

A Study of Antibacterial and Antioxidant Activities of Bee Products: Propolis, Pollen and Honey Samples

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Abstract

Background: The medicinal use of products made by bees is called apitherapy. Apitherapy has become popular as an alternative treatment in recent years. Pharmaceutical properties of bee products depend on biological activities such as antioxidant and antibacterial activities.

Objective: This study was undertaken to comparatively evaluate the bee products for their antioxidant and antibacterial activities against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157: H7 and *Salmonella* Enteritidis.

Methods: The agar well diffusion method was used for the determination of antibacterial effect of bee products. The samples were evaluated for antioxidant capacity by ELISA using Total Antioxidant Status (TAS) assay kit.

Results: All tested honey samples exhibited a measurable antibacterial activity against all of the tested bacteria with different values. Also, two of the propolis extract showed inhibitory effect only against *L. monocytogenes*. Four pollen extracts inhibited the growth of *S. aureus* and *L. monocytogenes* with different values. The propolis extracts showed the highest antioxidant capacity.

Conclusions: The results of this study demonstrated that the antibacterial and antioxidant properties of the bee product of Turkey origin seems to be promising to be used for food preservation and prevention of human health against diseases and disorders. [*Ethiop. J. Health Dev.* 2018;32(2):116-122]

Key words: Antibacterial activity, antioxidant property, bee products

Introduction

Honey, propolis, pollen and other bee products have been used for pharmaceutical properties since the early days in human history (1). Among bee products, propolis, pollen and honey have bacteriostatic and bactericidal effects on both pathogenic and food spoilage bacteria and fungi (2-4). Several studies have suggested that bee products may be recognised as potent antioxidants (5, 6).

The antibacterial effect of honey is associated with its high osmolarity, low pH and hydrogen peroxide content which is called inhibin factor. Non-peroxide antibacterial substances such as aromatic acids, phenolics, flavonoids are other groups of compounds of antibacterial effects of honey (7). Honey is most commonly used as an agent for the treatment of wounds, burns infections and ulcers (8). Honey comprises antioxidant properties which include flavonoids, carotenoid compounds, phenolic acids, and amino acids. Enzymes like catalase and glucose oxidase, ascorbic acid, variable organic acids and Maillard reaction products are also believed to be found in honey (8-10).

Propolis is a flavonoid-rich product derived from plants by bees. It is mixed with beeswax and salivary enzymes. It has medicinal properties such as

antibacterial, antioxidant and anti-inflammatory activities. Its food preservative effectiveness is also high. In addition, propolis stimulates the oral hygiene and protects regeneration of dental pulp (11, 12). The antioxidant and antibacterial effect of propolis is due to various substances such as flavonoids, cinnamic acid and caffeic acid phenethyl ester which depend on the botanical origin of propolis (13-15).

Pollen, another bee product, is recognized as an important part of traditional medicine in several countries. Because of its nutritional and therapeutic properties, pollen is considered as a functional food in the food industry. The phenolic compounds such as gallic, caffeic and trans-cinnamic acid are the components of pollen that are responsible for antibacterial and antioxidative effects. Equally important components of pollen are flavonoids like quercetin, flavones and catechin derivatives, steroids, carotenoid derivatives, and terpenoids (16-18).

In Turkey, there are suitable climate conditions, topographical structures and rich plant flora for honey, propolis and pollen production (19). There are some studies on the chemical composition and biological activities of Turkish bee products (3, 20). However, limited data is available on a comparison of antioxidant and antibacterial properties of bee products. The aim of

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this study was, therefore, to compare the antibacterial and antioxidant activities of different bee products (propolis, pollen and honey samples) produced in Turkey.

Materials and Methods

A total of 30 bee product samples including 5 unifloral chestnut honey (UCH), 5 unifloral pine honey (UPH),

10 multifloral honey and 5 multifloral honey from Central Anatolia Region (MCAH) were taken as samples. In addition, 5 multifloral honey from Black Sea Region (MBSH) and 5 pollen and 5 propolis were obtained. Unifloral chestnut honey (UCH) and Unifloral Pine Honey (UPH) were purchased from supermarkets while the other honey samples were directly obtained from beekeepers (Table 1).

Table 1: List of analysed bee products in the study

Sample	N	Location of Sample	Specified
Propolis	5	Beekeepers in the Central Anatolia Region	Hive location
Pollen	5	Beekeepers in the Central Anatolia Region	Hive location
Multifloral Honey (MCAH)	5	Beekeepers in the Central Anatolia Region	Hive location
Multifloral Honey (MBSH)	5	Beekeepers in the Black Sea Region	Hive location
Unifloral Chestnut Honey (UCH)	5	Supermarkets	Label information
Unifloral Pine Honey (UPH)	5	Supermarkets	Label information

The floral source for multifloral honeys was a combination of nectar producing plants including *Solidago virgaurea* L. subsp. *virgaurea*, *Lamium amplexicaule* L., *Rubus canescens* DC., *Astragalus* L., *Salvia* sp., *Trifolium campestre* Schreb., *Xeranthemum annuum* L., *Vicia sativa* L., *Thymus longicaulis* C. Presl., ect. in the Central Anatolia and Black Sea Regions in Turkey. All multifloral samples were from hives of *Apis mellifera anatoliaca*. All honey samples were transported in glass containers and placed in dark places that had a room temperature of 21 °C until they were analysed.

Chestnut honey samples were examined for pollen analysis using DIN 10760 method and the electrical conductivity of pine honey samples was determined using conductivity meters. Propolis samples were obtained from directly beekeepers by scraping and were stored in a dark environment until they were analysed. Pollen samples were collected by beekeepers using pollen trap. Afterwards, they were pre-dried, cleaned and put in the refrigerator (-18oC).

Preparation of Honey Samples: To prepare honey samples, ten grams of honey samples were put in 20 ml sterile volumetric flask and diluted with sterile distilled water (5).

Extraction of Propolis Samples: Extraction of Propolis Samples involved diluting thirty grams of propolis in 100 ml ethanol (70%) at room temperature. The extracts were filtered after a week and evaporated under vacuum at 50°C (21).

Extraction of Pollen Samples: Two grams of crushed pollen was diluted in 15 ml ethanol (70%), which stayed in a water bath at 70°C, for 30 min. Then the samples were filtered and evaporated under vacuum at 50°C (17).

Bacterial Strains: The antibacterial activities of honey, pollen and propolis samples were tested against

Escherichia coli O157:H7 (NCTC 12900), *Salmonella* Enteritidis (ATCC 13311), *Staphylococcus aureus* (ATCC 29213) and *Listeria monocytogenes* (ATCC 15313).

Antibacterial activity test: Antibacterial activity of the samples was determined by an agar well diffusion assay (22) with some minor modifications. Briefly, bacterial strains were inoculated in Mueller Hinton Broth and were incubated at 37°C for 24 h. After incubation, bacterial strains were diluted in 0.9% saline, equivalent to a 0.5 McFarland standard (approximately 10⁸ CFU/ml). One hundred µL of bacterial suspensions were inoculated on to Mueller Hinton Agar (Oxoid, CM0337) and spread by sterile cotton swabs. Following this, 8 mm diameter wells were cut into the surface of the agar using a sterile cork borer. One hundred µL of propolis, pollen and honey samples were added to each of the wells. *Escherichia coli*, *Salmonella* Enteritidis and *Staphylococcus aureus* plates were incubated at 37°C for 24 h under aerobic conditions, whereas *Listeria monocytogenes* plates were incubated at 37°C for 24 h under 10% CO₂ atmosphere. After incubation, the diameters (mm) of the inhibition zones were measured using a Vernier caliper. Each assay was carried out in duplicate (22).

Determination of Total Antioxidant Status (TAS): Total antioxidant status was measured by a commercially available TAS assay kit (Cat. #: RL0017 Rel Assay Diagnostics, Gaziantep, Turkey) using ELISA technique. Briefly, 500 µL of reagent 1 and 30 µL standards and samples were poured into separate microtiter wells and measured at 660 nm for the first absorbance point by an ELISA (ELX800, Bio-Tek Instruments, USA). Afterwards, 75 µL of reagent 2, mixed gently and incubated at 37°C for 5 min, was added to each well. The absorbance was measured a second time at 660 nm. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent.

TAS was calculated as follows. TAS: $[(\Delta\text{Abs Std1}) - (\Delta\text{Abs Sample})] / [(\Delta\text{Abs Std1}) - (\Delta\text{Abs Std2})]$ and the data were expressed Trolox equivalent per 1gram of extract ($\mu\text{mol TE/g}$).

Statistical Analysis: The software SPSS for Windows version 14.01 (SPSS inc. Chicago I.L) was used in the statistical analysis of data. Antioxidant values of bee product were compared to each other, using analysis of variance (ANOVA) and Student Newman Keuls (SNK) multiple range test as post hoc test. Comparisons between antimicrobial values of the multifloral honey samples from the beekeepers and the unifloral honey samples from the supermarkets were performed with the Mann-Whitney U test. In addition, antioxidant activity of multifloral honey samples from the beekeepers and the unifloral honey samples from the supermarkets were compared using Student's t-test.

Results

Antibacterial Activity: In this study, the antibacterial activity evaluations of bee products were carried out against two Gram-positive and two Gram-negative

bacteria. A significant amount of the samples showed a measurable antibacterial activity, as shown in Table 2. Gram-negative bacteria were found to be more resistant to the antibacterial compounds of honey and honey products than Gram-positive bacteria. *S. aureus* among the Gram-positive and *E. coli* among Gram-negative bacteria were more resistant than *L. monocytogenes* and *S. Enteritidis* respectively to the bee products tested. Furthermore, *L. monocytogenes* was found to be a more sensitive agent than all the other bacteria examined and all the other bee products tested, considering the size of inhibition zone (Table 2). With respect to their inhibition zone diameters, no significant difference was observed between the antibacterial activity of the beekeeper honeys and the supermarket honeys ($P > 0,05$, Table 4).

None of the propolis samples were shown to exhibit inhibitory effect against *S. aureus* *E. coli* O157: H7 and *S. Enteritidis*. The pollen samples were observed to display antibacterial effect only on Gram positive (*S. aureus* and *L. monocytogenes*) bacteria tested.

Table 2: The zone diameters of inhibition (ZDIs) of bee products

	Bee Product	N	Mean \pm SD (mm)			Min ^a (mm)	Max ^b (mm)
L.monocytogenes	Pollen	4	4,8	\pm	1,3	3	6
	Propolis	2	6,5	\pm	2,1	5	8
	MCAH	4	8,8	\pm	3,9	3	12
	MBSH	3	6,0	\pm	1,7	5	8
	UCH	4	5,3	\pm	2,2	3	8
	UPH	4	2,8	\pm	0,5	2	3
S.aureus	Pollen	4	4,8	\pm	2,4	3	8
	Propolis	0				ND	ND
	MCAH	4	9,3	\pm	2,2	7	12
	MBSH	4	6,8	\pm	2,2	4	9
	UCH	4	9,5	\pm	3,9	4	13
	UPH	4	7,8	\pm	2,1	5	10
E.coli O157:H7	Pollen	0				ND	ND
	Propolis	0				ND	ND
	MCAH	2	3,0	\pm	1,4	2	4
	MBSH	4	2,3	\pm	0,5	2	3
	UCH	5	2,8	\pm	0,8	2	4
	UPH	5	2,2	\pm	0,8	1	3
S. Enteritidis	Pollen	0				ND	ND
	Propolis	0				ND	ND
	MCAH	3	3,7	\pm	1,2	3	5
	MBSH	5	4,0	\pm	1,2	2	5
	UCH	5	4,2	\pm	0,8	3	5
	UPH	5	2,0	\pm	0,7	1	3

SD: Standart Deviation, N: Number of positive samples, ND: Not detected

^a: The minimum zone of antibacterial effect was observed extract of bee products against the Gram positive and negative microorganism

^b: The maximum zone of antibacterial effect was observed extract of bee products against the Gram-positive and negative microorganism

MCAH: Multifloral honey from Central Anatolia Region,

MBSH: Multifloral honey from Black Sea Region, UCH: Unifloral chestnut honey, UPH: unifloral pine honey

Antioxidant Activity of Tested Samples: The mean value of the total antioxidant activity of honey samples ranged from 2.84 ± 0.07 to 3.01 ± 0.01 $\mu\text{mol TE/g}$. In addition, the mean value of the propolis and pollen samples was found to be 8.53 ± 0.39 $\mu\text{mol TE/g}$, 5.56 ± 0.33 $\mu\text{mol TE/g}$, respectively (Table 3).

Table 3: Total antioxidant activity of bee products

	N	Mean± SEM ($\mu\text{mol TE/g}$)		Min ($\mu\text{mol TE/g}$)	Max ($\mu\text{mol TE/g}$)
Propolis	5	8,53	± 0,39 ^a	7,3	9,45
Pollen	5	5,56	± 0,33 ^b	4,31	6,2
MCAH	5	3,01	± 0,01 ^c	2,98	3,06
MBSH	5	3,00	± 0,01 ^c	2,98	3,02
UCH	5	2,84	± 0,07 ^c	2,63	3,04
UPH	5	3,01	± 0,02 ^c	2,96	3,05
Statistical Significant		P<0,001* F:120,54			

ANOVA

a,b,c: Different supers cripts within the same column demonstrate significant differences.

According to Student Newman Keuls (SNK) multiple range test, differences between the lines with different letters are statistically significant (P>0,05)

SEM: Standart error of mean

MCAH: Multifloral honey from Central Anatolia Region,

MBSH: Multifloral honey from Black Sea Region,

UCH: Unifloral chestnut honey,

UPH: unifloral pine hone

Antioxidant Activity Levels Between Bee Products:

Total antioxidant activity of honey samples were significantly lower (P < 0.001) than that of propolis and pollen extracts as evaluated by ANOVA and SNK tests (Table 3). The error bar graph showed 95% confidence intervals for differences of antioxidant

activity of propolis and pollen extracts (Figure 1). In connection with the antioxidant properties, no statistically significant results were observed between the honey samples from beekeepers and the samples from the market (P>0,05, Table 4).

Table 4: Results of the comparisons between antioxidant and antimicrobial activity values of honeys from beekeepers and supermarkets

	N	Antioxidant Properties Median (%25,%75 Quarter)	Antimicrobial Activities Mean±SEM
Beekeepers	10	4 (2;8)	3,00±0,01
Supermarkets	10	3 (2;5) P=0,406*	2,92±0,04 P=0,112** F:13,45

* Student T test

** Mann Whitney U test

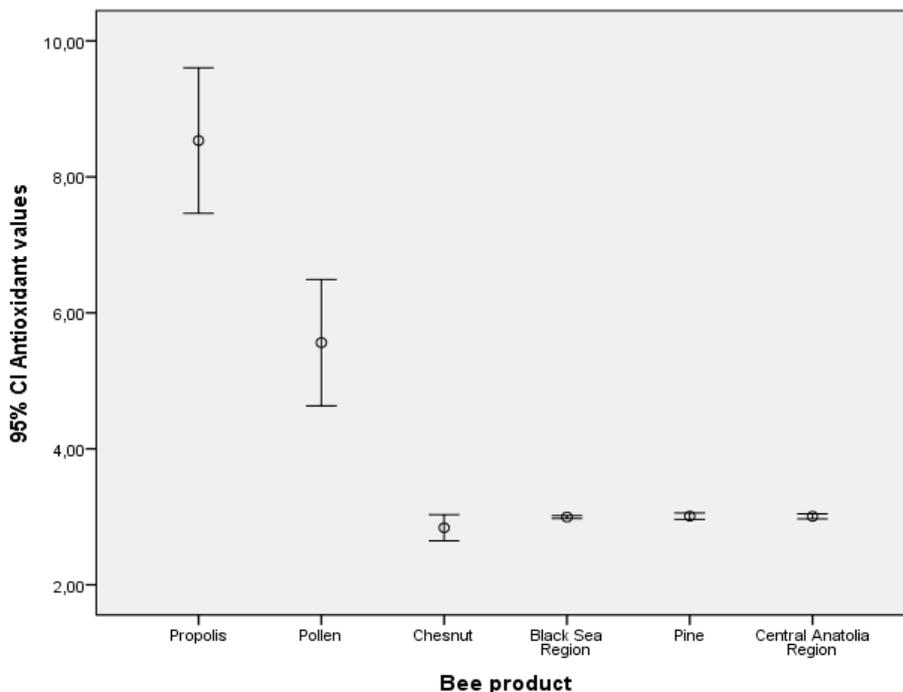


Figure 1: The error bar graphics of antioxidant activity of bee product

Discussion

Previous studies claimed that the antioxidant and antibacterial activities of bee products might be associated with their specific bioactive compounds obtained from different botanical origins (3, 4, 6, 7, 16). In the present study, propolis samples did not show antibacterial activity against *E. coli* O157: H7 and *S. Enteritidis* similar to Yaghoubi et al. (14). In some studies, investigators reported that propolis extracts show antibacterial activity against *E. coli* (15) and *S. Enteritidis* (13). Other studies revealed that the growth of Gram negative bacteria such as *E. coli* and *S. Enteritidis* are inhibited by higher propolis concentration (3, 23). Previous studies reported that Gram positive bacteria are more sensitive to ethanol extract of propolis than Gram negative bacteria (3, 14, 23). In this study, not all propolis extracts had antibacterial effect on *S. aureus* isolates while two propolis extracts showed inhibitory effects on *L. monocytogenes* with 5 to 8 mm inhibition zone. Afrouzan et al. (24) and Ozkalp and Ozcan (25) observed antibacterial effect of propolis extract against *S. aureus* isolates. Similar to the present results, Ozkalp and Ozcan (25) reported inhibitory effect of propolis on *L. monocytogenes*.

The results of other studies have indicated that antibacterial activity of propolis extracts depends on extract concentration (3), type of propolis and type of bacteria tested (15). In addition, some researchers have reported that the antibacterial effect of propolis depends on the differences in chemical composition of plants and their geographical regions (3, 12). According to a study by Temiz et al. (3), the antibacterial activity of the Central Anatolia propolis samples is lower than that of the West and the North Anatolia propolis. In this study, propolis samples were collected from the Central Anatolia.

In this study, four pollen samples inhibited the growth of *S. aureus* and *L. monocytogenes* with an average of ZDIs (3 mm- 8 mm) and (3 mm-6 mm), respectively. Similar to the findings of the present study, studies by Graikou et al. (16), Kacáňiová et al. (26) and Khider et al. (18) reported that *S. aureus* is inhibited by extracts of Greek, Slovakia and Egyptian pollen respectively. Kacáňiová et al. (26) and Khider et al. (18) have also observed antibacterial activity of pollen against *L. monocytogenes*. This finding agrees with the finding of the present study. In the present study, pollen extracts had no antibacterial effect on the *E. coli* O157:H7 and *S. Enteritidis*.

Graikou et al. (16) have noticed that *E. coli* isolates showed resistance to extracts of pollen. This is similar to the result reported here. However, Khider et al. (18) documented that Egyptian pollen extracts showed antibacterial activity on *S. Enteritidis* with an average of ZDIs (3 mm- 8 mm). Many investigators have observed that antibacterial activity of pollen extracts could be ascribed to the high content of phenolic compounds. Examples of such phenolic compounds include p-coumaric, caffeic and ellagic acids, galangin,

pinocembrin and tectochrysin and flavonoids. These are found in pollen such as glucosides, quercetin and kaempferol. All are variable depending on their floral source (16, 17).

The present study demonstrated that most samples of honey had an in vitro antibacterial activity against *S. aureus*, *L. monocytogenes*, *S. Enteritidis*, *E. coli* O157:H7 with 1 to 13 mm inhibition zone. Antibacterial activity of different honey samples was reported by various studies (4, 19, 27, 28). Turkish pine honey is produced by bees from nectar of *Marchalina hellenica* which lives in *Pinus brutia* (pine) only in the forests in Turkey (Aegean region) and Greece (27). There was no investigation on antibacterial effect of Turkish red pine honey. Alnaimat et al. (4) reported that Greek red pine honey samples had antibacterial effect against *E. coli* and other bacteria. Kolayli et al. (19) have documented that Turkish chestnut honey has antibacterial effect on *S. aureus*, but not on *E. coli*. In the present study, Turkish chestnut honey samples showed antibacterial activity against both *S. aureus* and *E. coli* O157:H7.

In this study, the propolis extracts had the highest and the honey samples had the lowest antioxidant activity among the bee products. Similarly, many investigators have reported propolis extracts to possess strong antioxidant effect (5, 6). Nagai et al. (5) and Najakima et al. (6) have also reported that propolis extracts are the most powerful antioxidant among bee products (propolis, pollen, honey and royal jelly).

Studies have postulated that the total phenolic content, flavonoid composition (like quercetin, flavones, isoflavones, flavonones), terpenes, steroids, aldehydes, ketones and ascorbic acid are responsible for the antioxidative activity of propolis (29, 30). As noted in the previous studies (16, 17), the present study also demonstrated that pollen extracts had very strong antioxidant effect. Other investigators demonstrated that the antioxidant activity of pollen extracts is correlated with phenolic content such as gallic, protocatechuic, p-hydroxybenzoic, caffeic, syringic, and p-coumaric. Antioxidant activity of pollen extracts is also reported to correlate with phenolic content such as benzoic, o-coumaric and trans-cinnamic acid and flavonol glycosides. Flavonoids like quercetin, isoquercetin, flavones, isoflavones, luteolin, kaempferol, isorhamnetin, and catechin derivatives were also reported to correlate with antioxidant activity of pollen extracts. (16, 31).

In this study, all honey samples showed antioxidant activity. Antioxidant activities have also been documented for Turkish red pine honey (32), Portugal honey (33) and Slovenian honey (34). Other investigators have observed that antioxidant activity in honey is based on the strong correlation with pH, color, electrical conductivity and total soluble solid (19, 33, 34).

In this study, however, no significant difference was observed between honey samples obtained from beekeepers and supermarkets in terms of antioxidant capacity and antibacterial effect. These results agree with the findings of Malika et al., (28) and A-Rahaman et al., (35). Some researchers have, however, reported the existence of significant differences in antioxidant capacity and antibacterial effect between honeys of different origins. The differences vary based on the processing, storage conditions and seasonal and environmental factors (9, 19, 27, 32, 34, 36).

In addition, other studies have observed that the antioxidant and antibacterial activity of darker honey samples like buckwheat, chestnut, anzer and linen vine are higher than that of lighter honey samples (2, 19, 36). Our darker honey samples (chestnut and pine) were bought from supermarkets. Thus the difference in antioxidant and antibacterial activity might be due to the differences in storage, environment and time (37).

In conclusion, the present research has shown that bee products tested in this study have antibacterial and antioxidant activity, propolis being the best displayer of antioxidant properties. The most sensitive micro-organism was *L. monocytogenes* against the tested bee products in this study. The results of the study demonstrated that the antibacterial and antioxidant properties of the bee products of Turkey origin seem to be promising to be used for food preservation and human diseases and disorder prevention.

References

1. Bankova V, de Castro SL, Marcucci MC. Propolis: recent advances in chemistry and plant origin. *Apidologie*. 2000;31:3–15.
2. 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.
3. Temiz A, Sener A, Ozkok TA, Sorkun K, Salih B. Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two foodborne pathogens, *Salmonella* Enteritidis and *Listeria monocytogenes*. *Turk J Biol*. 2011; 35:503-11.
4. Alnaimat S, Wainwright M, Al'Abri K. Antibacterial potential of honey from different origins: A comparison with Manuka Honey. *J Microb Biotech Food Sci*. 2012;1:1328-38.
5. Nagai T, Sakai M, Inoue R, Inoue H, Suzuki N. Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chem*. 2001;75:237–40.
6. Najakima Y, Tsuruma K, Shimazawa M, Mishima S, Hara H. Comparison of bee products based on assays of antioxidant capacities. *BMC Complement. Altern Med*. 2009;9:4.
7. Bogdanov S, Jurendic T, Sieber R, Gallmann P. Honey for nutrition and health: A review. *J Am Col Nutr*. 2008;27(6):677–89.
8. Molan PC, Betts JA. Clinical usage of honey as a wound dressing: an update. *J Wound Care*. 2004;13:353–6.
9. 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.
10. Brudzynski K, Miotto D. The relationship between the content of Maillard reaction-like products and bioactivity of Canadian honeys. *Food Chem*. 2011;124:869–74.
11. Castaldo S, Capasso F. Propolis, an old remedy used in modern medicine. *Fitoterapia*. 2002;73(1): 1-6.
12. Miorin PL, Levy NCJ, Custodio AR, Bretz WA, Marcucci MC. Antibacterial activity of honey and propolis from *Apis mellifera* and *Tetragonisca angustula* against *Staphylococcus aureus*. *J Appl Microb*. 2003;95:913-20.
13. Orsi R, Sforcin JM, Rall VLM, Funari SRC, Luciano B, Fernandes JRA. Susceptibility profile of *Salmonella* against the antibacterial activity of propolis produced in two regions of Brazil. *J Venom Anim Toxins Incl Trop Dis*. 2005;11:109-16,
14. Yaghoubi SMJ, Ghorbani GR, Soleimani ZS, Satari R. Antimicrobial activity of Iranian propolis and its chemical composition. *DARU*. 2007;15: 45-48.
15. Ivancajic S, Mileusnic I, Cenic-Milosevic D. In vitro antibacterial activity of propolis extracts on 12 different bacteria in conditions of 3 various pH values. *Arch Biol Sci*. 2010;62:915-34.
16. Graikou K, Kapeta S, Aligiannis N, Sotiroudis G, Chondrogianni N, Gonos E, et al. Chemical analysis of Greek pollen - Antioxidant antimicrobial and proteasome activation properties. *Chem Cent J*. 2011;33:1-9.
17. Fatrcová-Šramková K, Nůžková J, Kačániová M, Máriássyová M, Rovná K, Stričík M. Antioxidant and antimicrobial properties of monofloral bee pollen. *J Environ Sci Health Part B*. 2013;48:133–8.
18. Khider M, Elbanna M, Mahmoud A, Owayss AA. Egyptian bee pollen as antimicrobial, antioxidant agents, and dietary food supplements. *Food Sci Biotechnol*. 2013;22:1-9.
19. Kolayli S, Aliyazicioglu R, Ulusoy E, Karaoglu S. Antioxidant and antimicrobial activities of selected Turkish honeys. *Hacettepe J Biol Chem*. 2008;36: 163-72.
20. Ozkalp B, Ozcan MM. Antibacterial activity of pollen and propolis extracts. *J Food Agric Envir*. 2010;8:17- 9.
21. Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *J Ethnopharmacol*. 2005;99(1):69-73.
22. N al Somal NA, Coley KE, Molan PC, Hancock BM. Susceptibility of *Helicobacter pylori* to the

- antibacterial activity of Manuka honey. *J R Soc Med.* 1994;87:9-12.
23. Nieva Moreno MI, Isla MI, Cudmani NG, Vattuone MA, Sampietro AR. Screening of antibacterial activity of Amaicha del Valle (Tucumán, Argentina) propolis. *J Ethnopharmacol.* 1999;68:97-102.
 24. Afrouzan H, Cyrus AC, Mirhadi SA, Ebadollahi A, Vaseji N, Tahmasbi G. Evaluation of antimicrobial activity of propolis and nanopropolis against *Staphylococcus aureus* and *Candida albicans*. *Afr J Microbiol Res.* 2012;6:421-5.
 25. Ozkalp B, Ozcan MM. Antibacterial activity of pollen and propolis extracts. *J Food Agric Envir.* 2010;8:17-19.
 26. Kacániová M, Vuković N, Chlebo R, Haščík P, Rovná K, Čuboň J, et al. The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. *Arch Biol Sci.* 2012;64:927-34.
 27. Ozkok A, D'arcy B, Sorkun K. Total phenolic acid and total flavonoid content of Turkish pine honeydew honey. *J Api Product Api Medical Sci.* 2010;2:65-71.
 28. Malika N, Mohamed F, Chakib EA. Antimicrobial activities of natural honey from aromatic and medicinal plants on antibio-resistant strains of bacteria. *Int J Agri Biol.* 2004;6:289-293.
 29. Bankova V. Chemical diversity of propolis makes it a valuable source of new biologically active compounds. *Journal of ApiProduct and ApiMedical Science.* 2009;1:23-8.
 30. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. Functional properties of honey, propolis, and royal jelly. *J Food Sci.* 2008;73:117-124.
 31. Ulusoy E, Kolaylı S. Phenolic composition and antioxidant properties of Anzer bee pollen. *J Food Biochem.* 2014;38:73-82.
 32. Akbulut M, Ozcan MM, Coklar H. Evaluation of antioxidant activity, phenolic, mineral contents and some physicochemical properties of several pine honeys collected from Western Anatolia. *Int J Food Sci Nutr.* 2009;60:577-89.
 33. Estevinho L, Pereira A, Moreira L, Dias L, Pereira E. Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food Chem Toxicol.* 2008;46:3774-9.
 34. Bertonec J, Doberšek U, Jamnik M, Golob T. Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chem.* 2007;105:822-8.
 35. A-Rahaman NL, Chua LS, Sarmidi MJ, Aziz R. Physicochemical and radical scavenging activities of honey samples from Malaysia. *Agric Sci.* 2013;4:46-51.
 36. Taormina PJ, Niemira BA, Beuchat LR. Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *Int J Food Microbiol.* 2001;69(3):217-225.
 37. Wang XH, Gheldof N, Engeseth NJ. Effect of processing and storage on antioxidant capacity of honey. *J Food Sci.* 2004;69:96-101.