CHEMICAL ANALYSIS OF PROPOLIS SAMPLES FROM DIFFERENT PHYTO-GEOGRAPHICAL REGIONS OF TURKEY



ÖMÜR GENÇAY[1], KADRİYE SORKUN[1], BEKİR SALİH[2]

[1]Department of Biology, Hacettepe University, Faculty of Sciences, 06800, Ankara-Turkey

[2] Department of Chemistry, Hacettepe University, Faculty of Sciences, 06800, Ankara-Turkey

ABSTRACT

Propolis is a sticky dark colored material and honey bees use it in the construction and adaptation of their nests. The action against micro-organisms is an essential characteristic of propolis and it has been used by human beings since ancient times for its pharmaceutical properties. The chemical composition of propolis is very complex and the composition of propolis varies according to the local flora and flowering, climatic comditions and amount of resin on the buds. The aim of the present work is to study the organic chemical composition of Turkish propolis. Turkey has a geography characterized by different climatic conditions in three phyto-geographical regions (Mediterranean, Irano-Turanian and European-Siberian). Owing to this rich plant constituent, the content of propolis varies with respect to plant type, time and area. The propolis samples were collected from these three phyto-geographical regions. Six samples from European-Siberian, three samples from Irano-Turanian and one sample from Mediterranean phyto-geographical region. Type and the content of the organic compounds of these samples were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Compound found in all samples in high ratios were mostly 2-propen-1-one,1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl that was a kind of flavanoid. It was found in a sample from Irano-Turanian phyto-geographical region with the highest ratio (Erzincan-19.05%) compare to the other samples. The other kind of flavanoid frequently observed in the samples was Pinocembrin and it was found in a sample from European-Siberian phyto-geographical region with the highest ratio (Bursa15.49%). Chrysin was one of the most important flavonoid that had a lot of biological activities was found in a sample from Mediterranean phyto-geographical region with the highest ratio (Hatay-7.04%). It has been examined in this study whether the differences observed in the propolis samples belong to the different phyto-geographical regions of Turkey or not.

Keywords: *Turkish propolis, GC-MS, phyto-geographical region, Chemical Composition, Organic Compound*

INTRODUCTION

Propolis, is a resinous hive product collected by honeybees from various plant sources. It has a pleasant aromatic odor and yellow-green to dark brown color depending on its source and age. It possesses several biological activities such as anti-inflammatory, immunostimulatory, antiviral and antibacterial. Ghisalbetti reports that propolis was used for medical purposes in the last quarter of the18th century (Ghisalberti 1979). Since then many studies have been conducted on propolis and published in prestigious periodicals (Walker and Crane 1987; Volpert and Elstner 1993; Barberan et al. 1993; Koo and Park 1997; Bankova and Marcucci 2000; Orsolic and Basic 2000). In Fig. 1 some propolis samples are shown.

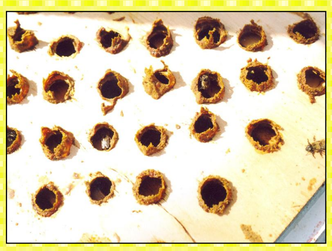




Figure 1. Some propolis samples.

MATERIAL AND METHODS

Propolis samples

Geographical origin and symbols of the samples are listed in Table 1. As seen from Table 1, three phyto-geographical regions of Turkey were studied and also different locations of each phyto-geographical region were scanned. The locations of collecting areas are given in Figure 2 and Figure 3.

Figure2. The cities where propolis samples were collected.

Figure 3. Some photograhs of the cities where propolis samples collected.

Table 1. The collecting areas of propolis samples.

COLLECTİNG AREA	SOLUBILITY IN ETHANOL
European-Siberian Phyto geographical Region	
Black Sea Region	
Artvin	%6
Kastamonu (İnebolu)	%17.6
Rize (Anzer)	%11
Marmarian Region	
Bursa (Tahtaköprü)	%11.54
Kırklareli	%11
Tekirdağ	%13.6
Irano-Turanian Phyto geographical Region	
Central Anatolia Region	
Ankara	%6.8
East Anatolia Region	
Erzincan	%9.8
Tunceli	%6.2
Mediterrranean Phyto geographical Region	
Mediterranean Region	
Hatay	%12.33

Extraction and sample preparation

100 grams of frozen propolis is grained and dissolved in 300 ml ethanol (% 96). This mixture was kept in the incubator at 30 C for two weeks in a bottle closed tightly. After incubation procedure, supernatant was filtered twice with Whatman No 4 and 1 filter papers. The final filtered concentrated solution (1:10, w/v) called Ethanol Extracts of propolis (EEP) was evaporated until it became fully dry. 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 analysis

A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS5973, Hewlett-Packard) was used for the analysis of EEP samples. Experimental conditions of GC-MS system was as follows: DB 5MS column (30 mx 0.25mm and 0.25 µm of film thickness) was used and flow rate of mobile phase (He) was set at 0.7 ml/min. In the gas chromatography part, temperature was kept for 1 min at 50 C and then increased to 150 C with 10 C/min heating ramp. After this period, temperature was kept at 150 C for 2 min. Finally, temperature was increased to 280 with 20 C/min. heating ramp and then kept at 280 C for 30 min.

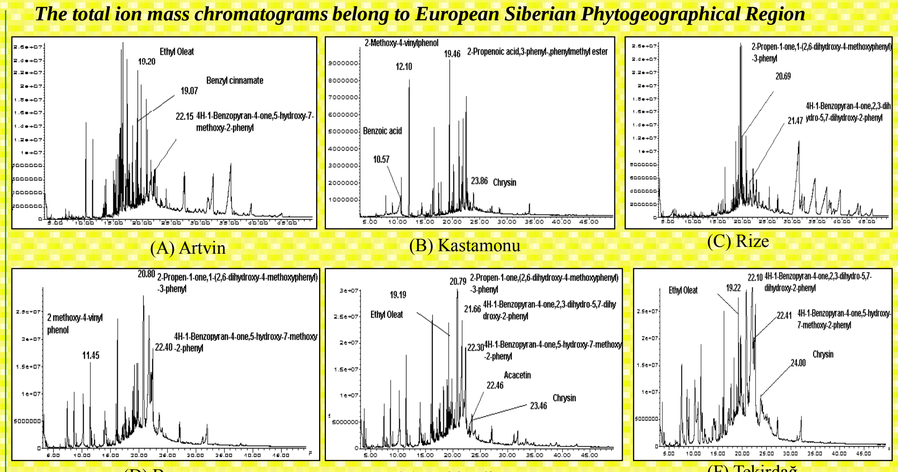
Organic compounds in propolis samples were identified using standard Willey and Nist Libraries available in the data acquisiton system of GC-MS, if the comparison scores were obtained higher than 95%. Otherwise fragmentation peaks of the compounds were evaluated and the compounds were identified using our memorial background for the identification of the compounds appeared in GC-MS chromatograms. For the quantification of the compounds in the ethanol extract, no internal and external standard was used. Only percentage reports of the compounds in the sample were used. This was the standard way to quantify the many organic compounds in the propolis samples. In this case, the relative error could not be higher than 5%.

The Gas Chromatograhy and Mass Spectrometry used in this study is shown in Figure 4.The results of the GC-MS analysis are given as chromatograms in Figure 5,6 and 7.

Figure 4. Gas Chromatography-Mass Spectrometry System.

RESULTS

The total ion mass chromatograms belong to European Siberian Phytogeographical Region

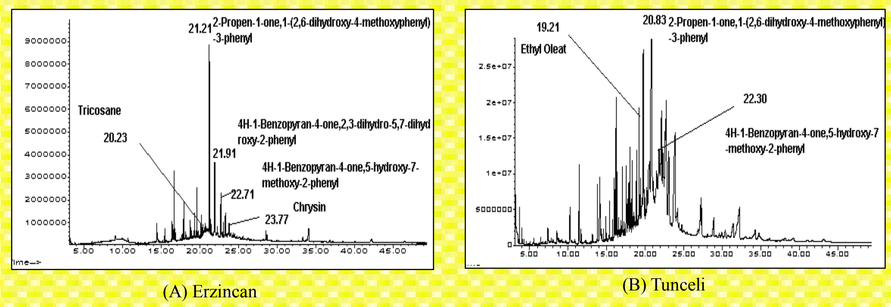


(A) Artvin (B) Kastamonu (C) Rize

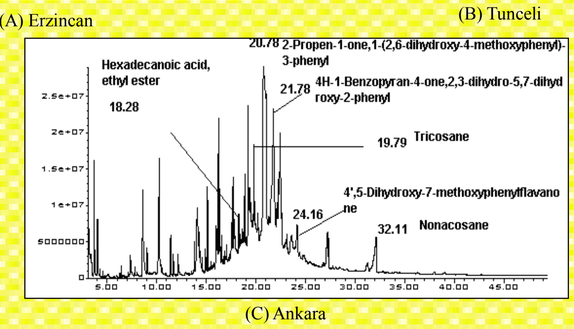
(D) Bursa (E) Kırklareli (F) Tekirdağ

Figure 5. The total ion chromatograms of propolis samples from European-Siberian Phytogeographical Region.

The total ion mass chromatograms belong to Irano-Turanion Phytogeographical Region



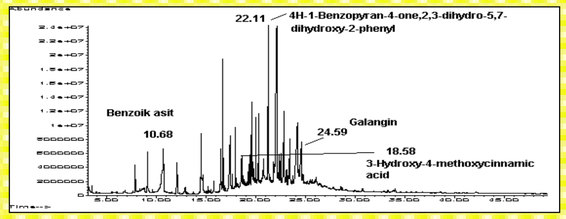
(A) Erzincan (B) Tunceli



(C) Ankara

Figure 6. The total ion chromatograms of propolis samples from Irano-Turanian Phytogeographical Region.

The total ion mass chromatograms belong to Mediterranean Phytogeographical Region



a) Hatay

Figure 7. The total ion chromatograms of propolis samples from Mediterranean Phytogeographical Region.

DISCUSSION

Table 2. Chemical compound groups and percent ratios identified in propolis samples.

Compounds	**European-Siberian Phyto-geographical Region**						**Irano-Turanian Phyto-geographical Region**			**Mediterrranean Phyto-geographical Region**
	Black Sea Region			**Marmarian Region**			**Central Anatolia Region**	**East Anatolia Region**		**Mediterrranean Region**
	Artvin	**Kastamonu**	**Rize**	**Bursa**	**Kırıkkale**	**Tekirdağ**	**Ankara**	**Erzincan**	**Tunceli**	**Hatay**
Aldehydes	1.12	0.33	0.3	0.43	0.47	0.19	-	-	0.76	0.14
Alifatic acids and their esters	3.61	0.15	0.74	4.19	2.32	2.9	7.38	3.73	3.29	0.19
Alcohols	0.64	6.64	0.2	6.55	6.24	7.67	6.56	6.02	4.38	5.45
Esters	-	-	-	-	-	-	0.44	-	0.13	-
Phenolic Compounds	-	-	-	-	-	0.27	-	-	-	-
Flavanoids	12.74	14.09	3.6	37.38	34.36	37.3	35.77	40.12	23.02	31.79
Hydrocarbons	8.48	6.53	6.21	6.32	10.23	6.72	9.24	2.21	7.19	3.14
Carboxylic acid and its esters	0.11	8.15	0.92	0.34	3.39	11.27	0.65	-	0.11	7.96
Ketones	0.08	-	0.06	0.16	0.16	0.3	1.71	2.61	1.82	0.4
Cinnamic acids and their esters	2.54	5.18	-	0.49	0.92	0.48	-	-	-	3.47

More important group compounds in propolis samples are flavanoids. As seen in Table 2, the samples from the Marmarian Region of European-Siberian Phytogeographical region and Irano-Turanian Phytogeographical region include high ratios of flavanoids.In our study from flavanoids *2-propen-1-one,1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl* was found mostly in highest ratios. It was found with a ratio of 19.05% in Erzincan sample, 16.27% in Ankara sample, 11.82% in Kırklareli sample,11.72% in Bursa sample, 9.24% in Tekirdağ sample, 8.21% in Tunceli sample, 6.40% in Kastamonu sample, 5.43% in Hatay and 3.53% in Artvin sample. We couldn't determine this compound only in Rize sample. As understood from these results we found this compound in Irano-Turanian phytogeographical region with highest ratios compare to the other two phytogeographical regions.An another kind of propolis we determined is pinocembrin. Pinocembrin is the most abundant compound in propolis(Bankova et al, 1982). It has been proven to have antimicrobial, antioxidant and anti-imflammatory activities. In this study the highest pinocembrin content was about 15.49% in Bursa sample from European-Siberian phytogeographical region.Chrysin, a naturally wide distributed flavanoid, has been reported to have many different biological activities such as anti-oxidant, anti-virus, anti-diabetogenic activity and anti-axiolytic effect.The chrysin cotents of the samples were lower compare to the other flavanoids. Chrysin contents of studied propolis were found between 2.68% and 7.04%.Aromatic acids, especially cinnamic acids in propolis are really important because of their different activity in the living organisms as widely mentioned in the literature. The content of cinnamic acid in propolis samples studied were between 0.49% and 10.10%.

As a result flavanoid content of Turkish propolis considerably high. The high amount of flavanoids gives activity to propolis so as to decrease the radicalic reactions in the living organizms as an anti-oxidant.

REFERENCES

Bankova,V.S.,Popov,S.S.,Marekov,N.L.,1982.High Performance Liquid chromatographic analysis of flavanoids from propolis.J.Chromatogr.242,135-143

Ghisalberti E. 1979. Propolis: A review. Bee World , 60, 59-84

Walker P., Crane E. 1987. Constituents of propolis. Apidologie, 18 (4), 327-334.

Volpert R., Elstner F.R.Z. 1993. Biochemical activities of propolis extracts, standardization and antioxidative properties of ethanolic and aqueous derivatives. Z Naturforsch, 48c, 851-857.

Barberan T., Garcia C., Olivier P., Ferreres F. 1993. Phytochemical evidence for the botanical origin of tropical propolis from Venezuela. Phytochemistry, 34, 191-196.

Koo M.H., Park Y.K. 1997. Investigation of flavonoid aglycones in propolis collected by two different varieties of bees in the same region. Biosci. Biotech. Biochem.,61 (2), 367-369.

Bankova V., Marcucci M.C.2000. Standardization of propolis: present status and perspectives.Bee World , 81 (14), 182-188 .

Orsolic N., Basic I. 2003. Immunomodulatory action of a water-soluble derivative of propolis, caffeic acid and caffeic acid phenethyl ester: Nitric oxide and macrophage function. Mellifera , 3 (6), 56-64 .