HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY
Research Article
Hacettepe J. Biol. & Chem., 2008, 36 (2), 163-172

Antioxidant and Antimicrobial Activities of Selected Turkish Honeys

Sevgi Kolaylı[1,*], Rezzan Aliyazıcıoğlu[2], Esra Ulusoy[1], Şengül Karaoğlu[3]

[1]Karadeniz Technical University, Faculty of Arts & Sciences, Department of Chemistry, Trabzon, Turkey

[2]Karadeniz Technical University, Vocational School of Health Sciences, Trabzon, Turkey

[3]Rize University, Rize Faculty of Arts & Sciences, Department of Biology, Rize, Turkey

Abstract

The purpose of this study was to investigate antioxidant and antimicrobial activities of selected honeys, chestnut (n = 15), Bayburt (n = 8) and Anzer (n = 7) from Turkey. Antioxidant activity was evaluated by the following three methods: scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals, total phenolics and ferric reducing/antioxidant power (FRAP). Antimicrobial activity was studied by agar diffusion method by using eight bacteria and two fungi. The antioxidant activities were found to be related to the sample concentrations. The phenolic contents of the honeys were found to be significantly related with antioxidant activities with a correlation coefficient of $R^2 = 0.77$. All samples showed moderate antimicrobial activity, especially against *Helicobacter pylori*, *Staphylococcus aureus* and *Klepsiella pneumonia*. No activity was observed against the two fungi, *Candida albicans* and *Candida tropicalis*.

Key Words: Anzer, antioxidant, antimicrobial, honey.

Abbreviations

(FRAP) ferric reducing/antioxidant power, (DPPH) 2,2-diphenyl-1-picrylhydrazyl, (Fe^{+3}-TPTZ) ferric-tripyridyl triazine, (BHT) butylated hydroxytoluene; (TCA) trichloro acetic acid, (TEAP) Trolox equivalent antioxidant power, (GAE) Gallic acid equivalent, (BHI) Brain Heart Infusion, (E.coli) *Esherichia coli*, (Kp) *Klepsiella pneumonia*, (Yp) *Yersinia pseudotuberculosis,* (Pa) *Pseudomonas auroginosa,* (Hp) *Helicobacter pylori* (Ef) *Enterococcus feacalis*, (Sa) *Staphylococcus aureus*, (Bc) *Bacillus cereus,* (Ca) *Candida albicans*, (Ct) *Candida tropicalis*.

* Correspondence to: Sevgi Kolaylı

K.T.U. Fen-Edebiyat Fakültesi, Kimya Bölümü, 61080, Trabzon, Turkey

Tel: +90462 377 24 87 Fax: +90462 325 31 96

E-mail: skolayli61@yahoo.com

INTRODUCTION

Honey, rich in antioxidants such as vitamin C, phenolic acids, flavonoids, is a valuable natural product, and has been used since the earliest times in history [1,2]. The composition of honey is variable owing to differences in plant types of flower origin, environmental conditions, climate, and the contribution of the beekeeper [3,4]. The role of honey in prevention of diseases is quite well known, but the type of honey with respect to antioxidant constituents and effectiveness against various diseases form the basis of many investigations [2].

Several scientists reported that reactive oxygen species (ROS), such as hydroxyl ($OH^{-\bullet}$), superoxide ($O_2^{\bullet-}$,) nitric oxide (NO•) radicals and hydrogen

peroxide (H_2O_2), are damaging for most biomolecules and cause many diseases, and that these compounds should be scavenged. The agents that scavenge or inhibit the formation of these radicals are called antioxidants [5,6]. The antioxidant compounds in natural products are believed to prevent cancer and several diseases by removing or scavenging radicals. Epidemiological studies have demonstrated that consumption of natural products, such as fresh fruit, vegetables, and honey reduce cancer incidence [7]. Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be effective nutrients in the prevention of oxidative stress related diseases [8,9].

Antioxidants can scavenge reactive oxygen/nitrogen species, stop radical chain reactions, or inhibit reactive oxidants from being formed in the first place. Several methods have been developed in recent years to evaluate the antioxidant capacity of biological samples. The most widely used antioxidant methods involve generation of radical species, and the radical concentration is monitored as the present antioxidants scavenge them. The method of FRAP reflects the total antioxidant capacity [10]. Free radical scavenging activity of the samples has widely been investigated by DPPH scavenging method [11].

Turkey, which is the fourth largest honey producing country in the world, has a rare mix of suitable conditions for honey production like climate, topographical structure and richness of plant flora. Turkey's annual honey production in 2006 was estimated at 80.000 tons. Turkey produces a large variety of unifloral (chestnut, pine, rhododendron, acacia, thyme, astragalus, citrus, and sun flowers) and heterofloral honeys. Among unifloral honeys, chestnut (*Castania sativa* L.) has a darker colour. About 1000 tons of chestnut honey is produced every year. It is believed to be a good ethno-remedy for asthma, respiratory diseases, and cancer [12,13]. The second honey type tested, called Anzer honey, is the most famous honey in Turkey and is exported to many countries of the world. Anzer honey is the most expensive, and is believed to have curative effects against many illnesses such as farangitis, tonsillitis, ulcerations, heart and vascular diseases, infertility, cancer, anemia and also in skin care [14]. The heterofloral Anzer honey is produced in Anzer plateau near Ikizdere, and Rize in the East-Black Sea Region of Turkey. The third honey type and the second heterofloral honey tested is collected from Bayburt plateaus. It is produced from the largest variety of flowers in Turkey.

There are number of research studies on the chemical composition and biological activities of several honeys [15-17]. However, there is few researchs on antioxidant and antimicrobial properties of Anzer and other Turkish honeys. Therefore, the purpose of the study is to evaluate the biological and medicinal properties of Turkish honeys.

MATERIALS AND METHODS

Chemicals

Gallic acid (GA), catechine, quercetin, methanol and ethanol were obtained from Sigma Aldrich, Chemie GmbH, Germany. BHT was supplied by Applichem (Darmstadt, Germany). Folin-Ciocalteu's phenol reagent and trichloroacetic acid (TCA) were supplied by Merck (Darmstadt, Germany). DPPH radical and Trolox were purchased from Fluka Chemie GmbH (Switzerland).

Preparation of Honey Extracts

All honey samples were collected during the harvest

time in August-September 2004. Chestnut honeys (n = 15) were collected from the Black Sea Region of Turkey. The Anzer flower honeys were collected from Anzer plateau near İkizdere town of Rize province. Other flower honeys grouped together as Bayburt flower honeys were collected from Bayburt plateaus. The tests were performed within two months of collection of the samples.

Honey samples were dissolved in methanol with continuous stirring at room temperature for 24 h (single extraction). The suspensions were filtered and concentrated under reduced pressure at 40°C in a rotary evaporator to obtain the crude extract in paste form and kept in a dry and dark place.

Determination of Antioxidant Capacity

The antioxidant capacity of honey samples was determined by comparing them with that of known antioxidants, BHT, catechine and Trolox, by employing the following two complementary *in vitro* assays: FRAP assay [18] and DPPH free radical scavenging activity [19]. Total phenolic content was also measured as a representative of total antioxidant capacity.

Determination of Total Phenolic Substance

Total soluble phenolic contents of the samples were determined through Folin-Ciocalteu's reagent method by using gallic acid as the calibration standard [20]. Briefly, 0.1 mL of honey solution (1.0 mg/mL) was diluted with 5.0 mL distilled water. About 0.5 mL of 0.2 N Folin-Ciocalteu's reagent was added, and the contents were vortexed. Following three-minute incubation, 1.5 mL of Na_2CO_3 (2%) was added. After vortexing, the mixture was incubated for 2 h at 20ºC with intermittent shaking. The absorbance was measured at 760 nm at the end of the incubation period. The concentration of total phenolic compounds was calculated as microgram of gallic acid equivalent (GAE) by using a standard graph.

FRAP Assay

The FRAP reagent was prepared by mixing 300 mM of 25 mL acetate buffer, pH 3.6 (3.1 g sodium acetate and 16 mL glacial acetic acid per liter of buffer solution) with 2.5 mL of 10 mM TPTZ solution (0.031g of TPTZ in 10 mL of 40 mM HCl) and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution (3.24 g of ferric chloride in 1000 mL of distilled water). Freshly prepared reagent was warmed at 37°C. Aliquots (100 µL from each extract) were mixed with 3 mL of freshly prepared FRAP reagent. The FRAP values were obtained by comparing the absorbance change of blue colored ferrous- tripyridyltriazine complex at 593 nm in diluted extracts of honey and propolis with those reference solutions containing ferrous ions of known concentrations [18].

Aqueous solutions of known ferrous sulphate concentrations in the range of 100–1000 µM were used for calibration. In order to make a comparison, Trolox was also tested under the same conditions as a standard antioxidant compound. FRAP value was calculated from the following formula:

FRAP value = $\Delta A_{593\ nm}$ test sample / $A_{593\ nm}$ standard x FRAP value of standard (1000 µM)

DPPH Radical Scavenging Activity

1500 µL of 100 µM DPPH in methanol was mixed with equal volume of honey solution in methanol, mixed well, and kept in the dark for 50 minutes. The absorbance at 517 nm was monitored in the presence of different concentrations of honey samples (5%, 2.5%, 1.25%, 0.625%, 0.3125%, and 0.156%). A blank experiment was also carried out to determine the absorbance of DPPH without any sample [199. The antioxidant capacities were expressed as SC_{50} (mg/mL), representing the concentration of the compounds that cause 50%

scavenging of the available DPPH radicals. There is a reverse correlation between SC_{50} values and free radical scavenging activity.

Antimicrobial Activity

All the test microorganisms, eight bacteria and two yeast-like fungi, were obtained from Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey) and were as follows: *Esherichia coli* ATCC 25922, *Klepsiella pneumonia* ATCC 13883, *Yersinia pseudo tuberculosis* ATCC 911, *Pseudomonas aeroginosa* ATCC 10145, *Helicobacter pylori* ATCC 49503, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* 709 ROMA, *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 13803.

Simple susceptibility screening test by using the agar-well diffusion method was employed [21]. Each microorganism was suspended in Brain Heart Infusion (BHI) broth and diluted ca. 10^5-10^6 colony-forming unit (CFU) per mL. They were "flood-inoculated" onto the surface of BHI agar and Sabouraud Dextrose agar (SDA) (Difco, Detroit, MI) and then dried. For *C. albicans* and *C. tropicalis*, SDA was used. Five-millimeter diameter wells were cut from the agar by using a sterile cork-borer, and 50 µl of the solutions (50% in methanol) was delivered into the wells. The plates were incubated for 18 h at 35°C. Antimicrobial activity was evaluated by measuring the zone of inhibition of the growth of the test microorganism. The tests were carried out in duplicate. Ceftazidime (Fortum) (10 µg) and Triflucan (5 µg) were standard antibacterial and antifungal agents, respectively. Water was used as solvent control. The results were expressed in terms of the diameter of the inhibition zones: (-), < 5.5 mm, inactive; (+), 5.5-10 mm, very low activity: (++), 11-15 mm, low activity, (+++), ≥ 16 mm, high activity.

Statistical Analysis

Results were presented as mean values and standard deviations (mean ± SD). Data were tested using SPSS (version 9.0 for Windows 98, SPSS Inc.). Statistical analysis of the results was based on Kruskal–Wallis test and Pearson correlation analyses. Significant differences were statistically considered at the level of $p < 0.05$ otherwise given.

RESULTS AND DISCUSSION

The three authentic honey samples were studied by performing antioxidant and antimicrobial activities performed in triplicate. The results of the colorimetric analysis of total phenolics based on the absorbance

Table 1. The results of total phenolics, FRAP and DPPH radical scavenging tests of the selected honeys from Turkey.

	Chestnut (n = 15)	Anzer (n = 7)	Bayburt (n = 8)	BHT	Catechine	Trolox
Total polyphenol (mg GAE/100 g honey)	430 ± 68	240 ± 52	170 ± 35	-	-	-
DPPH (SC_{50}) (mg/g)	66 ± 17	57 ± 11	42 ± 15	9.8 ± 0.2[a]	2.5 ± 0.3[a]	2.5 ± 0.2[a]
FRAP values (TEAP*)	90 ± 4	93 ± 3	44 ± 4	-	-	-

Values are expressed as mean ± SD

a: µg/mL, GAE:Gallic acid equivalent, SC_{50}: Amount of honey causing 50 percent scavenging of the available DPPH·: 2,2-diphenyl-1-picrylhydrazyl radicals, FRAP: ferric reducing/antioxidant power,

*TEAP: 1000 µM Trolox equivalent antioxidant power.

values of the extract solutions reacted with Folin–Ciocalteu's reagent, expressed as gallic acid equivalents, are given in Table 1. The lowest value was determined for Bayburt honey, with an average value of 170 mg/100 g honey. Anzer honey was second with an average value of 240 mg/100 g honey and the highest phenolics content was found in chestnut with an average value of 430 mg/100 g honey. The phenolic content of chestnut honeys was two times higher than that of Anzer honey and three times higher than that of Bayburt honey.

DPPH assay was used to estimate the total free radical scavenging. It was applied by determining SC_{50} values of the honey extracts. DPPH is a stable free radical, and any molecule that can donate an electron or hydrogen to it can react with it and thereby bleach the DPPH absorption at 517 nm. The results of the DPPH test of the honey samples are given in Table 1. Bayburt flower honeys showed the highest DPPH radical scavenging activity, although the difference was not significant ($p > 0.05$).

In order to determine the total antioxidant capacity, we used the FRAP assay. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{+3}-TPTZ) to its ferrous, colored form (Fe^{2+}-TPTZ) in the presence of antioxidants and measuring the absorbance at 593 nm. The FRAP values have been calculated by comparing the absorbance values of test samples containing serially diluted Trolox at 593 nm. The antioxidant activities of honey extracts by FRAP assay are shown in Table 1. The increased FRAP value is an indication of higher reducing/antioxidant power in this method. Among the samples, the Anzer and the chestnut honeys showed nearly the same FRAP activity, but Bayburt flower honeys showed two times lower FRAP values.

The correlation between FRAP activities and total polyphenol contents in 30 honey samples are analyzed in this study and the results are presented in Figure 1. A significant positive result was observed between FRAP values and total phenol contents ($r = 0.77$, $p < 0.01$).

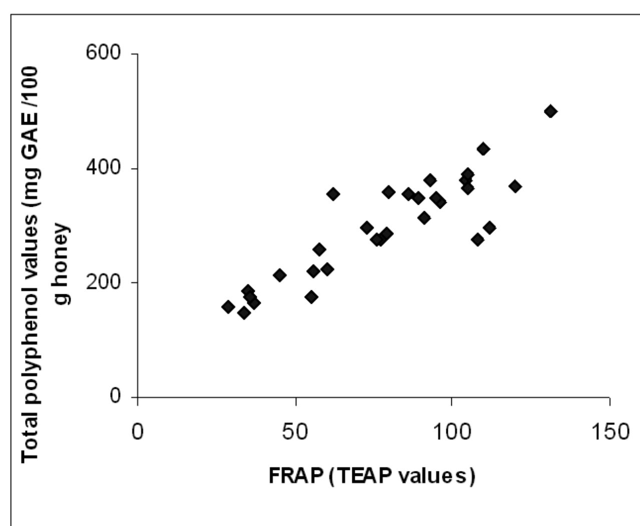

Figure 1. Correlation between FRAP values and total polyphenol contents in honey samples ($R^2 = 0.77$, $p < 0.05$).

The antimicrobial activity of the honey samples was tested *in vitro* by using the agar-well diffusion method with the selected eight bacteria and two fungi (Table 2). Aqueous honey solutions of 50% concentration were used in the antimicrobial activity tests. All honey samples showed moderate inhibition against five bacteria. The effect of Anzer honeys on *H. pylori* was more pronounced. On the other hand, Bayburt flower honeys showed weaker antimicrobial activity on the same bacteria. No antimicrobial activity was observed against the other five microorganisms tested, four bacteria (*E. coli*, *Y. pseudotuberculosis*, *P. auroginosa*, *B. cereus*), and two fungi (*C. albicans* and *C. tropolis*). Honey naturally contains a number of components known to act as antioxidants. Some examples are coumaric acid, ferulic acid, cinnamic acid, caffeic acid, syringic acid and other phenolic compounds [3,12].

The present study was designed to assess total antioxidant capacity, total phenol concentration, free

Table 2. Screening results for antimicrobial activity of the selected honeys from Turkey (1 mg/mL).

	Microorganisms and inhibition									
Honey Code	Ec	Kp	Yp	Pa	Hp	Ef	Sa	Bc	Ca	Ct
401 B	-	+	+	-	-	-	++	-	-	-
404 B	-	+	-	-	++	-	-	-	-	-
403 B	-	-	-	-	+	-	-	-	-	-
417 B	-	+	-	-	-	-	-	-	-	-
425 B	-	++	-	-	+	-	++	-	-	-
426 B	-	++	-	-	+	-	++	-	-	-
428 B	-	+	+	-	-	-	++	-	-	-
430 B	-	+	-	-	-	-	+	-	-	-
406 A	-	+	-	-	++	-	+	-	-	-
407 A	-	+	-	-	++	-	+	-	-	-
405 A	-	+	-	-	++	-	+	-	-	-
408 A	-	++	-	-	++	-	+	-	-	-
409 A	-	+	-	-	++	-	-	-	-	-
410 A	-	+	-	-	++	-	-	-	-	-
413 A	-	+	+	-	++	-	+	-	-	-
411 C	-	-	-	-	+	-	-	-	-	-
312 C	-	++	-	-	+	-	+	-	-	-
414 C	-	++	-	-	+	-	+	-	-	-
415 C	-	++	-	-	++	-	++	-	-	-
416 C	-	++	+	-	+	-	+	-	-	-
402 C	-	++	-	-	++	-	++	-	-	-
418 C	-	++	-	-	+	-	++	-	-	-
419 C	-	++	+	-	+	-	++	-	-	-
420 C	-	+	-	+	+	-	++	-	-	-
421 C	-	++	+	-	+	-	++	-	-	-
422 C	-	++	-	-	+	-	++	-	-	-
423 C	-	++	+	-	+	-	++	-	-	-
424 C	-	+	-	-	+	-	-	-	-	-
427 C	-	+	-	+	+	-	+	-	-	-
429 C	-	++	-	-	+	-	++	-	-	-
Ceftazidime (10 µg)	+++	+++	+++	+++	+++	+++	+++	+++	-	-
Triflucan (5 µg)	-	-	-	-	-	-	-	-	+++	+++

Results were interpreted in terms of the diameter of the inhibition zones: (-) inactive, < 5.5 mm; (+), 5.5-10 mm; very low activity, (++), 11-15 mm; low activity, (+++), ³16 mm high activity. B: Bayburt honey, Code number-A: Anzer honey, Code number C: Chestnut honey. Ec: *Esherichia coli*, Kp: *Klepsiella pneumonia*, Yp: *Yersinia pseudotuberculosis,* Pa: *Pseudomonas auroginosa,* Hp: *Helicobacter pylori,* Ef: *Enterococcus feacalis*, Sa: *Staphylococcus aureus*, Bc: *Bacillus cereus,* Ca: *Candida albicans*, Ct: *Candida tropicalis*.

radical scavenging activity and antimicrobial ability of three different floral honey samples from Turkey. The antioxidant activities of the natural samples were examined by comparing them with known antioxidants such as BHT, catechine, Trolox by employing the following three complementary in vitro assays: total phenolic content, FRAP, and DPPH radical scavenging activity.

Table 3. Test statistics[a,b] of the honey samples for their 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH·), ferric reducing/antioxidant power (FRAP), total polyphenol contents ($p < 0.05$).

	DPPH·	FRAP	Total Polyphenol
Chi-Square	9.605	17.700	23.135
df	2	2	2
Asymp. Sig.	0.008	0.000	0.000

a. Kruskal Wallis Test

b. Grouping variable: Anzer, Chestnut, Bayburt honey samples

The significantly of total phenolics, FRAP and DPPH radical scavenging tests of the honey samples were given in Table 3. The total phenolic content of the chestnut honeys was higher than Anzer and Bayburt honeys. Similar findings were reported [22,23] studied by the same method.

Most plants contain an extensive number of polyphenols and each plant tends to have a distinct profile. The concentration and type of polyphenolic substances depend on the floral origin of honey. Polyphenols are the main components responsible for biological activities, including antioxidant, antimicrobial, antiviral, and anticancer activities [23]. There are many different antioxidant components of any natural sample. It is relatively difficult to measure the activity of each separately. Several methods have been employed to determine antioxidant activity of biological samples, and the results are compared with those of reference antioxidant standards [9,24]. The reducing ability of polyphenols, as determined by the FRAP assay, seems to depend on the degree of hydroxylation and the extent of conjugation of the phenolic compounds. The test is considered to be a good indicator of antioxidant capacity [9,18]. The increased absorbance is an indication of higher reducing power in this method. Among the samples, Bayburt honey showed the lowest reducing power. The order of reducing power for the honeys is as follows: Anzer honey ≥ chestnut honey > Bayburt honey. The total reducing power is the sum of the reducing powers of individual compounds present in a sample. The results of the total reducing power correlated well with those of the total phenolic concentration. However, a positive correlation was found between total reducing power and total phenolic substance. A similar correlation was reported by some workers [25,26]. A direct relationship has also been reported widely between the phenolic content and antioxidant capacity of plants in literature [27,28]. The results of total reducing power in this study correlated well with those of total phenolic concentration. The positive correlation was in agreement with the findings of other authors [10,12].

There are many different experimental methods by which free radical scavenging activity can be estimated. One such method is applied by determining the efficiency of antioxidants to scavenge DPPH radicals as in the current study. Chestnut honeys showed the lowest DPPH radical scavenging activity but the difference was not significant ($p > 0.05$). All honeys showed the same DPPH radical scavenging activity. But we did not find any correlation between DPPH activity and total polyphenol content. DPPH radical scavenging activity is determined by individual compounds present in the sample. It is well known that the type and position of the substituents of polyphenols, such as flavonoids and anthocyanins, affects of radical scavenging activity considerably [12].

In this study, eight different bacteria and two yeast species were used to screen the antimicrobial activity of honey samples. The different floral honey samples exhibited antimicrobial activity against *K. pneumonia*, *H. pylori* and *S. aureus*. The effect of Anzer flower honeys on *H. pylori* was more pronounced and evident for its use against gastric infections. On the other hand, Bayburt flower honeys showed weaker antimicrobial activity on the same bacteria. However, no antimicrobial activity was observed against the five microorganisms tested, four bacteria (*E. coli*, *Y. pseudotuberculosis*, *P. auroginosa*, *B. cereus*), and two fungi (*C. albicans* and *C. tropolis*). *H. pylori* cause stomach ulcers and *S. aureus* causes skin wounds and respiratory infections. *S. aureus* is one of the most common gram positive bacteria causing food poisoning [10, 27].

Some scientists reported that different floral honey and propolis samples showed a good inhibitory activity against *S. aureus* [29-31]. Their findings are in good agreement with the present results. A possible role for honey in the treatment of wounds colonized by antibiotic-resistant bacteria is indicated. The antibacterial activity of the honey samples might be attributed to the components of the honey's floral origin [32,33].

In conclusion, among the studied samples, three different Turkish honeys had high levels of polyphenols and exhibited high antioxidant activity in the two antioxidant assays, and did not possess any pro-oxidant character. Antimicrobial activity tests showed that all the honey samples moderately inhibited *K. pneumonia, H. pylori*, and *S. aureus*. Anzer and chestnut honeys showed higher antimicrobial activity against the three bacteria. No antimicrobial activity was observed against the two yeast-like fungi. The honey samples proved to be a good source of antioxidants, and antimicrobial agents, which could serve as a good dietary source of antioxidants. They could be used to fight against several diseases. It could also be used in the treatment of wounds, gastritis and ulcer.

ACKNOWLEDGEMENTS

This study was supported by Karadeniz Technical University Research Fund (Project No: 2003.111.002.6). We also thank Mr. Mustafa Lezgioğlu for providing the authentic Anzer honeys.

REFERENCES

1. Lusby, P.E., Coombes, A. and Wilkinson, J.M., Honey: A potent agent for wound healing? J. Wound Care Ostomy Continence Nurs. 29, 295, 2002.
2. Schramm, D.D., Karim, M., Schrader, H.R., Holt, R.R., Cardetti M. and Keen, C.L., Honey with high levels of antioxidants can provide protection to healthy human subjects, J. Agric. Food Chem. 51, 1732, 2003.
3. Anklam, E., A review of the analytical methods to determine the geographical and botanical origin of honey, Food Chem. 63, 549, 1988.
4. Azeredo, L.C., Azeredo, M.A.A., De Souza, S.R. and Dutra, V.M.L., Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins, Food Chem. 80, 249, 2003.
5. Halliwel, B., Oxidative stress in cell culture: an under-appreciated problem. FEBS Letters, 540, 3, 2003.
6. Yaylacı, F., Kolaylı, S., Kuçük, M., Karaoğlu, S.A. and Ulusoy, E., Biological activities of trunk bark extract of five tree species from Anatolia, Turkey, Asian J. Chem. 19, 2241, 2007.

7. Halliwel, B., The antioxidant paradox, Lancet 355, 1179, 2000.

8. Halliwel, B., Dietary phenolics; Good, bad, or, indifferent for your health? Cardiovasc. Res. 73, 341,.2007.

9. Huang, D., Ou, B. and Prior, R.L., The Chemistry behind antioxidant capacity assays, J. Agric. Food Chem. 53, 1841, 2005.

10. Beratta, G., Granata, P., Ferrero, M., Orioli, M. and Maffei Facino, R., Standardization of antioxidant properties of honey by a combination of spectrophotometric / fluorimetric assays and chemometrics, Anal. Chim. Acta 533, 185, 2005.

11. Chen, L.A., Mehta, M., Berenbaum, A.R., Zanger, I. and Engeseth, N.J., Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates, J. Agric. Food Chem. 48, 4997, 2000.

12. Küçük, M., Kolaylı, S., Karaoğlu, S., Ulusoy, E., Baltacı, C. and Candan, F., Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem. 100, 526, 2007.

13. Tüzen, M., Silici, S., Mendil, D. and Soylak, M., Trace element levels in honeys from different regions of Turkey, Food Chem. 103, 325, 2006.

14. Ozkırım, A. and Keskin, N., A survey of nosema apis of honey bees (*Apis mellifera* L.) producing the famous Anzer honey in Turkey, Z. Naturforsch C-A J. Biosci. 56, 918, 2001.

15. Mckibben, J. and Engeseth, N.J., Honey as a protective agent against lipid oxidation in ground Turkey, J. Agric. Food Chem. 50, 592, 2002.

16. Sorkun, K., Doğan, C. and Başoğlu, N., Physicochemical, Characteristics and Composition of *Eucalypthus camaldulensis* Dehnh. honey produced in Turkey, Apiacta 36, 182, 2001.

17. Tananaki, C.H., Thrasyvoulou, A., Giraudel, J.L. and Montury, M., Determination of volatile characteristics of Greek and Turkish pine honey samples and their classification by using Kohonen self organizing maps, Food Chem. 101, 1687, 2007.

18. Benzie, I.F.F. and Strain, J.J., The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP assay, Anal. Biochem. 239, 70, 1996.

19. Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J. and Qian, M., Free radical scavenging properties of wheat extracts, J. Agric. Food Chem. 50, 1619, 2002.

20. Slinkard, K. and Singleton, V.L., Total phenol analysis: automation and comparison with manual methods, Am. J. Enol. Viticult. 28, 49, 1977.

21. Perez, C., Pauli, M. and Bazerque, P., An antibiotic assay by the agar well diffusion method, Acta Biol. Med. Exp. 15, 113, 1990.

22. Kumazawa, S., Hamasaka, T. and Nakayama, T., Antioxidant activity of propolis of various geographic origins, Food Chem. 84, 329, 2004.

23. Ahn, M.R., Kumazawa, S., Usui, Y., Nakamura, J., Matsuka, M., Zhu, F. and Nakayama, T., Antioxidant activity and constituents of propolis collected in various areas of China, Food Chem. 101, 1383, 2007.

24. Rice-Evans, C.A., Miller, N.J. and Paganga, G., Antioxidant properties of phenolic compounds, Trends Plant Sci. 2, 152, 1997.

25. Higdon, J.V. and Frei, B., Tea catechine and polyphenols: Health effects, metabolism and antioxidant functions, Crit. Rev. Food Sci. Nutr. 43, 89, 2003.

26. Pulido, R., Bravo, L. and Saura-Calixto, F., Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay, J. Agric. Food Chem. 48, 3396, 2000.

27. Al-Mammary, M., Al-Meeri, A. and Al-Habori, M., Antioxidant activities and total phenolics of different types of honey, Nutr. Res. 22, 1041, 2002.

28. Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P. and Glover, W., Phenolic compounds and their role in oxidative processes in fruits, Food Chem. 66, 401,1999.

29. Moirin, P.L., Levy Junior, N.C., Custodio, A.R., Bretz, W.A. and Marcucci, M.C., Antibacterial activity of honey and propolis from *Apis melliafera* and *Tetragonisca angustula* against *Staphylococus aureus,* J. Appl. Microbiol. 95, 913, 2003.

30. Oradifiya, L.O., Adesina, S.K., Igbeneghu, O.A., Akinkunmi, E.O., Adetogun, G.E. and Salau, A.O., The effect to honey and surfactant type on the antibacterial properties of the leaf essential oil of *Ocimum gratissimum* Linn. Against common wound–infecting organisms, Inter. J. Aromatherapy 16, 57, 2006.

31. Cooper, R.A., Molan, P.C. and Harding, K.G., The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds, J. App. Microbiol. 93, 857, 2002.

32. Weston, R.J., The contribution of catalase and other natural products to the antibacterial activity of honey: a review., Food Chem. 71, 235, 2000.

33. Ceylan, N. and Uğur, A., Investigation of in vitro antimicrobial activity of honey, Rev. Biol. 94, 363, 2001.