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Phenolic Characterization of Some Propolis Samples of Anatolia

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Abstract

Propolis is an important substance that honeybees collect from the resins of plants and form them with their own enzymes. Propolis has many biological activities thanks to more than 300 active compounds it contains. These active compounds in the content of propolis vary depending on the plants that are the source of propolis, the region where the bees are and the season. The aim of this study is to determine the phenolic compound content and levels of organic propolis obtained from Anatolian soils. For this purpose, 25 phenolic compounds were examined in the propolis samples with LC-MS / MS device. Among these compounds, Acetohydroxamic acid, 2-Hydroxy-1, 4-naphthoquinone, Thymoquinone, Alizarin could not be detected in the propolis sample. The highest phenolic compounds detected were Hydoxycinamic acid (16.85 ppm) and Quercetin (14.49 ppm). Other compounds that came out high following these compounds were Kaempferol (8.48 pmm) and Vanillic acid (4.5 ppm) compounds. The lowest phenolic compounds detected were Protocatechuic acid (0.05 ppm) and Curcumin (0.05 ppm) compounds. As a result of this study, the phenolic compound levels contained in some propolis samples of Anatolia were determined. In addition, our study results will provide information about the flora of the area where the propolis was taken.

Keywords: Propolis, phenolic compound, LC-MS/MS

Anadolunun Bazı Propolis Örneklerinin Fenolik Karakterizasyonu

Öz

Propolis, bal arılarının bitkilerin reçine ve salgılarını toplayıp kendi enzimleri eşliğinde oluşturdukları önemli bir maddedir. Propolisin içerdiği 300'den fazla aktif bileşik sayesinde birçok biyolojik aktivitesi mevcuttur. Propolisin içeriğinde bulunan bu aktif bileşikler propolisin kaynağı olan bitkilere, arıların bulunduğu bölgeye ve mevsime bağlı olarak değişmektedir. Bu çalışmanın amacı da Anadolu topraklarından alınan propolisin fenolik bileşik içeriğinin ve düzeylerinin tespit edilmesidir. Bu amaç ile propolis örneklerinde LC-MS/MS cihazı ile 25 fenolik bileşik bakıldı. Bu bileşiklerden Acetohydroxamic acid, 2-Hydroxy-1, 4-naphthoquinone, Thymoquinone, Alizarin propolis örneğinde tespit edilemedi. Tespit edilen en yüksek fenolik bileşikler Hydoxycinamic acid (16.85 ppm) ve Quercetin (14.49 ppm) bileşikleriydi. Bu bileşikleri takiben yüksek çıkan diğer bileşikler ise Kaempferol (8.48 pmm) ve Vanillic acid (4.5 ppm) bileşikleriydi. Tespit edilen en düşük fenolik bileşikler ise Protocatechuic acid (0.05 ppm) ve Curcumin (0.05 ppm) bileşikleriydi. Yapmış olduğumuz bu çalışmanın sonucunda Anadolunun bazı propolis örneklerinin içerdiği fenolik bileşik düzeyleri belirlenmiş oldu. Çalışma sonuçlarımız Türk propolisinin karakterizasyonu ve standardizasyonu için yardımcı olacaktır. Ayrıca propolisin alındığı bölgenin florası hakkında bilgi sağlamış olacaktır.

Anahtar Kelimeler: Propolis, fenolik bileşik, LC-MS / MS

Introduction

Propolis is a resinous material that bees gather from varied plant exudates and use to load cavities and to signet pieces of the hive. Within the types of chemical materials found in propolis are waxes, resins, balsams, aromatic and ethereal oils, pollen and other organic substances. Propolis or bee glue consists of a mixture of oils, pollen, special resin and waxy substances collected from the cones and barks of trees, buds and sprouts of plants; It is an adhesive substance with a very strong antiviral (Bufalo et al., 2009), antibacterial (Mercan

et al., 2006), antifungal (Marcucci, 1995), antioxidant (Kumazawa et al., 2004), anticarcinogenic effect (Kumova, 2002).

Bees use propolis, to close the hive holes and cracks, to repair the honeycombs, to stick the honeycombs to each other and to narrow the hive entrance. Bees use their hind legs and upper jaws to make propolis to extract the gummy resinous leak found in the buds and shoots of the plants. It softens it by moisturizing it in their mouth. The first studies on propolis we could reach were published by Ghisalberti in 1978 (Ghisalberti et al., 1978). The most important plant resources in propolis production are pine, birch, poplar and its species, oak, horse chestnut, willow, ash tree, fir, plum, black tree (Kumova, 2002; Soylu and Bayram, 2020). Knowing the plant sources where propolis is collected is important in terms of establishing chemical standardization as well as scientific aspects. It contains about 150 chemical compounds, more than 20 mineral substances, beeswax, resin and pollen with the phenolics presence the most plenty compounds (Bankova et al., 2000; Çakır et al., 2018). This study focused on phenolic identification and quantification of Anatolian propolis using LC-MS/MS.

Material and Methods

The propolis samples were collected from the propolis producer in Anatolia (Eastern Anatolia, Black Sea, Central Anatolia and Marmara), mixed, and analyzed into a single sample.

Extraction

Propolis extraction for LC-MS/MS analysis was carried out using the method of Iurlina et al. With slight modifications (2009). 25 g of propolis was mixed well with 5 parts of acidic water (pH 2, with HCl) until it was completely liquid and filtered through cotton to remove solid particles. The filtrate was then passed through a (50 x 5 cm) glass column filled with Amberlit XAD-4 resins with an average pore size of 4 nm. Thus, phenolic compounds remained in the column, sugars and other polar compounds were washed with aqueous solvent. The column was washed with acidic water (100 mL) and then distilled water (300 mL). The whole phenol fraction was then washed with methanol (400 mL) and concentrated under reduced pressure (40 °C). The residue was re-dissolved in 5 mL of water and extracted with diethyl ether (5 mL x 3). The ether extracts were combined and concentrated under reduced pressure at 30 °C in a rotary evaporator. The dried residue was taken in 0.5 mL of methanol and passed through a 0.45 μm membrane filter ready for LC-MS / MS analysis.

Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis of phenolic compounds

Analysis of the samples was carried out with a UHPLC device connected to a double MS device. 25 different phenolic compounds were analyzed in the samples. These components; acetohydroxamic acid, vanilic acid, resveratrol, fumaric acid, gallic acid, caffeic acid, fluoridzinhydrate, oleuropein, 4-hydrocinamic acid, ellagic acid, myricetin, protocathetic acid, silymarin, 2-hydroxy 1.4 naphthoquinone, butein, narinogen, lutroleolin, kurmine, timoquinone, alizarin, hydroxyben, quercetin, catechinhydrate and salicylic acid.

Results and Discussion

Phenolic contents of propolis sample are given in Table 1. and Figure 1.

Table 1. Phenolic compounds analyzed in propolis sample and their quantities

Phenolic Compound	Quantity (ppm)	Phenolic Compound	Quantity (ppm)
Catechin hydrate	0.73	Myricetin (ppm)	0.88
Acetohydroxamic acid	N.D.	Protocatechuic acid	0.05
Vanillicacid	4.50	Silymarin	0.33
Resveratrol	0.75	2-Hydroxy-1, 4-naphthoquinone	N.D.
Fumaricacid	1.19	Butein	0.2
Gallicacid	0.34	Naringenin	1.61
Caffeic acid	1.12	Luteolin	2.28
Phloridzin dehydrate	0.32	Kaempferol	8.48
Oleuropein	0.36	Curcumin	0.05
Hydoxycinamic acid	16.85	Thymoquinone	N.D.
Ellagicacid	2.95	Alizarin	N.D.
Hydroxybenzoic acid	0.60	Quercetin	14.49
Salisylic acid	0.58		

N.D: Not Detected

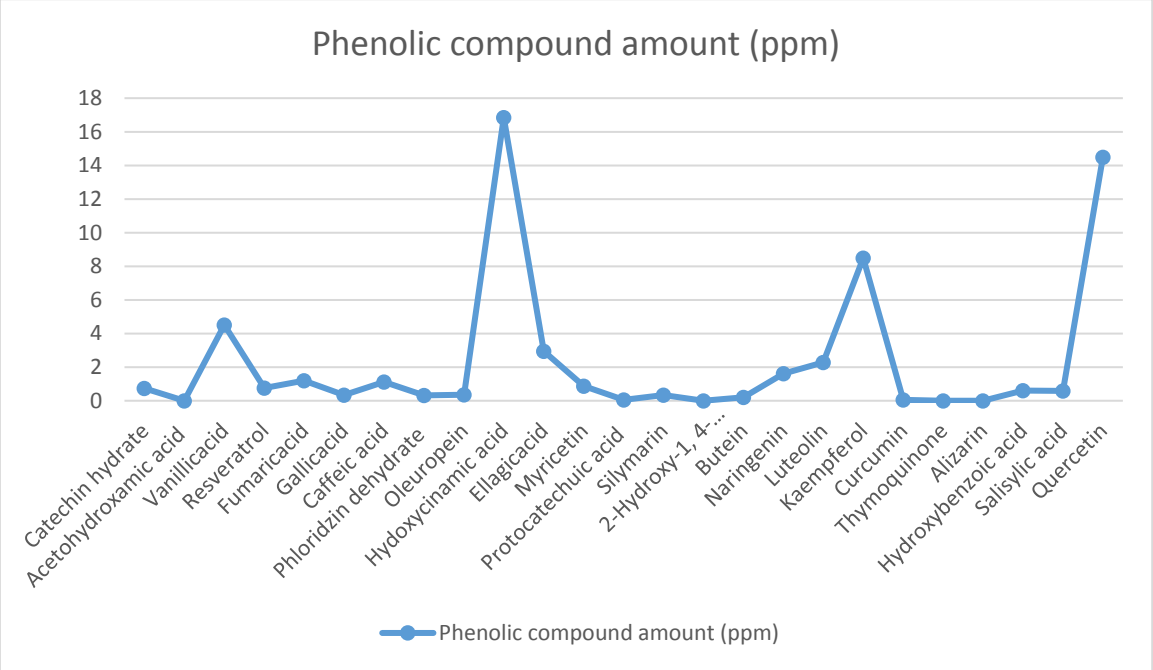

Figure 1. Phenolic composition of Anatolian propolis

In this study, 25 phenolic compounds in propolis were examined. As a result of the analysis made on the propolis sample, 4 phenolic compounds were not detected. Phenolic compounds that have not been detected are Acetohydroxamic acid, 2-Hydroxy-1, 4-naphthoquinone, Thymoquinone, Alizarin. The highest phenolic compounds detected in propolis sample are Hydoxycinamic acid and Quercetin. The determined amounts of these phenolic compounds are 16.85, 14.49 ppm, respectively. The lowest phenolic compounds detected are Protocatechuic acid and Curcumin. The amount determined in these two compounds is 0.05 ppm.

Propolis has many biological effects depending on the amount and variety of active compounds it contains. Phenolic compounds in propolis are also the most important group

that gives propolis biological activity. Since the chemical composition of propolis varies depending on the region, plant, season, colony and propolis collection techniques, the phenolic compound compositions of each propolis differ (Oruç et al. 2017; Karlıdağ and Genç, 2019).

Many studies have been conducted to determine the phenolic compound composition of propolis. Bayram et al. (2018) examined the phenolic compound content of 64 propolis samples collected from four different districts of Hakkari. This propolis has been reported to exhibit a rich chemical content in flavonoids, including coumarins and furocoumarins. In a study conducted to investigate the phenolic / flavonoid content of Marmara region propolis, Sorucu and Oruç (2019) reported that propolises in this region are rich in phenolic acids such as ferulic acid, caffeic acid, gallic acid and falvonoids such as pinosembrin, galangin, rutin, and apigenin. Aliyazıcıoğlu et al. (2013) investigated the chemical components of propolis samples obtained from 10 different cities in Turkey. In their study, it has been reported that compound levels such as ferulic acid, quercetin, caffeic acid, benzoic acid and coumaric acid are high, but the levels of vanilic acid, chlorogenic acid, epicatechin, rutin, syringic acid and p-coumaric acid are low.

Coşkun et al. (2018) have 86 different propolis samples collected from 25 cities in Turkey in the work they have done to determine the composition and characterization of bioactive Turkish propolis. They also compared propolis in Turkey with other countries. As a result of the analysis, Genistein, CAPE, Caffeic Acid, Kaempferol, pCumaric Acid, Ferrulic Acid, Quercetin, M-Coumaric Acid, 3.4-Dimethoxy Cinnamic Acid, trans-Cinnamic Acid, Pinobanxin, Naringenin, Galangin Luteolin, Hesperetin, Apigenin. They concluded that Pinocembrin, Chrysin, is mainly found in Turkish propolis. Turkish propolis when compared with other countries reported that they differ from other types of propolis flavonoid contents of propolis produced in Turkey. Mercan et al. (2006) extracts of propolis collected from different regions of Turkey; They reported that it contained krisin, apigenin, flavonoid, flavanone, naringenin, 3-4-dimethoxycinnamic acid and 9-octadecenoic acid.

Gençay and Salih (2009) reported that Turkish propolis contains a higher rate of flavanone than Japanese and Brazilian propolis samples. Erdogan et al. (2010) in all propolis samples collected from different regions of Turkey; basic polyphenols; gallocatechin, catechin, epicatechingalate, caffeic acid, chlorogenic acid and myrrhetin. Üzel et al. (2005) in Turkey in their study on propolis collected from different regions; reported that flavonoids such as pinosembrin, pinostrobin, isalpin, pinobanksin, quercetin, naringenin, galangin and krisin were at high levels in propolis. In our study, it was observed that propolis is rich in Hydoxycinamic acid and Quercetin, Kaempferol and Vanillic acid.

Numerous studies have been conducted to date in determining the total phenolic content of propolis. Keskin and Kolaylı (2019) reported that the total phenolic substance amount of Anatolian propolis ranged between 16.13-178.34 mg GAE / g. Ozdal et al. (2019) reported that the total phenolic substance amount of propolis obtained from different regions of Anatolia varies between 2748 mg GAE / 100 g and 19969 mg GAE / 100 g. Keskin et al. (2020) reported that the total phenolic substance amount of propolis samples collected from different provinces of Anatolia ranged from 28 mg GAE / mL to 80 mg GAE / mL. Döner (2020) reported that the phenolic compound amounts of propolis collected in Bingöl provinces and districts varied between 27.28 mg GAE / g -199.69 mg GAE / g. Sarıkaya et al. (2009) reported that they found the total phenolic substance content in Turkish propolis as 313-476 mg GAE / g. Ahn et al. (2007) reported that the phenolic compound amount of propolis obtained from India was between 159-269 mg GAE / g. Kumazawa et al. (2004) reported that the total phenolic substance amounts of propolis samples collected from 16 different regions of the world were at the lowest 31 mg GAE / g in Thailand and the highest

299 mg GAE / g in China. Mohammadzadeh et al. (2007) reported that the total phenolic substance amount of propolis collected from three different regions of Iran ranged from 30.8 mg GAE / g to 84.60 mg GAE / g. Da Silva et al. (2018) reported that the total phenolic content of propolis collected in the southwest of Brazil ranged from 5.294-50.41 mg GAE / g. Andrade et al. (2017) reported that the phenolic compound amounts of brown, green and red propolis collected from the northeastern region of Brazil were 55.74, 90.55, 91.32 mg GAE / g, respectively. As can be seen in all these studies and in our study, it has been observed that the pheneolic compound content and amount of propolis is different for each region.

Hydoxycinamic acid, one of the phenolic compounds found in plants, stands out with its antioxidant properties. Hydoxycinamic acid is especially abundant in grains (Gallardo et al., 2006). In this study we have done, the highest amount of phenolic compound is Hydoxycinamic acid. Plants containing Hydoxycinamic acid are thought to be high in the flora of the region where this propolis is obtained. In addition, the fact that Hydoxycinamic acid is high in our propolis sample, it is thought that this propolis will show high antioxidant properties.

Quercetin is one of the most important phenolic substances. Quercetin is substances that are commonly found in the bark of many plants and gives color to plants. Quercetin is abundant in hazelnuts, tea, blueberries, apples, tomatoes, cherries, beans, raspberries, onions, and red wine (Coşkun et al., 2005; Lee et al., 2011). Quercetin is important for human health as it has many important functions such as antioxidant, anticarcinogenic, antiviral, antithrombotic, anti-ischemic, anti-inflammatory and antiallergenic properties. (Elik et al., 2007; Kelly, 2011). In this study we have done, among the phenolic compounds of propolis, the level of Quercetin was the highest after Hydoxycinamic acid. Therefore, fruit crops are considered to be dominant after cereal crops in the flora of the region. In addition, the fact that Quercetin is high in our propolis sample is thought to have many beneficial effects on health.

Kaempferol is found in the seeds, leaves, fruits and flowers of plants (tea, broccoli, cabbage, cabbage, beans, chicory, leek, tomato, strawberry and grape etc.). It is also found in herbs or botanical products commonly used in traditional medicine (Rajendran et al., 2014; Sharifi-Rad et al, 2018). Kaempferol; It has been reported that it is cardioprotective, neuroprotective, anti-inflammatory, antidiabetic, antioxidant, antimicrobial, antitumor and has anticancer activities (Calderon-Montano et al., 2011). In this study, we have done, Kaempferol level among the phenolic compounds of propolis has been determined at the highest level after Hydoxycinamic acid and Quercetin. Therefore, it is thought that the propolis we analyzed will show many beneficial effects on health thanks to the Kaempferol phenolic compound.

Vanillic acid is a natural component of phenolic compounds. Vanillic acid is found in high amounts in Angelica sinensis, Euterpe oleracea fruit, wine and vinegar roots and has been used in medicine in China for years. Vanillic acid has a variety of pharmacological effects, including antidiabetic, anti-inflammatory, anti-metastatic, antioxidant, antioxidant, cardioprotective, and anti-apoptotic effects. It also has antifilarial agent and respiratory stimulant effects (Baniahmad et al., 2020; Mirza and Panchal, 2020). In our study, Vanillic acid level among the phenolic compounds of propolis was found to be the highest level after Hydoxycinamic acid, Quercetin and Kaempferol. It is thought that the high Vanillic acid in the propolis sample we analyzed, and our propolis sample will have many beneficial effects on health thanks to this compound.

Conclusion

As a result, the phenolic compound levels and total phenolic compound amounts of propolis in our country and other countries vary. The phenolic composition of each propolis varies depending on the region, plant, season, colony, propolis collection techniques. The phenolic compound levels in this propolis that we analyzed were found to be different from other studies. The extracts of propolis, which are also used as supplements, need to be standardized in the preparation and placing on the market. The results of this study will be helpful for the characterization and standardization of the region's flora.

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