Major Constituents of Different Propolis Samples

Farklı Propolis Örneklerinin Ana Bileşenleri

Research Article

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ABSTRACT

Propolis is a resinous material collected from plant by honey bees. The aim of this study is to investigate the chemical composition of Hakkari (Turkey) propolis. For this, eight propolis samples collected from different geographical locations were extracted in ethanol and then chemical content of samples were detected by Gas Chromatography and Mass Spectrometry (GC-MS). As a result, hydrocarbons, aliphatic acids and their esters, carboxylic acids and their esters, cinnamic acids and their esters, flavonoids, alcohols and terpens were determined in propolis samples. It was found that total flavonoid content of propolis 6 (P6) was significantly higher than other samples. Also, 'Ethyl oleate' compound was identified in all samples with different amounts.

Key Words
Propolis, chemical composition, GC-MS, ethyl oleate.

ÖZ

Propolis bal arıları tarafından bitkilerden toplanan reçinemsi bir maddedir. Bu çalışmanın amacı Hakkari (Türkiye)'den toplanan propolisin kimyasal içeriğini incelemektir. Bu amaçla farklı coğrafik özelliklere sahip yerlerden toplanan sekiz propolis örneğinin ilk olarak etanol özütleri hazırlandı ve daha sonra kimyasal bileşenlerinin yoğunluğu gaz kromatografisi ve kütle spektrometresi (GC-MS) aracılığıyla belirlendi. Sonuç olarak, hidrokarbonlar, alifatik asit ve esterleri, karboksilik asit ve esterleri, sinnamik asit ve esterleri, flavonoidler, alkoller ve terpenler propolis örneklerinde tespit edildi. Propolis 6'nın total flavonoid içeriği diğer örneklere kıyasla daha yüksek oranda bulundu. Ayrıca 'Etil oleat' bileşiği bütün propolis örneklerinde farklı miktarlarda saptandı.

Anahtar Kelimeler
Propolis, kimyasal bileşim, GC-MS, etil oleat.

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INTRODUCTION

Propolis is a natural honeybee product known to be beneficial for human health, with a complex chemical composition, highly dependent on the collection site [1]. Because of its popularity in folk medicine, propolis has become the subject of intense pharmacological and chemical studies for the last 30 years [2]. The chemical composition of propolis includes polyphenols, flavonoids, phenolic acid and their esters, caffeic acid and their esters, aldehydes, ketones, and terpenes several other compounds in trace amounts. Its physical characteristic as color, texture, and scent varies widely and is determined by many factors, including mainly its complex chemical composition. Raw propolis generally consists of 50% resin (flavonoid and related phenolic acids, known as the polyphenolic fraction), 30% wax, 10% essential oil, 5% pollen, and 5% other organic compounds. To date, more than 300 different constituents have been identified in propolis [3]. The propolis production occurs through the gathering of resinous and balsamic substances present in branches, leaves, flours and pollen, which are aggregated to bee salivary secretions and enzymes, originating the propolis [4].

The composition of propolis primarily depends upon the vegetation of the area from where it was collected. Sometimes bees may also collect hazardous substances, for example asphalt from road construction sites, and thus it is important to evaluate propolis chemically as well as biologically before it is used in food or beverages or in traditional medicine [5]. In this study, the chemical contents of the propolis samples collected from different localities were determined and the compounds which could be markers for the propolis of this region were identified.

MATERIALS and METHODS

The propolis samples were collected from eight different regions from Hakkari, Turkey. Raw propolis samples were stored at 4°C in the dark before analysis. Frozen propolis is grained and dissolved in ethanol (96%) and then sealed in a bottle at 30°C for two weeks. After two weeks, the supernatant was filtered twice with Whatman No. 4 and No.1 filter paper, respectively. The final solution, (1:10, w/v) was evaporated until complete dryness. About 5 mg of dry substance were mixed with 75 μl of dry pyridine and 50 μl bis (trimethylsilyl) trifluoroacetamide (BSTFA) heated at 80°C for 20 min and the final supernatant was analyzed by GC-MS [6].

GC-MS analysis of propolis samples were performed using a GC 6890N from Agilent (Palo Alto, CA, USA) coupled with mass detector (MS5973, Agilent) equipped with a DB-5 MS capillary column (30m x 0.25mm and 0.25 μm of film thickness). The column oven temperature was initially held at 50°C for 1 min, then programmed to rise to 150°C at a rate of 10°C/min and held for 2 min. Finally, temperature was increased to 280 with 20°C/min. heating ramp and kept at 280°C for 30 min. Helium was used as the carrier gas at a flow rate of 0.7 mL/min.The identification of various organic compounds present in propolis samples were carried out by computer search on Willey and Nist Libraries data, if the comparison scores were obtained higher than 95%. Otherwise, fragmentation peaks of the compounds in mass spectra were evaluated and the compounds were identified using our memorial background for the identification of the compounds appeared in GC-MS ion chromatograms. For the quantification of the compounds in the ethanol extract, no internal and external standards were used; only percentage reports of the compounds in the sample were used. This was the standard way to quantify most organic compounds in the propolis samples, thus reducing the relative error in < 5% [7].

RESULTS and DISCUSSION

The eight propolis samples collected from different regions in Hakkari were extracted, and analyzed. The chemical composition of propolis samples are identified and listed in Table 1. In different studies carried out to determine the chemical content of propolis samples obtained from different origins; compounds such as isoflavonoidler [8], fatty acids, organic acids, sugars, alcohols, phenolic acids, triterpenes, steroids [9] were determined in the chemical structure of propolis. In this study, the main chemical classes present in propolis are hydrocarbons, aliphatic acids and their esters, carboxylic acids and their esters, cinnamic acids and their ester, flavonoids, alcohols, terpens.

Table 1. Chemical content of propolis samples (% of total ion current).

	P1	P2	P3	P4	P5	P6	P7	P8
Hydrocarbons	3.88	0.31	0.69	-	-	0.98	2.19	0.74
Aliphatic acids and their esters	7.88	0.73	12.81	30.91	5.65	1.17	2.50	0.28
Carboxylic acids and their esters	-	-	-	2.62	-	-	-	1.18
Cinnamic acids and their esters	1.29	-	-	-	-	-	-	-
Flavonoids	5.52	-	0.81	-	7.28	34.81	-	8.08
Alcohols	0.75	-	0.11	-	-	1.02	-	9.81
Terpens	2.75	3.53	-	-	-	0.40	-	-

*P1: Propolis 1, P2: Propolis 2, P3: Propolis 3, P4: Propolis 4, P5: Propolis 5, P6: Propolis 6, P7, Propolis 7, P8: Propolis 8.

Compounds in the hydrocarbon group were found to be between 0.31% and 3.88% in other samples except P4 and P5. 17-Pentatriacontene, is a hydrocarbon, was defined as dominant compound in propolis samples.

This compound was determined in Hatay and Mersin propolis samples by Şahinler et al. (2009) [10] respectively 11.66% and 23.42%. In addition, Bayram et al. (2015) [11] reported that this compound was in the chemical content of propolis.

The level of ethyl oleate (30.91%) belong to aliphatic acids and their esters had the highest concentration. This compound was found in all sample for different amount (0.28-30.91%). 'Ethyl oleate' was found as the predominant compound by Mohammadzadeh et al. (2007) [12] in the Iranian propolis; and Mercan et al. (2006) [13] in propolis samples collected from different regions of Turkey. Similarly, this compound was determined as 8.1% the chemical content of propolis by Kayaoğlu et al. (2011) [14]. The compounds belonging to the carboxylic acids and esters were identified in P4 and P8, but not in the other six samples. In the propolis samples, only one compound (Benzoic acid, 4-hydroxy-) belonging to the this group was identified. Similarly, Girgin et al. (2009) [15]; Çelemli et al. (2012) [16], found this compound in a sample of Black Sea Region too. Cinnamic acid and its esters were detected only in P1 at 1.29%.

Propolis resin is mainly composed of flavonoids, phenolic acids and their esters, which often form up to 50% of the total ingredients [17]. However, studies suggest that the chemical concentration of these substances may be related to the characteristics of the local flora and seasonality, which ultimately affect the biological properties of propolis [18]. In this study, flavonoids were detected at different ratios in P1, P3, P5, P6 and P8 (5.52% 0.81%, 7.28%, 34.81%, 8.08%, respectively). Contrary to these samples, the flavonoid group compounds couldn't be observed in P2, P4, P7 samples. 'Pinostrobin chalcone' compound was detected in the highest ratio in the P6. Similar to our study, this compound was found in the samples of propolis collected from Tekirdağ [16].

The compounds belonging to the alcohol group were determined at different ratios in P1, P3, P6 and P8, and terpenes were determined to be in the rates of 2.75%, 3.53%, and 0.40% respectively in P1, P2 and P6. The following alcohol compounds were identified in the propolis samples; 2-Buten-1-ol, 3-methyl-;17-(1,5-Dimethyl-hex-4-enyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol; alpha.-Eudesmol; 2-Buten-1-ol, 3-methyl-,acetate; 2-methyl-3-isobutenyl-4-penten-2-ol.

The chemical composition of a given propolis sample varies considerably depending on its geographic origin, the plant of origin, climate factors and collection season, as well as the species of bee that collected it [17] and thus very difficult to analyze and standardize [19]. Also in this study, especially the therapeutic activity of P6, which has been identified as having high flavonoid content, might be considered to be higher. Furthermore, it can be said that the ethyl oleate compound detected in all the propolis samples is a marker compound for the propolis of this region.

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