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Determination of Chemical Contents of Propolis Extracts Obtained from Different Regions of Denizli Province, Turkey

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Propolis is a substance of honeybee origin with known chemical content effects. Its chemical composition varies by geographic location, climatic zone, and local flora. In the present study, the biological and chemical contents of the propolis samples from two different regions of Denizli were determined. This study aims to analyze the Servergazi and Sarayköy propolis contents and their use for therapeutic and pharmacologic purposes. Propolis is a resinous substance collected by honey bees (*Apis mellifera* L.) from plants. The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagents as gallic acid equivalents. The results for the propolis contents collected from the region were calculated as follows: the DPPH value of Sarayköy propolis was measured as 11.43 mg/ml, while the DPPH value of Servergazi propolis was measured as 232.778 mg/ml. As a result, the highest phenolic content was found as 223.102 mg GEA/100g in the Servergazi propolis content. According to the analysis, it was determined that the two propolis samples had rich chemical contents but the Servergazi propolis had a very rich content in all respects. In conclusion, two propolis samples of the region were very rich in phenolic substances and antioxidants; however, it was proved that the Servergazi propolis can be used for medical and pharmaceutic purposes**.**

Keywords: Antioxidant activity, total phenolic content, propolis, chemical content, *Apis mellifera* L.

INTRODUCTION

Propolis obtained from honey bees (*Apis mellifera*) has been widely used in cosmetics, medicine industry due to its versatile biological and chemical activities (Teles et al., 2015). Propolis is a hive product obtained from plants and it is normal that it contains antioxidant substances. Its chemical content is complex and consists of water, essential oils, phenolic matters, resin, and wax. The exact composition of propolis depends on the source plants (Ghisalberti et al., 1998). Moreover, propolis contains high amounts of vitamins and minerals (Matei et al., 2004). The chemical and biological composition of propolis is affected by climate conditions, the type of honey bee, and the plant flora. Propolis has different pharmacological and biological actions including antibacterial, antifungal, antitumoral (anticarcinogenic), anti-inflammatory, antioxidant, and immune modulatory effects (Carvalho et al., 2014). In a study on the subject in the literature, the antioxidant, antiparasitic and antimicrobial properties of Brazil propolis were investigated under in vitro conditions. The red Brazil propolis extract has the highest activity and has been shown to inhibit tumor cell growth (Silva et al., 2017). In a study by Silva et al., it was understood that the antimicrobial effect of propolis had changed seasonally. There are a great number of studies conducted on this subject. Many different methods have been developed for determining the chemical and biological components of propolis.

Mass spectrometry, gas chromatography, and HPLC have applied in the analysis of propolis extracts. Recently, LC-MS has also been employed for the identification of phenolic compounds in the samples (Yang et al., 2013; Pellati et al., 2011). High-performance liquid chromatography with DAD-HPLC is the most common method used to analyze flavonoids. The basis of the present study is to use this natural raw material therapeutically by determining the total phenolic substance content and antioxidant capacity of the propolis samples collected from two different regions in Denizli.

MATERIALS AND METHODS

The samples of propolis used in this study were collected from the Servergazi and Sarayköy counties of Denizli Province. The samples were kept at +4ºC in dark plastic bags until the analysis. Propolis samples were collected from Denizli Province of Turkey and were then kept and dried in dark until the process. The total phenolic substance, antioxidant substance,and biochemical analyses were performed according to various methods (Singleton 1965; Kiselev 2007). The samples were dissolved in absolute ethanol, shaken for 7 days under controlled speed, and were extracted (40 g of propolis completing the volume to 100 ml with 80 % ethanol) at room temperature. Then the propolis extracts were filtered through the Whatman No.1 filter paper. The filtrates were evaporated through the rotary evaporator (IKA RV 10, USA). The extracted water was completely removed by evaporation in a lyophilizer (Labconco Freezone 6, USA) and then the prepared propolis samples were stored in a dry condition. The extracts were filtered through a filter paper. Following the filtration, the evaporation process was carried out using a rotary evaporator. The total polyphenol content of propolis extracts was calculated by the Folin-Ciocalteu method. Then, the antioxidant properties of the samples were determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH mg/ml) free radical scavenging. The antioxidant activity was determined using the Folin-Ciocalteu reagents as gallic acid equivalents. An efficient, precise, and reliable method was developed for the quantification of propolis extractive solution using the HPLC (Shimadzu Prominence, HPLC, Tokyo, Japan) with the UV Perkin Elmer ICPOES Optima 8000 detection, Tokyo, Japan. All chemicals were from Merck, Germany.

RESULTS AND DISCUSSION

Plants produce some substances, insulate heat, and keep water in their control to survive. Honey bees receive extracts from plants, chew them with mouth digestive enzymes, add wax, and use them in the hives. This substance used is called propolis. The flavonoids present in the propolis, i.e. antioxidant activity, can clear free radicals. As for the biological activities of propolis, mainly flavonoids are components from this group (Mani 2006). The biochemical compositions of propolis samples from different regions depend on the geographical location they are taken. Their biological effects are naturally closely related to the site of flora (Park et al., 2002). The present study is based on the chemical and biological investigation of different propolis contents collected from two different regions in Denizli. While the phenolic compounds are very low in Sarayköy propolis, they are very high in Servergazi propolis sample. Kumazawa and Nakayama (2004) reported that the polyphenol contents of ethanolic extracts from European and Chinese propolis samples were 200 mg/g. In another study on red Brazil propolis, the total phenolic compound content was found to be 232 mg/g. It can be said that the contents of propolis samples taken from two different regions of Albania are different and the results of the present study are similar to those of the said research. In the total polyphenol content of the samples from two regions of Albania, the amounts of gallic acid in Ghardaia and Khanchla samples were measured as 493.49 and 1423.32 mg, respectively, and the highest antioxidant activity was found in the Khanchla sample. The phenolic contents and DPPH free radicals that have been used to test the free radical scavenging ability of various samples are given in (Table 1).

Table 1: The DPPH radical scavenging activities and total phenolic contents of ethanolic extracts of propolis

Samples (µg/g)	Servergazi Propolis	Sarayköy Propolis
Syringic	54.92	238.049
Apigenin	1224.345	921.373
Gallic	223.102	611.821
Catechol	13.879	387.498
Caffeic	31.939	436.91
Coumaric	43.186	322.241
Quercetin	214.007	213.945
Lutein	50.953	116.764
DPPH (µmol TE/g)	232.7782	11.43

*Total contents was determined by Folin-Ciocaltau method

The amount of gallic acid found in the

samples of Servergazi propolis was measured as 223.102 µg/g, while the value of gallic acid in the sample of Sarayköy propolis was measured as 166,573 µg/g. There is evidence about the chemical content activity of propolis and its relationship with total polyphenol content, especially the flavonoid concentration. Propolis may be used as functional foods because of their naturally high content potential. The TP chemical profile analysis performed by HPLC-DAD revealed the presence of 8 bioactive compounds, most of them belonging to the group of flavonoids. As a result, the highest phenolic content was found in the Servergazi propolis extract. These results indicate that the DPPH values of the propolis samples obtained in the region are high. Specifically, it is understood that the Servergazi propolis can be used as a free radical scavenger in medicine. The antioxidant phenolic compounds in propolis samples are important for nutritional purposes and their levels may vary by the geographic origin of the propolis. The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. The antioxidant activities of propolis samples are examined by comparing them with the known antioxidants (MacDonald-Wicks et al., 2006; Moon et al., 2009). DPPH scavenging is commonly used to test the free radical scavenging activity of several natural products (Ahn et al., 2007). In the present study, DPPH activity was very high in the Servergazi propolis and lower in the Sarayköy propolis and the total phenolic contents and the antioxidant quantifications of propolis samples were significant. These variations can be attributed to the geographic location and the floral source.

CONCLUSION

Natural products have been used lately as an alternative to different proposals. In this study, the antioxidant activities and phenolic contents of two different samples obtained from Denizli Province, Turkey were investigated. Propolis contains a wide-ranging spectrum of chemical compounds that have many biological actions. It is believed to be a useful product and is already used in alternative medicine and therapeutic treatment. Propolis samples have strong antioxidant activities and the highest antioxidant activities are found in the Servergazi propolis sample. The antioxidant capacity was measured at the highest rate in Sarayköy propolis sample. Due to its complex chemical structure and its pharmacological and healing properties, propolis is considered a very strong natural product produced by bees. The medical use of propolis is difficult due to the fact that its content is highly variable and its standardization is difficult. It should be kept in mind that propolis is not a medicine that heals all diseases. However, as stated in the present study, it is thought that the secondary content analysis of this natural product may contribute to studies in many fields, especially in medicine and pharmacy.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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This research was carried out in Pamukkale University, Science and Literature Faculty, Plant Physiology Laboratory.

AUTHOR CONTRIBUTIONS

Yeşim Kara: Conceived the idea, planned for the study and writing the article.

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