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Botanical origin and antioxidant activities of propolis from the Irano-Turanian region

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ABSTRACT

Propolis is a natural bioactive mix and a traditional medicine that has been used for treating several complications. The bioactive properties of propolis are dependent on its botanical origin. This study investigated the pollen composition, antioxidant activities, and the total phenol and total flavonoid content of a propolis sample from the Refahiye (Erzincan, Turkey) region. Melissopalynological analysis conducted according to the relevant literature revealed that the pollen profile of the sample primarily indicated the presence of the Fabaceae (38.4%), Asteraceae (20.2%), and Fagaceae (11.2%) families. The antioxidant ability of propolis extract was analyzed by the hydrogen peroxide scavenging activity (HPSA) (in terms of SC50), ferric reducing antioxidant power capacity (FRAP) (%), DPPH radical scavenging activity (in terms of SC50), metal-chelating activity (%), total phenol content (TPC), and total flavonoid content (TFC), which showed the following values: 11.72±0.04 µg/mL, 90.73% ± 0.24%, 18.34±0.08 µg/mL, 89.69% ± 0.12%, 10673.4±3.30 mg GAE/100 g of propolis sample (PS), and 170.65±1.12 mg QE/100 g of PS, respectively. These results were compared using butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol (TOC) as standard antioxidant compounds. The high biological activity of propolis from the Refahiye region could be attributed to its rich pollen composition. These results indicate that propolis is an important source in terms of its antioxidant activities.

Keywords: Bioactive properties, melissopalynological analysis, propolis, Refahiye (Erzincan)

INTRODUCTION

Propolis (bee glue) is a sticky dark-colored material that is collected from honeybee plants (buds and leaves), which is mixed with pollen as well as enzymes secreted by bees (Marcucci 1995). The term "propolis" has been derived from two Greek words, i.e., "pro" stands for "at the entrance to" and polis for "community" or "city" (Wagh 2013). Bees use propolis in their hives as a protection against predators and microorganisms, to repair damage, as a thermal isolator, and to build aseptic locals to prevent microbial infection of larvae (Bankova et al. 2000; Huang et al. 2014). Propolis has a wide spectrum of biological activities and has been used for various purposes by the people. Several studies have investigated the antibacterial (Sforcin et al. 2000; Hegazi and Abd El Hady 2001), antifungal (Ota et al. 2001; Herrera et al. 2010), anti-inflammatory (Borrelli et al. 2002), anticancer (Sawicka et al. 2012), antioxidant (Perveen and Qaiser 2007; Kalogeropoulos et al. 2009; Silva et al. 2013), and antitumor (Oršolić & Bašić 2003; Sobočanec et al. 2011) properties of propolis. The chemical ingredients of propolis have been reported to be highly variable and dependent on the native flora (Bankova et al. 2000; Kumazawa et al. 2004; Silva et al. 2008). Turkey has a great diversity of plants comprising more than 10,000 taxa with 173 families and about 2,650 endemic species (Davis 1965-1985; Özhatay 2013). However, propolis production has been generally ignored in Turkey, where several beekeepers focus on only the production of honey. The aim of the present study was to determine the quality of a propolis sample from the Irano-Turanian phytogeographic region of Turkey in terms of its antioxidant activity and botanical origin.

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MATERIALS AND METHODS

Reagents and standards

All the following reagents used were of proanalysis grade: 2,2-diphenyl-1-picrylhydrazyl, butylated hydroxyanisole, butylated hydroxytoluene, gallic acid, quercetin, α-tocopherol (Sigma), Folin–Ciocalteu reagent, and absolute ethyl alcohol (Merck). All other chemicals were of analytical grade.

Sample collection

Propolis sample was collected from the East Anatolia Region of Turkey, which covers the Irano-Turanian floral region (Davis 1965-1985).

Sample solution

The sample solution was prepared by mixing 1.33 g propolis with 100 mL absolute ethanol. This suspension was shaken at room temperature on a magnetic stirrer for 24 h. Then, the extract solution was filtered through a Whatman no. 4 filter paper and stored at -4°C.

Palynological identification

The study material was prepared for examination under the microscope according to the method of Warakomska and Maciejewic (1992). The sample was ground into powder, mixed with ethanol-ether-acetone (1:1:1), and then shaken. This mixture was filtered through a strainer with 0.3-mm holes. The suspension was then centrifuged at 3500–4000 rpm for 20 min, after which the supernatant was discarded. Then, using the residual sediment, two slides were prepared for each sample using basic fuchsin glycerin gelatin and were examined simultaneously for determining the pollen count.

The identification of the stages of pollen grains was performed using an optical microscope (Nikon Eclipse Ci, Japan) at 400× and 1000× magnifications.

Antioxidant analyses

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The DPPH radical scavenging activity was determined according to a previously reported method of Blois (1958) with few modifications. Serially diluted samples (3.0 mL) at different concentrations (10–100 µg/mL) were added to DPPH solutions (1.0 mL, 0.2 mM) with ethanol. The mixtures were vigorously shaken and allowed to stand at room temperature for 30 min. Then, the absorbance was recorded at 517 nm using a spectrophotometer, and the results were expressed as SC_{50} (the concentration required for scavenging 50% of DPPH) (µg/mL) by a linear regression analysis and represented as mean of the data.

Determination of hydrogen peroxide scavenging activity (HPSA)

The HPSA was determined according to the method described by Ruch et al. (1989). Briefly, the samples were dissolved in 0.04 M phosphate buffer (pH=7.4) and 3.4 mL of the sample was mixed with 0.6 mL of 40 mM H_2O_2 solution (prepared using the same buffer). The absorbance of the mixture was measured at 230 nm versus the blind sample after 10 min using a UV/VIS spectrophotometer. Phosphate buffer without hydrogen peroxide was used as blank. A decrease in the absorbance value indicated a high level of hydrogen peroxide scavenging activity. The results were expressed as SC_{50} values (µg/mL).

Ferric reducing antioxidant power (FRAP) assay

The reducing ability of the sample was investigated following a method using a ferric ion, with minor modifications (Güder et al. 2014). About 2.0 mL of the sample or standards was mixed with PBS (phosphate-buffered saline) (2.0 mL, 0.2 mol L^{-1}, pH 6.6) and potassium ferricyanide (2.0 mL, 1.0%). This mixture was incubated at 50°C for 20 min, followed by the addition of trichloroacetic acid (2.0 mL, 10%). Then, 2.0 mL of this solution was mixed with distilled water (2.0 mL) and $FeCl_3$ (0.5 mL, 0.1%). The Fe^{3+}/Fe^{2+} transformation was determined due to the presence of samples at 700 nm.

$$FRAP\ (\%) = (A_s/A_c) \times 100$$

Where, A_c is the absorbance of the control, and A_s is the absorbance of the sample or standards.

Determination of metal-chelating activity

The metal-chelating activities of the propolis extract and the standard antioxidant materials were estimated according to the method described by Dinis et al. (1994). Briefly, 0.05 mL of 2 mM $FeCl_2$ and 0.4 mL of the extract solution were mixed. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution. This mixture was vigorously shaken and kept at room temperature for 10 min, after which the absorbance of the mixture was measured at 562 nm using a UV/VIS spectrophotometer. A decrease in the absorbance value demonstrated a high level of metal-chelating activities of the extract solution and the standard antioxidant materials. The metal-chelating activities of the extract solution and the standard antioxidant material were calculated according to the following formula:

$$\text{Ferrous ion chelating activity } (\%) = [1 - (A_s/A_c)] \times 100$$

Where, A_c is the absorbance value of the control, and A_s is the absorbance value of the extract solution or the standard antioxidant material

Determination of total flavonoid content (TFC)

The TFC of the extracts was determined according to the colorimetric method described by Chung (2002) with minor modifications. Sample solutions (0.5 mL) were added to a tube containing 1.5 mL of absolute ethanol. $AlCl_3.6H_2O$ solution (0.1 mL, 10.0%) and potassium acetate (0.1 mL, 1.0 mol L^{-1}) were subsequently added to prepare the mixture. Distilled water was added to make up the total volume to 5.0 mL, and then the absorbance was read after 30 min at 415 nm. The TFC values were expressed as microgram of quercetin equivalent that was obtained from the standard graph ($R^2 = 0.9979$).

Determination of total phenolic content (TPC)

The TPC of the samples was determined by the Folin–Ciocalteu phenol reagent (Folin C) colorimetric method described by Slinkard and Singleton (1977). The sample solutions (0.5 mL) were mixed with 7.0 mL of distilled water and subsequently with Folin C reagent (0.5 mL). After 3 min, Na_2CO_3 solution (3.0

mL, 2.0%) was added to the mixture. The color developed after 1 h, and then the absorbance was measured at 760 nm using a spectrophotometer. Gallic acid was used as the standard, and TPC was expressed as microgram of gallic acid equivalent using an equation that was obtained from the standard gallic acid graph ($R^2 = 0.9995$).

RESULTS AND DISCUSSION

All the 18 pollen types (Table 1) belonging to 13 families were identified in the propolis sample. The identified pollen samples generally belonged to the Fabaceae (38.4%), Asteraceae (20.2%), and Fagaceae (11.2%) families (Figure 1). The pollen spectra of the sample were found to overlap with those of the Refahiye vegetation. Gençay and Sorkun (2006) stated that 32 different plant families have been identified by the pollen analysis of 30 propolis samples from Kemaliye (Erzincan), and Apiaceae, Asteraceae, Campanulaceae, Fabaceae, Fagaceae, Lamiaceae, Liliaceae, Pinaceae, Rosaceae, Salicaceae, Rhamnaceae, and Scrophulariaceae families were primarily determined as the botanical origins of propolis. Çelemli and Sorkun (2012) analyzed the pollen spectra of 92 propolis samples collected from Tekirdağ and reported that the frequently observed pollen grains belonged to the Asteraceae, Boraginaceae, Brassicaceae, Fabaceae, and Salicaceae families. These results are consistent with those of our study.

The HPSA, FRAP, DPPH radical scavenging activity, metal-chelating activity, TPC and TFC values are as follows: 11.72±0.04 µg/mL, 90.73% ± 0.24%, 18.34±0.08 µg/mL, 89.69% ± 0.12%, 10673.4±3.30 mg GAE/100 g of PS, and 170.65±1.12 mg QE/100 g of PS, respectively. These results were compared using butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol (TOC) as standard antioxidant compounds. The obtained results of the standards are presented in Table 2. Moreira et al. (2008) reported the DPPH radical scavenging activities of two propolis samples (Bornes and Fundão) as 6±3 and 52±3 µg/mL, respectively. In addition, they reported the TPC of the same samples as 32900 and 15100 mg GAE/100 g, respectively. Based on these literature data, our results showed an average DPPH radical scavenging activity but a lower TPC.

Popova et al. (2005) investigated the TPC of Turkish propolis samples (from Adana, Artvin, Erzurum, İzmir, Kayseri, and Yozgat) and reported TPC values of 8.2%–30.4%. In the Irano-Turanian samples (Erzurum, Kayseri, and Yozgat), the TPC values were 10.5%, 27.5%, and 26.4% respectively. Our results were found to be consistent with these literature data.

Lima et al. (2009) reported the TPC values of the methanolic propolis extract as 25700–39300 mg GAE/100 g. In addition, the TFC was found to be between 6600 and 13300 mg QE/100 g. Wali et al. (2015) analyzed the propolis samples collected from the Kashmir–Himalayan region using different extraction solvents (ethanol, water–ethanol, and water) and reported TPC and TFC values of 18000–26000 mg GAE/100 g and 4500–10500 mg QE/100 g, respectively. Ahn et al. (2007) studied the propolis samples collected from different parts of China and reported that the TPC values ranged from 42.9±0.8 to 302±4.3 mg GAE/g of samples and the TFC values ranged from 8.3±3.7 to 188±6.6 mg QE/g of samples. Choi et al. (2006) found the TPC value of Korean propolis samples collected from Yeosu to be 212.7±7.4 mg GAE/g, and Kumazawa et al. (2004) reported that the TPC and TFC values of propolis ranged from 31.2±0.7 to 299±0.5 mg GAE/g and from 2.5±0.8 to 176±1.7 mg QE/g, respectively, collected from different geographic regions. Laskar et al. (2010) showed that the TPC and TFC values ranged from 159.10±0.26 to 269.10±0.17 mg GAE/g and from 57.25±0.24 to 25.50±0.36 mg QE/g, respectively, in Indian propolis samples. The TPC and TFC values are comparable with the literature data because of the average contents. Furthermore, the DPPH radical scavenging activity and the HPSA were found to be 18.34 and 11.72 µg/mL, respectively. Gülçin et al. (2010) have also reported the DPPH radical scavenging activity and the HPSA of the lyophilized aqueous extract of propolis collected from

Table 1. Pollen types identified from Refahiye propolis

Taxa/Family	%	Taxa/Family	%
Apiaceae	1.2	Fagaceae	
Asteraceae		*Quercus*	11.2
Echinate type	9.2	Lamiaceae	2.6
Scabrate type	1.2	*Thymus*	1
Xanthium	2	*Phlomis*	0.6
Taraxacum	1.8	Rosaceae	1.8
Carthamus	4.2	*Sanguisorba*	0.6
Centaurea	1.8	Salicaceae	
Betulaceae		Salix	5
Betula	0.4	*Populus*	4
Boraginaceae	8.8	Pinaceae	
Onosma	0.6	*Pinus*	0.2
Brassicaceae	0.6	Poaceae	0.6
Campanulaceae	1	Unidentified	1.2
Fabaceae	20.2		
Astragalus	10.2		
Onobrychis	8		

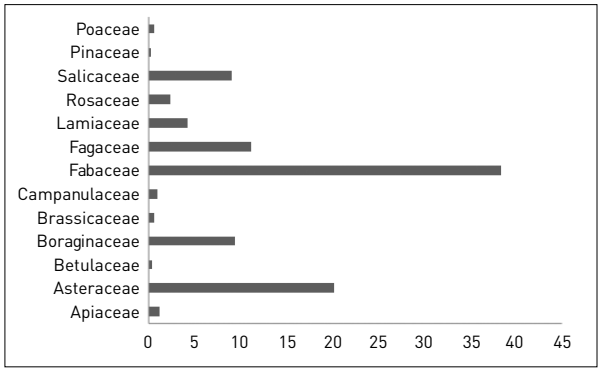

Figure 1. Distribution of families of which the pollens were identified from Refahiye propolis

Table 2. Antioxidant test results of the sample and standards

	HPSA[1]	FRAP[2]	DPPH[1]	TPC[3]	TFC[4]	MCA[2]
Propolis	11.72±0.04	90.73±0.24	18.34±0.08	10673.4±3.30	170.65±1.12	89.69±0.12
BHA	184.13±1.19	92.02±0.80	8.53±0.39	-	-	89.95±0.05
BHT	147.49±0.09	54.16±0.10	9.01±0.02	-	-	86.26±0.15
TOC	216.26±0.47	32.98±0.21	11.97±0.07	-	-	93.41±0.06

[1] SC_{50} (µg/mL)
[2] % activity
[3] mg GAE/100 g of PS
[4] mg QE/100 g of PS
BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; TOC: α-tocopherol

Erzurum as 31.81 and 6.54 µg/mL in terms of IC_{50} after analyzing the polyphenol contents and the antioxidant activity. Our sample showed a higher DPPH radical scavenging activity than that of the Erzurum sample, but lower HPSA. Moreira et al. (2008) studied two Portugal propolis samples collected from different regions and determined the DPPH radical scavenging activities to be 0.006 and 0.052 mg/mL, respectively (in terms of EC_{50}). Laskar et al. (2010) determined the DPPH radical scavenging activities of propolis in terms of IC_{50} values to be 0.05–0.07 mg/mL. The DPPH radical scavenging activities of Brazil propolis was found to be 3.17–8.79 mg/mL (Pontis et al. 2014). The DPPH radical scavenging activity of our sample was the highest among all the literature samples, except the Portugal propolis sample. Gülçin et al. (2010) found the FRAP activity to be 0.568 (absorbance value at 700 nm). Compare this value with our result, it was very lower than that of our sample. The metal-chelating activity of propolis was determined as 89.69%, and those of the standard compounds (BHA, BHT, and TOC) were found to be 89.95%, 86.26%, and 93.41%, respectively. Gülçin et al. (2010) calculated the metal-chelating activity using EDTA as a reference standard and reported a value of 12.04 µg/mL of Fe^{+2}-chelating activity for the Erzurum propolis sample, which was lower than those of the standard compounds (BHA, BHT, and TOC). However, our sample showed a similar activity as those of the standard compounds, especially BHA. Geckil et al. (2005) determined the metal-chelating activities of different extracts of Malatya propolis and reported values of 56%–70%. Geckil et al. (2005) also reported lower metal-chelating activities of propolis samples than those of the standard compounds (BHA and BHT). Subsequently, the Erzincan propolis sample demonstrated effective chelating activity than that by the Erzurum propolis sample. Therefore, the results of this study show that our propolis samples exhibited highly effective antioxidant activities.

CONCLUSION

This study showed that the propolis sample collected from the Refahiye (Erzincan, Turkey) region has an average antioxidant activity in comparison with the literature data. Therefore, it can be used as a natural source in the medicine and food industry. Especially, the active components in the propolis sample can be isolated and characterized. In this context, these active components can be used as potential treatment agents for certain diseases.

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