Determination of Chemical Composition of Turkish Propolis

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Turkish Propolis, Chemical Composition

The aim of the present work is to study the chemical composition of Turkish propolis. Propolis samples were collected from different regions of Turkey (Bursa, Erzurum-Askale, Gumushane-Sogutagil and Trabzon-Caglayan) in 1999. Ethanol extracts of propolis (EEP) were prepared for chemical analysis, using gas chromatograph coupled with mass spectrometry (GC-MS). Our findings show that propolis samples from Trabzon and Gumushane region have a similar chemical composition. In both samples aromatic acids, aliphatic acids and their esters, and also ketone derivatives are the main compound groups. The chemical composition of the single sample that was collected from Erzurum region shows a very different pattern than the other two samples. In this propolis, the main compounds are aromatic acid esters and alcohols. However, it contains a high amount of amino acids compared to the other samples. The other samples collected from three different region of Bursa City are rich with flavavones, aromatic acids and their esters, terpenoids, flavones and ketones.

Introduction

Propolis is a kind of resinous bee product, collected by Worker honeybees from the buds and leaves of the plants, trunk wounds and trees (eg, *Castanea sativa, Populus* spp., *and Aesculus hippocastanum*). The bees attach the propolis on their hind legs, and carry it back to their colony, where it is combined with beeswax and used by worker "hive" bees to seal and sterilize the colony nest.

Propolis is used in various folkloric and medical applications for its antiseptic (Grange and Davey, 1990), antimycotic (Arkan *et al.,* 1997), anti-inflammatory (Khayyal *et al.,* 1993) effect and other beneficial properties in many part of the world (Gallo and Savi, 1995). Its chemical composition is very complex and contains many different organic compounds (e.g., aliphatic and aromatic acids and their esters, alcohols, ketones, aldehydes, chalcones, flavanoids, amino acids, sugar, inorganic metal ions, and the other compounds) (Papay *et al.,* 1987). Chemical composition of propolis is changed by different parameters such as, climate, location and years. In order to determine the plant sources and the composition of propolis sample many studies were done (Barberan *et al.,* 1993). Since the different chemical compounds in propolis may affect different biological reactions in living organisms, determination of chemical composition and amount of the each component is important for the use.

In Turkey, Sorkun and Bozcuk (1994) published the first report on propolis. Subsequently, a lot of studies related to propolis in Turkey were published (Ozturk *et al.,* 2000; Orhan *et al.,* 1999; Velikova *et al.,* 2000; Velikova *et al.,* 2001). This communication is one of the first detailed studies on chemical properties of Turkish propolis system.

Material and Methods

Sampling

The propolis samples which were provided by Civan Beekeeping farm at Bursa and Beekeeper's Association of Trabzon province were collected in October 1999 from Bursa (three samples), Erzurum-Askale, Gumushane-Sogutagil and Trabzon-Caglayan. The sampling location of propolis is shown in Fig. 1.

All group samples collected from different regions in Turkey belonged to *Apis mellifera* colonies. The hives were located at Bursa province in Marmara region, at Erzurum and Gumushane provinces in East-Anatolian region and at Trabzon province in Central North Anatolian region in Turkey.

The samples were obtained by scraping the walls, frames and other hive parts. Also samples were collected using a propolis trap.

Preparation of ethanol extracts of propolis (EEP)

The hardened propolis was ground and 100 g of sample were dissolved in 300 ml ethanol (% 96). This mixture was preserved for two weeks in a bottle closed tightly and kept in the incubator at

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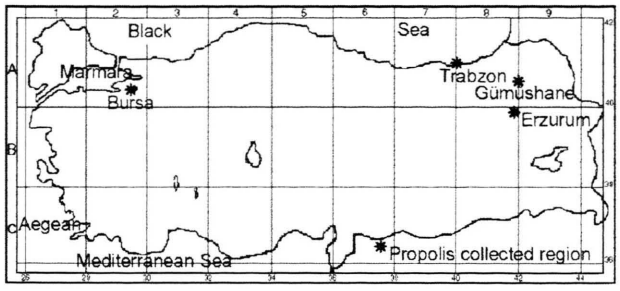

Fig. 1. Various locations where propolis samples were collected from Turkey.

30 °C. After incubation procedure, supernatant was filtered twice with Watman No. 4 and No. 1 filter papers. The final filtered concentrated solution (1:10, w/v) called **Ethanol Extracts of Propolis (EEP)** was evaporated to dryness. About 5 mg of residue were mixed with 75 µl of dry pyridine and 50 µl bis(trimethylsilyl)trifluoroacetamide (BSTFA), heated at 80 °C for 20 min and then the final supernatant was analyzed by GC-MS.

GC-MS system

A GC 5890, from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS 5972, Hewlett-Packard) was used for the analysis of ethanol extracts of propolis samples. Experimental conditions of GC-MS system was as follows: HP-1 column (25 m × 0.2 mm and 0.02 µl of film tickness) was used and flow rate of mobile phase (He) was set at 1.0 ml/min. In the gas chromatography part, temperature was kept for 1 min at 50 °C, then increased to 200 °C 15 °C/min heating ramp. After this period, temperature was kept at 200 °C for 5 min. Finally, temperature was increased to 280 with 25 °C/min heating ramp and then kept at 280 °C for 10 min.

Results and Discussion

1 µl of EEP extract was injected to the GC-MS system to screen the sample and identify the compounds present in each propolis sample. Compounds, such as amino acids, aliphatic acids and their esters, aromatic acids and their esters, alcohols, aldehydes, flavones, flavavones, hydrocarbons, ketones and terpenoids, and other compounds, in each sample were identified by computer search using reference Wiley Library (HP commercial library) and mass spectra patterns. It was found that all six samples could be divided into three main sample groups. In the first group were three samples collected at different places in Bursa City. First group samples mainly contain high content flavanones, flavones, aromatic acid and esters, ketones and terpenoids (Table I). In the second group samples that were collected in Trabzon and Gumushane Cities contain mainly flavavones, alcohols, ketones and aromatic acid esters (Table II). In the second group samples, terpenoid content was very low. There was only one sample in the third group collected at Erzurum region. Aromatic acid esters were the main component in the sample. The flavanone content of this sample was extremely lower than the flavanones content of the other two groups. On the other hand, this sample contained a high amount of amino acid of about 4.46 percent (Table II). The *Castanea* propolis and the propolis of Erzurum region are very different from each other because of the origin of two propolis is different. One of them was collected from *Castanea sativa* and the second one was collected from grass.

The climatic condition and flora of Bursa, Trabzon, Gumushane and Erzurum are absolutely different from each other. Flora of first three prov-

Table I. Chemical composition of three propolis collected from Bursa region.

Compound	Bursa (I) (%)	Bursa II (%)	Bursa (III) (%)
Alcohols	4.40	1.71	6.34
Aliphatic acids	2.75	2.22	6.41
Amino acids	Very low	Very low	Very low
Aromatic acid esters	Very low	13.14	3.10
Aromatic acids	7.54	1.52	18.15
Aromatic aldehyde	3.91	–	1.86
Flavonoids	47.40	31.8	37.55
Ketones	11.20	24.74	6.95
Others	20.88	20.87	10.09
Terpenoids	1.92	4.50	7.70
Vitamin A	–	–	1.84

Table II. Chemical composition of propolis collected from Erzurum, Gumushane and Trabzon regions.

Compound	Erzurum (%)	Gumushane (%)	Trabzon (%)
Alcohols	21.73	11.30	15.03
Aliphatic acids	1.96	0.98	3.1
Amino acids	4.46	–	–
Aromatic acid esters	31.86	5.52	5.12
Aromatic acids	1.32	2.18	3.35
Aromatic aldehydes	2.24	1.05	0.95
Flavonoids	4.72	50.55	43.55
Ketones	8.19	11.11	21.30
Others	20.21	13.31	7.87
Terpenoids	3.31	very low	very low

inces namely Bursa, Trabzon and Gumushane matches with the flora of Euro-Sibiria. The other flora, Erzurum, shows the properties of step flora. Propolis sample of Bursa II was collected from a district where *Castanea sativa* was found very density. Therefore, propolis that was collected from Bursa II region is different than the other two propolis from Bursa I and Bursa III regions. The main observations of three different propolis groups could be concluded as follows: (a) flavonoids content of propolis that were collected Bursa region is high. One propolis sample from *Castanea sativa* region has a reasonable high vitamin A content; (b) propolis from Gumushane and Trabzon regions have the highest content of flavonoids compared to the other propolis samples; (c) propolis from Erzurum region showed a different pattern in contrast to the other samples. This propolis has a high content of amino acids of about 4.46 percent. This is due to the particular vegetation in the Erzurum region.

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