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Antioxidant Variability of Propolis Collected from Different Zones in Hives

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Abstract

Propolis is biologically highly active honey bee product. The popularity of propolis is increasingly growing because of its contribution to human health. Propolis composition is highly variable depending on its sources. Different honey bees subspecies can collect propolis having different anti-bacterial effect. Honey bees, collect propolis for a couple of different purposes such as narrowing the entrance of their own hives and airflow isolation. In this study, propolis collected from entrances (EC) and top of deep supper (FC) of six different hives, and the antioxidant properties of these propolis samples were compared. Average values of total phenolic content were 68.5 and 62.6 mg GAE/g propolis extract, IC_{50} value of DPPH were 0.14 and 0.16 mg/mL, and FRAP value were 43.5 and 38.4 mg TE/g propolis extract samples collected from EC and FC, respectively. Statistically significant differences have not been found in terms of antioxidant activity analysis between EC and FC collected propolis (P>0.05).

Introduction

Propolis (bee glue) is collected from variable plant sources and the name came from the Greek, pro-for or in defence, and polis- the city, and means is defence of the city (or the hive) (Ghisalberti, 1979). Propolis is used by bees for versatile purposes such as covering holes and cracks, repairing combs, sticking the border combs, narrowing the entrance of the hive for easy defending, and embalming the aliens (Ghisalberti, 1979). The composition of propolis is highly variable depending on collected sources, and the most important botanical propolis sources are poplars (Populus spp.), briches (Betula spp.), willows (Salix spp.), chestnut tree (Aesculus hippocastanum L.), elms (Ulmus spp.), pine trees (Pinus spp.), oaks (Quercus spp.), spruces (Picea spp.) and ashes (Fraxinus spp.) (Bonvehí & Coll, 1994; Greenaway, Scaysbrook, & Whatley, 1990). Marcucci (1995) and Bankova, Christov, Kujumgiev, Marcucci, and Popov (1995) identified that propolis have more than 300 constitutes. It is reported that-variable biological activities of the ethanolic extract of propolis such as hepatoprotective effect (González et al., 1994), antitumor activity (Mitamura et al., 1996), antioxidative activity (Matsushige, Kusumoto, Yamamoto, Kadota, & Namba, 1995), antimicrobial activity (Bankova et al., 1995), and anti-inflammatory effect (Krol et al., 1996).

Honey bees accumulate propolis to entrances of their hives for narrowing, to top of deep supper the hives for air isolation, between the combs for sticking the combs, and some more hive region. There are various research indicate that the composition of propolis not only be affected from environmental and botanical origin factors and this can be affected from the race of bees and bees diseases as well (Popova,

Antonova, & Bankova, 2017; Silici & Kutluca, 2005). Honey bees can consciously prefer to choose the different resource of propolis in nature. Bees accumulate propolis to the entrance of hive for narrowing the entrance and maybe grooming themselves with propolis, but bees accumulate propolis to top of deep supper for airflow isolation. Hypothesis of this study is that the antioxidant effect of propolis collected from the entrance of the hive and top of deep supper the hives can be different due to bees collect this propolis for different purposes.

Materials and Methods

Propolis collected from 6 colonies in Apicultural Research Institute apiary. The raw propolis were collected from two different zones in hives; the entrance (EC) of the hives and the top of deep supper the hives (FC). Collected raw propolis extracted by using 70% ethanol solution. Samples placed to erlenmeyer flasks and ethanol solutions were added. Propolis/solvent ratio was 3:10 (m/V). Maceration lasted 7 days at room temperature. Samples were filtered through the filter page after maceration and were held at 4ºC for one day. Samples were filtered again with filter page, then ethanol vaporized at the rotary evaporator. Thus, extracted propolis obtained.

Extraction of propolis samples

0.1 g from each propolis sample weighed and ethanol added up to 10 mL in falcon tubes. Samples left at 4ºC for one day, then all samples centrifuged 10 min at 5000 rpm. The supernatant was used for antioxidant analysis (Elmastas, Isildak, Turkekul, & Temur, 2006; Lachman, Orsak, Hejtmankova, & Kovarova, 2010).

Determination free radical scavenging activity (DPPH)

Trolox was used as standard for free radical scavenging activity analysis and activity was determined by reading absorbances of compounds at 517 nm which reacted with 1,1-diphenyl-2-picryl-hydrazil (DPPH). 3 mL ethanol and 1 mL DPPH were added to 80 µL sample solution. All absorbances obtained after holding of all samples at room temperature for 30 minutes (Shimada, Fujikawa, Yahara, & Nakamura, 1992).

Determination total phenolic content (TPC)

200 µL from supernatants of each sample were transferred to tubes. 0.1 mL Folin-Cicoalteu reactive and 0.3 mL 2% Na_2CO_3 solution added and all tubes filled up to 5 mL with distilled water. A standard curve obtained by reaction of gallic acid with Folin-Cicoalteu. Absorbances of samples were read at 760 nm and results were calculated as mg GAE/g propolis extract (Gülçin, Şat, Beydemir, Elmastaş, & Küfrevioğlu, 2004; Slinkard & Singleton, 1977).

Determination ferric reducing ability of plasma (FRAP)

80 µL samples were transferred to tubes for determining FRAP value of each sample and 1.25 mL sodium phosphate buffer (0.2 M, pH 6.6) and 1.25 mL 1% potassium ferro cyanide solution were added, and solutions left for incubation at 50ºC for 30 minutes. 1.25 mL 10% 3-chloro acetic acid solution and 0.25 mL 1% $FeCl_3$ solution were added after incubation. Trolox was used to obtain standard curve and all samples read at 700 nm at spectrophotometer (Oyaizu, 1986).

Statistical Analysis

IBM SPSS Statistics 20 program was used for statistical analysis. Kruskal Wallis h test was used to compare mean ranks of all values of 2 groups.

Results

The value of total phenolic content of propolis extract between 47.9 and 88.1 mg GAE/g propolis extract showed in Table 1. Even though the propolis were collected from different zones of the hive, there are no statistical differences between the groups (P>0.05). The result of DPPH was expresed IC_{50} values that ranged between 0.12-0.19 mg/mL and while average value for propolis EC collected was 0.14 mg/mL, average value for propolis FC collected was 0.16 mg/mL. IC_{50} values of all samples can be seen in Table 1. DPPH values of propolis samples collected from different areas of hives were not different statistically (P>0.05). FRAP values ranged between 32.9-58.9 mg TE/g propolis extract and average values propolis samples EC and FC collected were 43.5 and 38.4 mg TE/g propolis extract respectively. FRAP values of samples were also indifferent statistically (P>0.05).

Discussion

Free radical scavenging activity analysis (DPPH)

IC_{50} value of DPPH analysis expresses the concentration of antioxidant compound which required for scavenging 50% of free radicals found in medium. Marghitas, Dezmirean, Moise, Mihai, and Laslo (2009) obtained IC_{50} values between 0.3-5.6 mg/mL in the study which propolis samples collected from Romanian. Mercan et al. (2006) indicate that IC_{50} value range from 3.4 to 4.6 mg/mL. IC_{50} values calculated in this study were lower in contrast with values of these works. Talla et al. (2017) compare DPPH value of Cameroonian propolis and vitamin C. The IC_{50} value of Cameroonian is found 0.30 mg/mL. That value is higher than our findings. The possible explanation of this non-overlapping is that our propolis sample were collected Black See region of Turkey, but Talla et al.'s (2017) study propolis samples were collected from Cameroon.

Table 1. The TPC, DPPH and FRAP analysis results of propolis collected from FC and EC.

Samples	TPC	DPPH (IC_{50})	FRAP
FC1	57.0±3.76	0.17±0.03	33.3±2.45
FC2	60.0±2.60	0.13±0.04	37.9±2.52
FC3	54.0±3.21	0.13±0.03	36.2±1.60
FC4	68.2±4.05	0.19±0.04	34.0±1.04
FC5	64.8±3.33	0.17±0.02	43.6±3.23
FC6	71.4±5.49	0.14±0.04	45.5±3.21
Avg.	62.6±2.51	0.16±0.01	38.4±1.24
Min.	54.0±2.21	0.13±0.03	33.3±2.45
Max.	71.4±5.49	0.19±0.04	45.5±3.21
EC1	76.8±3.18	0.17±0.03	39.0±3.53
EC2	47.9±2.64	0.13±0.03	37.0±2.89
EC3	75.3±2.89	0.13±0.25	44.8±3.09
EC4	49.6±3.23	0.18±0.03	32.9±2.71
EC5	73.3±3.09	0.17±0.26	48.4±2.43
EC6	71.4±3.04	0.14±0.03	58.9±2.37
Avg.	68.5±5.29	0.14±0.06	43.5±3.78
Min.	47.9±2.64	0.12±0.03	32.9±2.71
Max.	88.1±4.01	0.18±0.03	58.9±2.37

FC: Propolis collected from top of deep supper the hives. EC: Propolis collected from entrance of the hives. All analysis were performed three times. TPC (mg GAE/g propolis extract). DPPH (IC_{50} mg/mL) and FRAP (mg TE/ g propolis extract) of propolis collected from FC and EC. ±standart error of mean.

Total phenolic content (TPC)

Total phenolic content was illustrated with gallic acid equivalency (GAE). The average of the total phenolic content of propolis EC and FC collected are 68.5 and 62.6 mg GAE/g propolis extract respectively (Table 1). There appears to be no previous research exploring about comparison propolis collected from EC and FC. Socha, Galkowska, Bugaj, and Juszczak, (2015) indicate that the total phenolic content of propolis range from 150.05 to 197.14 mg GAE/g propolis extract which are higher than our total phenolic content. The possible explanation is that Socha et al. (2015) collect propolis from Polland, but we collected our propolis from Turkey Black Sea region. Aliyazıcıoglu, Sahin, Erturk, Ulusoy, and Kolayli, (2013) study show that the phenolic content of the propolis collected from different part of Turkey is between 115 and 210 mg GAE/g. The potential explanation of these differences is that propolis collected region. Propolis collected from Black Sea region in this study, but in Aliyazıcıoglu et al. (2013) study propolis collected from different parts of Turkey. Also, different honey bees subspecies can be caused by this difference because it is well known that different honey bee species collect propolis from different propolis sources (Silici & Kutluca, 2005).

Ferric reducing ability of plasma analysis (FRAP)

Trolox equivalent (TE) value of FRAP analysis show the concentration of antioxidant compounds. Average FRAP value was 156.59 mg TE in Ozdal, Sari-Kaplan, Mutlu-Altundag, Boyacioglu, and Capanoglu (2018) study which samples collected from different regions of Turkey. Barlak, Değer, Ucar, and Çakıroğlu (2015) calculated average FRAP value as 59.5 mg TE in a similar work. Regional differences and use of different honey bee species may be caused these FRAP value differences. Ahmed et al. (2017) research indicate that FRAP value of propolis is calculated 62.5 TE/g that is compatible with our findings.

Conclusion

In this study, it is hypothesized that honey bees can behave selectively at propolis collection for different purposes in hives. Antioxidant activity of propolis collected from different zones of hives could be different due to this selective behaviour. However, there are no differences at antioxidant values of propolis collected from different zones. Similar future works from different areas which are rich for plant sources and contain additional analysis such as volatile compounds composition and phenolic composition have potential for exploring differences at propolis samples collected for particular purposes.

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