), comprising 6.7 mM sodium chloride. The samples were dissolved in DMSO and blended with starch solution. The reagent (DNS) solution contained 96 mM of 3, 5-dinitrosalicylic acid (20 mL) and deionized water (12 mL) and 5.31 M potassium tartrate sodium in 2 M sodium hydroxide (8 mL).

On the other hand, 0.04 mL of the enzyme solution and 0.56 mL of the extract-starch solution were blended and hold for 15 minutes at 37°C. Then, 0.6 mL of DNSA was added, and the test tube was incubated in the water bath (85°C, for 15 minutes), after that; samples absorbance was read at 540 nm (11). The control presented 100% enzyme activity and replaced extracts with DMSO. Acarbose was used as a standard. All experiments were carried out 3 times. The percentage of α-amylase inhibition was determined by the subsequent equation:

$$\alpha\text{-amylase inhibition \%} = 100 - ((\Delta A_{cotrol} - \Delta A_{sample}) / \Delta A_{control}) \times 100$$
$$\Delta A_{control} = A_{test} - A_{blank}$$
$$\Delta A_{sample} = A_{test} - A_{blank}$$

Evaluation inhibition of α-glucosidase

Alpha-glucosidase inhibitors were assessed by the method stated by McCue et al (12). The enzyme solution is composed of phosphate buffer (125 μL, pH 6.9, 0.1 M) and 5 μL of α-glycosidase (25 unit/mL). 4-Nitrophenyl α-D-glucopyranoside in the mentioned buffer (pH 6.9) was applied as a substrate. Twenty microliters of the various concentrations of the samples were blended in microplate wells with enzyme solution and hold at 37°C for 15 minutes. The reaction was begun by adding 20 μL of substrate solution and incubated for an extra 15 minutes. The reaction was retarded by adding 0.2 M of sodium carbonate solution (80 μL).

The absorbance of the samples was determined at 405 nm by a microplate reader. The system without extracts was used as a control. The reaction system without

α-glucosidase was applied as a blank, and acarbose was used as a positive control. All measurements were performed 3 times. The rates of enzyme inhibition of the samples were estimated by the subsequent equation:

$$\text{Inhibition \%} = [(\text{control absorption} - \text{sample absorption}) / \text{control absorption}] \times 100$$

Statistical analysis

The obtained results were presented as the mean± SD of three replicates. ANOVA (one-way analysis of variance) and post test Tukey were applied to calculate the differences among the means. *P* values ≤ 0.05 are considered as significant differences.

Results

Among honey samples, Gavan honey had the highest DPPH scavenging (IC$_{50}$=2 ± 3.09 mg/mL). In this regard, the IC$_{50}$ of quercetin as an antioxidant standard was shown in Table 1. Besides, Bahare honey had the highest NO scavenging (IC$_{50}$= 0.04 ± 0.0009 mg/mL) among honey samples and quercetin (IC$_{50}$=0.07 ± 0.0016 mg/mL, Table 1).

In FRAP method, Meymand honey had the highest antioxidant potential (IC$_{50}$=0.0018 ± 0.000003) in comparison with quercetin (IC$_{50}$=0.009 ± 0.00003 mg/mL).

As shown in Table 2, Gavan honey had the highest amount of phenolic (3817 ± 1.52 mg GAE/100 g) and flavonoids (3.1 ± 0.005 mg QE/100 g honey, Table 2). Bahare honey possessed the lowest amounts of phenolic (58 ± 1.06 mg GAE/100 g honey) and flavonoids (1 ± 0.0015 mg QE/100 g honey).

All of the samples, except Bahare, inhibit α- amylase. By increasing the concentration of honey, α-amylase inhibition was decreased. Moreover, as shown in Table 3, Gavan honey revealed the highest inhibition of α-amylase (23 ± 0.1 % in 2.5 mg/mL). After isolation of sugar, as shown in Table 3, Gavan honey presented the highest inhibition of α- amylase (31.2 ± 0.1% in 25 mg/mL); however after isolation of sugar, α-amylase inhibition was increased in honey samples depended on the concentrations. As shown in Table 3, acarbose as a standard inhibits α- amylase (100± 0.18 %) in 2.5 mg/mL.

Table 1. Antioxidant potentials of 4 honeys phenolic extracts by different methods compared with antioxidant standards

Samples	DPPH radical scavenging (IC50, mg/mL)	Nitric oxide scavenging ability% (200 mg/mL)	Antioxidant potential by FRAP method (IC50, mg/mL)
Gavan, bee	2 ± 0.003	0.054 ± 0.002	0.652 ± 0.002
Zataria	>3.200	0.045 ± 0.0017	0.294 ± 0.0014
Bahare	>3.200	0.0403 ± 0.0009	>3.200
Meymand	>3.200	0.05 ± 0.0014	0.0018 ± 0.000003
Quercetin	0.0265 ± 0.00006	0.07 ± 0.0016	0.009 ± 0.00003

Results are given as mean± SD values.

Table 2. Phenolic and flavonoid compounds of four honeys phenolic extracts

Samples	Phenolic content (mg GAE/100 g honey)	Total flavonoids (mg QE/100 g honey)
Gavan	3817 ± 1.52	3.1 ± 0.005
Zataria	102 ± 1	2.3 ± 0.015
Bahare	58 ± 1.06	1 ± 0.0015
Meymand	866 ± 1.15	2.7 ± 0.005

GAE: Gallic acid equivalent; QE: Quercetin equivalent.
All values are expressed as mean ± SD of three parallel measurements ($P < 0.05$).

Table 4 shows the highest inhibition of α- glucosidase (46 ± 0.1%) for the Bahare sample. After isolation of sugar, the most inhibition of α-glucosidase was revealed by Zataria honey (54 ± 0.6% in 30 mg/mL). The percentage of α-glucosidase inhibition by different concentrations of acarbose is demonstrated in Table 4. The highest-level inhibition of α-glucosidase by acarbose was 75 ± 0.25% in concentration of 32 mg/mL (Table 4). Km and Vmax of α-glucosidase inhibition by Zataria honey were 3.56 ± 0.001 mM and 0.0008 ± 0.00001 μM/min, respectively. As presented in Table 5, in the absence of inhibitor, Km and

Vmax were 2.29 ± 0.005 mM and 0.0016 ± 0.00001 μM/min, respectively.

Discussion

The therapeutic capacity of honey is usually related to antioxidant property against free radicals. The use of the whole extract rather than special antioxidants allows evaluating interactive effects of varied phenolic complexes that exist in the extracts. Moreover, this property is more simply observed in the phenolic extracts rather than in the total honey. For isolation of sugar and purification phenolics of honey, Amberlite XAD-2 has been used as the solid phase (10).

Antioxidant potentials of 4 kinds of honey after isolation of sugar were evaluated by DPPH radical scavenging, NO radical scavenging, and FRAP method. Furthermore, the amounts of antioxidant compounds such as phenolic and flavonoid compounds were measured.

Following the separation of sugar from four honey samples, DPPH radical scavenging radicals were evaluated in lower proportion than quercetin as a standard (Table 1).

DPPH radicals scavenging measured the reduction in the absorption of DPPH after exposure to antioxidant

Table 3. Percentageof α-amylase inhibition by crude extracts and phenolic extracts of four honeys in comparison to acarbose

Samples	2.5 mg/mL	5 mg/mL	10 mg/mL	15 mg/mL	20 mg/mL	25 mg/mL	
Gavan (crude)	23±0.1	19.6±0.1	15.4±0.2	11±0.22	5.2±0.14	2.3±0.1	
Zataria (crude)	-	15±0.2	10.6±0.18	5.2±0.1	2.3±0.32	1±0.22	
Bahareh (crude)	0	-	-	-	-	-	
Meymand (rude)	11.2±0.2	10±0.1	8.4±0.24	6.3±0.15	4.7±0.3	3.2±0.16	
Gavan (phenolic extract)	5±0.1	7.2±0.14	15.4±0.22	18.7±0.22	22±0.16	31.2±0.1	
Zataria (phenolic extract)	0	0	0	0	0	0	
Bahareh (phenolic extract)	1.7±0.22	3.2±0.32	5.2±0.1	7±0.18	11.4±0.2	13.2±0.24	
Meymand (phenolic extract)	5.2±0.16	7.6±0.3	9.2±0.15	11.4±0.24	15±0.1	17.3±0.5	
Acarbose	**0.125 mg/mL**	**0.25 mg/mL**	**0.5 mg/mL**	**1 mg/mL**	**1.5 mg/mL**	**2 mg/mL**	**2.5 mg/mL**
	5±0.1	14±0.15	28±0.14	44±0.2	56±0.1	75±0.25	100±0.18

Table 4. Percentage of α-glucosidase inhibition by crude extracts and phenolic extracts of four honeys in comparison to acarbose

Samples	2.5 mg/mL	5 mg/mL	10 mg/mL	15 mg/mL	20 mg/mL	25 mg/mL	30 mg/mL
Gavan (crude)	29±0.1	32±0.14	34±0.2	31±0.2	32±0.16	28±0.2	33±0.18
Zataria (crude)	18±0.2	29±0.32	30±0.1	34±0.18	38±0.20	0	0
Bahareh (crude)	35±0.16	43±0.3	44±0.15	45±0.24	46±0.1	20±0.2	25±0.5
Meymand (crude)	0	0	0	0	0	0	0-5
Gavan (phenolic extract)	-5	-7	-11	-30	ND	ND	ND
Zataria (phenolic extract)	10.5±0.18	22.35±0.2	35±0.16	36.2±0.26	40.45±0.5	45.24±0.34	54±0.6
Bahareh (phenolic extract)	0	1.4±0.45	3.6±0.16	7.7±0.2	14.1±0.44	27.6±0.2	35.5±0.56
Meymand (phenolic extract)	0	0	1.3±0.2	3.8±0.6	8.6±0.26	12.54±0.46	14.4±0.16
Acarbose	**0 mg/mL**	**1 mg/mL**	**2 mg/mL**	**4 mg/mL**	**8 mg/mL**	**16 mg/mL**	**32 mg/mL**
	0	15±0.1	24±0.2	48±0.15	64±0.1	56±0.18	75±0.25

Table 5. Kinetic parameters of α-glucosidase inhibition by Zataria honey after separation of sugars

Samples	Km mM	Vmax μM/min
α-glucosidasewithout inhibitor	2.29±0.005	0.0016±0.0000
Zataria	3.56±0.001	0.0008±0.00001

IC_{50} α-glucosidase inhibition= 11± 0.19 mM.

compounds.

Phenolic extract of Gavan sample presented the highest potential (IC_{50}= 2±0.003 mg/mL, Table 1) of DPPH radical scavenging. Moreover, in other research, the IC_{50} of DPPH radical scavenging was reported 90.78±5.10 mg/mL, 168.44±1.99 mg/mL, and 204.24±0.63 mg/mL in phenolic extracts of light honey, amber honey, and dark honey, respectively (13).

However, all of the honey samples could scavenge NO radical and in this regard, Bahare honey (IC_{50}= 0.0403 ± 0.0009 mg/mL in 200 μg/mL) presented the highest antioxidant potential which was more than quercetin as an antioxidant standard (IC_{50}= 68.73±1.6 μg/ mL, $P <$ 0.001, Table 1).

In the determination of reducing power by FRAP method, meymand extract has the greatest antioxidant potential (IC_{50}=0.0018±0.000003 mg/mL) which was more than querctin (IC_{50}=0.009±0.0003 mg/mL, $P <$0.0001, Table 2). However, in FRAP method the conversion of a Fe3+/ferri cyanide complex to the ferrous form was evaluated. The phenolics was detected using Folin-Ciocalteu method (8).

In the present study, phenolic extract of Gavan honey showed the highest amounts of phenolic (3817± 1.52 mg GAE/100 g, $P <$0.001, Table 2) and flavonoid compounds (3.1±0.005 mg QE/100 g, Table 2). The correlation coefficient between phenolic levels and scavenging of NO was 1; it means may be phenolic compounds were responsible for scavenging of NO radical. Also, it was reported that phenolics of honey may be responsible for its different biological activities (14).

For determination of flavonoids, a spectrophotometric method based on the formation of an aluminum chloride compound was used (8).

A proportionately high flavonoids compound was existed in rosemary honey (2.35 mg QE/100 g) from Spain (16). However, in the present study, Gavan honey and Meymand honey possessed 3.1±0.005 mg QE/100 g and 2.7±0.005 mg QE/100 g of flavonoids respectively, which were higher than Spanish rosemary honey (Table 2).

In this study, a significant difference was noted between phenolic and flavonoid contents of different samples ($P <$ 0.001).

To evaluate the anti-diabetic effects of honey samples, inhibition of α-amylase and α-glucosidase were evaluated. After isolation of sugar, Gaven honey possessed the highest inhibition of α- amylase (Table 4).

In other research, the inhibition rate of α-amylase was reported between 88.8% and 30.5% from the greatest to the smallest concentration (4 μg/mL) (17).

By increasing the honey concentrations, inhibition of α- glucosidase also increased. Accordingly, Bahare sample presented the most inhibition of α-glucosidase (46±0.1% in 20 mg/mL, Table 4). In addition, after isolation of sugar, inhibition of α-glucosidase was increased by increasing the concentrations. Consequently, Zataria honey with 30 mg/mL concentration presented the highest inhibition of α-glucosidase (54±0. 6%, Table 4).

In Gavan honey, after isolation of sugar in 1.25, 2.5, 5, 10, and 15 mg/mL, α-glucosidase was activated in amounts of 5.1%, 7.2%, 7.2%, 11.4%, and 80.1% respectively (Table 4). It was reported that increase of α-glucosidase activity decreased ROS generation (18), and ROS was the origin of different diseases such as diabetes.

The α-glucosidase inhibitors, which include acarbose and miglitol (12), reduce the absorption rate of carbohydrate in the small intestine.

The results presented that inhibition mode of Zataria honey (after isolation of sugar) may be un-competitive (mixed) inhibitions (Table 5). Un-competitive inhibition may demonstrate an increase in Km value while the inhibitor favors binding to the free enzyme. It is probable that Zataria honey cannot bind the active site of the enzyme and presents a broader of inhibition in comparison with acarbose as a competitive inhibitor (19). It means that higher concentrations of acarbose (20) are needed to present the same effect of Zataria honey.

Conclusion
The samples of honey possessed antioxidant properties especially NO radical scavenging and anti-diabetic effects by inhibition of α-amylase, and α-glucosidase. Zataria honey, after isolation of sugar, presents the highest α-glucosidase inhibition with uncompetitive mode of inhibition compared to acarbose as a competitive inhibitor.

Authors' contribution
MM and SM designed the study. FF, SR and ZS performed the experiments. SM wrote and revised the final version of the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest for publication of this manuscript.

Ethical issues
The Ethics Committee of Faculty of Medicine, Shiraz University of Medical Sciences, Iran approved this study. Moreover, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

References

1. Allsop KA, Miller JB. Honey revisited: a reappraisal of honey in pre-industrial diets. Br J Nutr. 1996;75:513-20.doi: 10.5455/ja.20170208043727.
2. McKibben J, Engeseth NJ.Honey as a protective agent against lipid oxidation in ground Turkey. J Agric Food Chem. 2002;50:592-95. doi: 10.1021/jf010820a.
3. White JWJ, Riethof ML, Subers MH, Kushnir I. Composition of American Honeys. 1ᵗʰ ed. Washington: Technical Bulletin; 1962. p. 1261
4. Enefe N G, Ebueih OA. Antioxidant Enzymes and lipid peroxidation in alloxan-induced diabetes rabbits. Am J Anim Vet Sci. 2018;6:27–34. doi: 10.11648/j.avs.20180602.12.
5. Hung TM, Manh HD, Minh PTH, Youn UJ, Na MK, Oh WK. α-Amylase and protein tyrosine phosphatase 1B inhibitory of some Vietnamese medicinal plants used to treat diabetes. Nat Prod Sci. 2007;13:311-16.
6. Pinheiro L, Florack A, Faustino C. Honey Inhibitory Effect on Cancer. Novel Techniques in Nutrition and Food Science. 2019;4:315-16.
7. Devarajan S, Venugopal S. Antioxidant and α-amylase inhibition activities of phenolic compounds in the extracts of Indian honey. Chin J Nat Med. 2012;10:255-59.
8. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal plants and aromatic plant extract. Food Chem. 2004;85: 231-37. doi: 10.1016/j.foodchem.2003.05.007.
9. Lee SH, Lee J B , KW Lee, Jeon Y J. Antioxidant properties of tidal pool microalgae, *Halochlorococcum porphyrae* and *Oltamannsiellopsis unicellularis* from Jeju Island, Korea, Algae. 2010; 25: 45-56. doi: 10.4490/algae.2010.25.1.045.
10. Mockute D, Bernotiene G. The main citral- geraniol and carvacrol chemotypes of the essential oil of *Thymus pulegioides* L. growing wild in Vilnius district (Lithuania). J Agric Food Chem. 1999;47:3787-90. doi: 10.1021/jf980987c.
11. Ademiluyi AO, Oboh G. Phenolic-rich extracts from selected tropical underutilized legumes inhibit α-amylase, α-glucosidase, and angiotensin I converting enzyme in vitro. J Basic Clin Physiol Pharmacol. 2012;23:17-25. doi: 10.1515/jbcpp-2011-0005.
12. Mccue P, Kwon YI, Shetty K. Anti-amylase, anti-glucosidase and anti-angiotensin I-converting enzyme potential of selected foods. J Food Biochem. 2005;29:278-94. doi: 10.1111/j.1745-4514.2005.00020.
13. Ferreira IC, Aires E, Barreira JC, Estevinho LM. Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. Food Chem. 2009;114:1438-43.
14. Małgorzata D,˙Zugan, ID, Tomczyk M, Sowa P, Lejko DG. T. Antioxidant Activity as Biomarker of Honey Variety. Molecules. 2018;23:2069. doi: 10.3390/molecules23082069.
15. Voravuthikunchai S, Howe P. Report on the Fifth International Conference on Natural Products for Health and Beauty (NATPRO 5); May 6–8; Multidisciplinary Digital Publishing Institute; Thailand; 2014.
16. Pontis JA, Costa Lamad, Silva SJRd, Flach A. Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. J Food Sci Technol. 2014; 34: 69-73.
17. Mahmoud AM, Geslevich J, Kint J, Depuydt C, Huysse L, Zalata A, et al. Seminal plasma alpha-glucosidase activity and male infertility. Hum Reprod. 1998:13:591-95. doi:10.1080/19396360902801935.
18. Inzucchi SE: Oral anti-hyperglycemic therapy for type 2 diabetes: scientific review. J Am Med Assoc. 2002;287:360–72. doi:10.1001/jama.287.3.360
19. Zhang H, Wang G, Dong J. Inhibitory properties of aqueous ethanol extracts of propolis on alpha-glucosidase.J Evid Based Complementary Altern Med. 2015;2015:5873838. doi: 10.1155/2015/587383.
20. Ghadyale V, Takalikar S, Haldavnekar V, Arvindekar A. Effective control of postprandial glucose level through inhibition of intestinal alpha glucosidase by *Cymbopogon martinii* (Roxb.). J Evid Based Complementary Altern Med. 2012;2012:372909. doi:10.1155/2012/372909.