More Insight into the Chemical Composition of Greek Propolis; Differences and Similarities with Turkish Propolis

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We aimed to determine the differences and similarities between Greek and Turkish propolis with respect to their chemical composition given that the two countries have many similarities in floral biodiversity. We observed that: a) Greek propolis is different from the European-type propolis, having a high terpene content; therefore we can definitely characterize it as a Mediterranean type; b) the Turkish propolis collected along the coast line of the Aegean Sea is similiar to the examined Greek propolis; c) the remaining Turkish samples, originating from the European part of Turkey, were found to be similiar to the European-type propolis, having a high flavonoid content. Finally, especially two compounds, β-elemene and totarol, were found in Greek samples in quite high amounts that are thought to have important biological properties.

Key words: Propolis, Greek, Turkish

Introduction

Propolis or bee glue is a sticky dark-coloured material that honey bees collect from plants and use in the hive: they apply it to seal the walls, to strengthen the borders of combs, to line all cells inside, and to embalm dead invaders (Bankova, 2005). It is well known that propolis possesses antibacterial, antifungal, and antiviral properties, and many other beneficial biological activities: anti-inflammatory, antiulcer, local anesthetic, hepatoprotective, antitumour, immunostimulating etc. (Bankova *et al*., 2000). Bees use it, therefore, as a protective barrier against their enemies (Burdock, 1998). Propolis has been used by mankind in traditional medicine since 3000 BC in Egypt.

The plant origin of propolis determines its chemical diversity. For example, in the temperate zone of the world, the main source of bee glue is the resinous exudate of the buds of poplar trees, mainly the black poplar (*Populus nigra*) (Bankova, 2005). For this reason, European propolis contains typical poplar bud phenolics: flavonoid aglycones (flavones and flavanones), phenolic acids and their esters. Poplar trees are common only in the temperate zone; they cannot grow in tropical or subtropical regions. As a result, propolis from tropical regions, although highly diverse in its chemical composition, has a chemical profile different from that of the poplar type (Bankova, 2005).

Recent studies have revealed a new type of European propolis: it is called Mediterranean propolis and is distinguished by its high content of diterpenoids. This propolis type was found in South Greece, in Sicily, and on some Croatian Adriatic islands (Popova *et al*., 2009). Melliou and Chinou (2004) isolated also a new flavanone derivative from Greek propolis, 7-prenylpinocembrin, which, together with totarol and 7-prenylistrobopinin, are regarded as antibacterial principles.

The Greek flora represents a high floral biodiversity, with a high percentage of endemic plants (Melliou *et al*., 2007). Owing to its rich plant cover it would not be suprising to find a highly variable chemical composition in Greek propolis. Previous studies on Greek propolis were limited and concentrated on South Greece as well as on Aegean Islands and Crete. There is therefore a gap in the knowledge of samples from the northern areas of Greece, where high mountains exist. Turkey is also a country with diverse geomorphological characteristics and a rich flora, and there is a shared border line between the north-east region of Greece and the European part of Turkey. Above this, there is a long coast line of Turkey, along the Aegean Sea, with many similarities with the Greek islands in terms of climatic conditions and floral diversity. Our aim was firstly to complete the chemical profile of Greek propolis including samples from the northern regions of the country, and secondly to investigate the chemical composition of some Turkish propolis samples for comparison. For the second aim, the Turkish samples were especially collected from areas of the European part of Turkey and areas along the coast line of the Aegean Sea.

Material and Methods

Collection of samples

Samples of propolis were collected during spring and summer 2009 and 2010. Special propolis traps were used in order to collect clean material. Samples were stored at 4 °C until chemical analysis.

The Turkish samples originated from two geographical regions of Turkey (Fig. 1). Samples TR1 and TR2 were collected from the European part of the Marmara region of Turkey, and the TR3 sample was collected from the Asian part of the Marmara region. Samples TR4 and TR5 were collected from the Aegean region of Turkey. The Greek samples were collected from western and northern regions of the country as well as from areas near the border line with Turkey and two Aegean islands (Fig. 1).

Extraction and sample preparation

Each frozen propolis sample was ground and suspended in ethanol (96%) in a ratio of 1:3 (w/v). The mixture was kept in a tightly closed

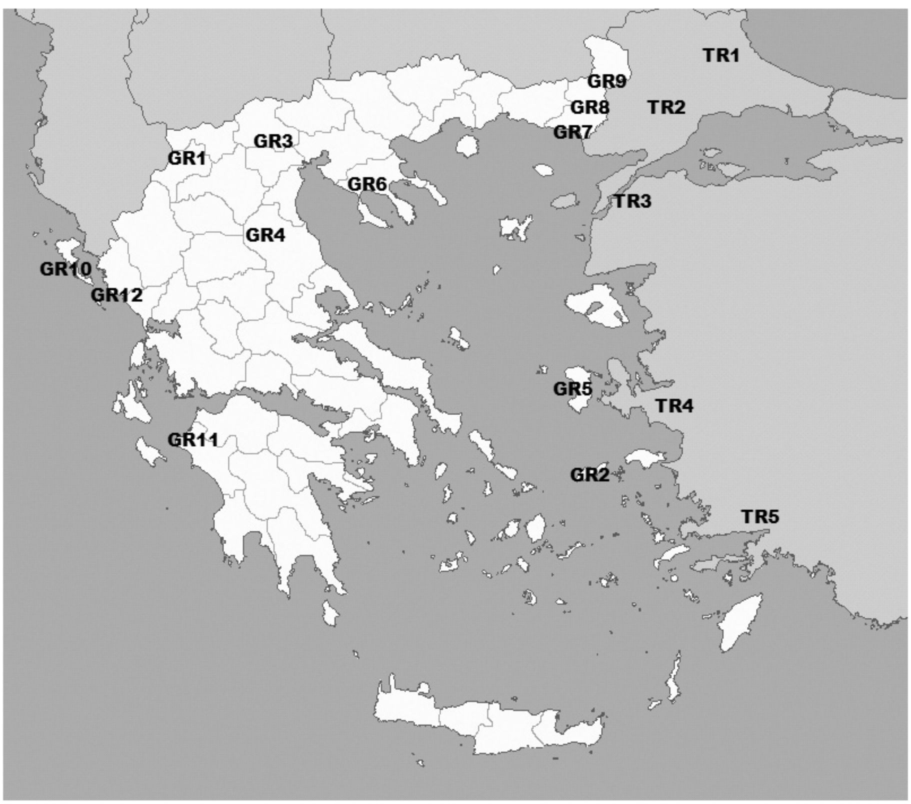

Fig. 1. Sampling areas in Greece (GR) and Turkey (TR): GR1, Kastoria; GR2, Ikaria; GR3, Veroia; GR4, Elassona; GR5, Chios; GR6, Nea Moudania; GR7, Alexandroupoli; GR8, Tichero; GR9, Didimoticho; GR10, Kerkyra; GR11, Nea Manolada; GR12, Igoumenitsa; TR1, Kırklareli; TR2, Tekirdağ; TR3, Çanakkale; TR4, Izmir; TR5, Muğla.

bottle in an incubator at 30 °C for two weeks. Then the supernatant was filtered twice through Whatman (Maidstone, England) No. 4 and No. 1 filter papers. The final filtered and concentrated solution (1:10, w/v), designated "ethanol extract of propolis" (EEP), was evaporated to 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 analysed by gas chromatography and mass spectrometry (GC-MS) (Gencay and Salih, 2009).

GC-MS analysis

A GC 6890N instrument from Agilent (Palo Alto, CA, USA) coupled with a mass detector (MS5973; Agilent) was used for the analysis of EEP samples. Experimental conditions of the GC-MS system were as follows: a DB 5MS column (30 m x 0.25 mm, 0.25 μm film thickness) was used, and the flow rate of the mobile phase (He) was set at 0.7 ml/min. In the GC part, the temperature was kept for 1 min at 50 °C and then increased to 150 °C at 10 °C/min intervals, followed by 2 min at 150 °C. Finally, the temperature was increased to 280 °C at 20 °C/min intervals and kept at 280 °C for 30 min.

Organic compounds in the propolis samples were considered identified in Wiley's NIST Mass Spectral Library, when the obtained comparison scores were higher than 95%. Otherwise, fragmentation peaks of the compounds were evaluated, and the compounds were identified using the memory background for the identification of the compounds that appeared in the GC-MS chromatograms. Contents of individual compounds in the ethanol extract are given in percent of the total compounds in the sample. This is the standard procedure to quantify most organic compounds in the propolis samples. Variations were not higher than 5%.

Principal component analysis (PCA)

PCA can compress data based on their similarities and differences. PCA was applied in order to determine which were the most characteristic volatiles for the propolis samples from different origins and to establish the relation between samples and volatile compounds (variables) (Cheng *et al.*, 2013), *i.e.* aldehydes, aliphatic acids and their esters, alcohols, benzoic acids and their derivatives, flavonoids, hydrocarbons, ketones, cinnamic acids and their ester, terpenes, to evaluate possible similarities and differences among propolis from the two countries. In this context, the cases were the different propolis samples, the variables were the identified volatile compound groups, and the input value in the matrix was the compound ratio.

Results

General

The GC-MS analysis of Greek and Turkish propolis samples revealed a total of more than 150 compounds, belonging to aldehydes, alcohols, aliphatic acids and their esters, benzoic acids and their derivatives, cinnamic acids and their esters, ethers, flavonoids, hydrocarbons, ketones, and terpenes. The chemical compositions of the EEPs, as percentage of total ion current (TIC), are presented in Table I for the Greek samples and in Table II for the Turkish samples.

We detected quite a number of compounds with known biological activities, such as chrysin and totarol, in both the Turkish and Greek propolis samples. Greek samples had a significantly high terpene content which distinguished them from the European-type propolis. They also had a relatively low flavonoid content compared to the European-type propolis.

Aldehydes

This type of compounds was found in minor amounts in three out of five Turkish samples (TR1, TR2, and TR5) and only in a single Greek sample (GR7).

Aliphatic acids and their esters

Aliphatic acids and their esters were observed frequently, ranging from 0.51 to 13.82% of TIC in all investigated Greek samples. Of these, oleic acid and hexadecanoic acid had also been observed in previous studies (Kalogeropoulos *et al.*, 2009; Velikova *et al.*, 2000).

Similiar to the Greek samples, most Turkish samples contain the ethyl esters of decanoic, hexadecanoic, linoleic, oleic, and octadecanoic acid, respectively, in minor concentrations. Only sample TR3 had a considerable content of oleic acid ethyl ester (9.20%).

Table I. Some important compounds of propolis extracts from Greece and their content in the 12 samples (% of total ion current).

Compound	GR1	GR2	GR3	GR4	GR5	GR6	GR7	GR8	GR9	GR10	GR11	GR12
Aldehydes												
Octadecanal							0.41					
Total							0.41					
Aliphatic acids and their esters												
9,12-Octadecadienoic acid (*Z*,*Z*)									1.35			
9-Octadecenoic acid (*E*)										0.34		
2-Methyl-butanoic acid			0.10									
Decanoic acid ethyl ester									0.57			
Oleic acid ethyl ester	0.30	1.52	1.32	1.25	2.43	0.77	0.90	1.78	2.23		5.85	1.01
Ethyl tridecanoate	0.26			0.54								
Hexadecanoic acid				0.22		0.27	0.55		1.67		0.96	
Hexadecanoic acid ethyl ester		0.86	0.20		1.78	0.31	0.53	1.02	2.07	0.17	5.37	0.49
Linoleic acid ethyl ester				0.41	0.47				2.53		0.90	
Octadecanoic acid ethyl ester		0.22	0.23	0.35							0.74	
Oleic acid						0.84						
Tetradecanoic acid												0.68
Total	0.56	2.6	1.85	2.77	4.68	2.19	1.98	2.8	10.42	0.51	13.82	2.18
Flavonoids												
3,5,7-Trihydroxyflavone										0.25		
4',5-Dihydroxy-methoxyflavanone	0.61			1.12								
5,7-Dihydroxyflavanone	3.75		1.19	6.66		0.24	1.68		1.96	7.94		2.69
5,7-Dihydroxyflavone	0.94		0.72	2.55						1.51		1.28
5-Hydroxy-7-methoxyflavone	4.26		2.03	6.45	1.92	0.58	1.80		2.55	1.79	1.85	2.46
Pinostrobin chalcone	16.93		5.89	15.98	3.46	1.12	5.98	10.82	7.80	6.72	7.23	6.53
Total	26.49		9.83	32.76	5.38	1.94	9.46	10.82	12.31	18.21	9.08	12.96
Cinnamic acids and their esters												
2,4-Dimethoxycinnamic acid										0.76		0.13
3,4-Dimethoxycinnamic acid methyl ester				0.19								
4-Hydroxy-3-methoxycinnamic acid										0.30		
Hydroxycinnamic acid				0.29								
Cinnamic acid				0.12						1.44		
Benzyl cinnamate										0.34		
3-Hydroxy-4-methoxycinnamic acid				0.21						0.23		
Hydrocinnamic acid										0.55		0.12
Hydrocinnamic acid ethyl ester												0.07
Total				0.81						3.62		0.32
Terpenes												
Monoterpenes												
α-Terpinene						0.07				0.18		0.08
β-Myrcene			0.04									
β-Phellandrene			0.13									
γ-Terpinene			0.10			0.08						
(1*R*)-α-Pinene			2.76		0.41		2.69					
2-β-Pinene										0.06		
3-Carene					0.04							
Sabinene						0.55						
α-Thujene			0.12		0.08							
β-Thujene					0.11							
(–)-β-Pinene			0.15			1.03						
Camphene						1.43						
Limonene			0.06									
l-Limonene										0.04		
Total			3.36		0.64	3.16	2.69			0.28		0.08

Table I continued.

Compound	GR1	GR2	GR3	GR4	GR5	GR6	GR7	GR8	GR9	GR10	GR11	GR12
Sesquiterpenes												
δ-Selinene					0.11					0.66		
(+)-Aromadendrene		4.79										
β-Pinene			0.08									
γ-Cadinene	1.23	0.49	0.05		0.04					0.13		0.15
Alloaromadendrene oxide-(1)			0.18									
Alloaromadendrene oxide-(2)			0.03			1.90				0.94		0.08
Caryophyllene					0.08							
β-Elemene			13.28								13.79	
γ-Muurolene			0.04	0.35						0.21		0.12
trans-Caryophyllene		0.58						0.45				
α-Muurolene	0.34											0.04
α-Cadinene	0.17											
α-Humulene				0.09								
γ-Cadinene	0.69									0.10		
Junipenene						0.25				0.32		0.05
Aristolone	0.73											
Caryophyllene oxide						0.11						
Cadina-1,3,5-triene	0.29		0.03	0.17								
Valencene			0.41			0.06	0.29			0.41		
1,2,4a,5,6,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene												0.10
β-Selinene			0.03									
Total	3.45	5.86	14.13	0.61	0.23	2.32	0.29	0.45		2.77	13.79	0.54
Diterpenes												
13-Epitorulosol			4.07									
Totarol		25.77	11.21	4.03	10.17		21.04	12.45	2.68	0.33	24.94	21.31
Ferruginol	0.35	0.94	0.99		0.85	0.80	7.92	8.38		0.51	1.07	0.98
Cadina-1,4-diene				0.18								0.10
Total	0.35	26.71	16.27	4.21	11.02	0.8	28.96	20.83	2.68	0.84	26.01	22.39
Triterpenes												
(5a,5b,8,8,11a,13b-Hexamethyl-3-prop-1-en-2-yl-1,2,3,3a,4,5,6,7,7a,9,10,11,11b,12,13,13a-hexadecahydrocyclopenta[a]chrysen-9-yl) acetate					4.90							
Lanosterol					1.48							
Keto-urs-12-ene	3.46								2.53			
4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2*H*-picen-3-one	4,09											
Total	7.55				6.38				2.53			

Table II. Some important compounds of propolis extracts from Turkey and their content in the 5 samples (% of total ion current).

Compound	TR1	TR2	TR3	TR4	TR5
Aldehydes					
Benzaldehyde	0.12	0.02			
Isovanillin	0.15				
Vanillin	0.11				
4-Methylene-5-hexenal					0.21
Cinnamaldehyde	0.09				
Total	0.47	0.02			0.21

Table II continued.

Compound	TR1	TR2	TR3	TR4	TR5
Aliphatic acids and their esters					
Oleic acid ethyl ester	2.32		9.20	3.04	
6-Octadecenoic acid methyl ester					0.58
Hexadecanoic acid ethyl ester		4.56	5.91		
Octadecanoic acid ethyl ester	0.86	3.21	0.67		
Oleic acid		7.52			0.98
Heptadecanoic acid ethyl ester	0.92	1.37			
E-11-Hexadecenoic acid ethyl ester			0.21		
Linoleic acid ethyl ester			1.14		
Decanoic acid ethyl ester			0.34		
5-Phenyl-4-pentenoic acid	0.35				
Total	4.45	16.66	17.47	3.04	1.56
Flavonoids					
5,7-Dihydroxy-4'-methoxyflavone	1.76				
Pinostrobin chalcone	11.82	7.10			
5,7-Dihydroxyflavanone	10.10	24.61	4.24		
5-Hydroxy-7-methoxyflavone	5.92				
4,5-Dihydroxy-7-methoxyflavanone	1,32				
5,7-Dihydroxyflavone	3.44				
Total	34.36	31.71	4.24		
Cinnamic acids and their esters					
Benzyl cinnamate	0.92				
Total	0.92				
Terpenes					
Monoterpenes					
β-Myrcene		0.05			
Total		0.05			
Sesquiterpenes					
trans-Caryophyllene					0.37
α-Selinene	0.08				
γ-Selinene		0.05			
1*R*-*α*-Pinene					3.48
γ-Muurolene	0.38				
Aromadendrene					0.46
β-Phellandrene					1.01
Junipene					0.20
Total	0.46	0.05			5.52
Diterpenes					
Totarol			7.27	24.47	11.18
Total			7.27	24.47	11.18

Benzoic acids and their derivatives

Of the compounds belonging to this group, benzoic acid was also found in previous studies of Greek and Turkish samples (Kalogeropoulos *et al.*, 2009; Velikova *et al.*, 2000).

Cinnamic acids and their derivatives

Similiar to Kalogeropoulos *et al.* (2009) and Velikova *et al.* (2000), we found cinnamic acid and 3-hydroxy-4-methoxycinnamic acid in minor amounts in Greek samples. 4-Hydroxy-3-methoxycinnamic acid is known to have hepatoprotective activity (Bankova, 2005); we found this compound only in GR10 with a content of 0.30%.

Unlike Greek samples, only one Turkish sample, TR1, contained just one kind of cinnamic acid ester (benzyl cinnamate), in minor amounts.

Flavonoids

Flavonoids are the compounds responsible for the antibacterial, anti-inflammatory, hepatoprotective, and antioxidant activities of propolis (Bankova, 2005). In Greek samples we found pinostrobin chalcone (1.12–16.93%), 5-hydroxy-7-methoxy flavone (0.58–6.45%), 5,7-dihydroxyflavone (0.72–2.55%), 3,5,7-trihydroxyflavone (0.25%), 5,7-dihydroxyflavanone (0.24–7.94%), and 4',5-dihydroxy-methoxyflavanone (0.61–1.12%). Kalogeropoulos *et al.* (2009) also found similiar amounts of 5,7-dihydroxyflavone (0.46–4.50%), pinostrobin (chalcone) (0.23–1.37%), and 3,5,7-trihydroxyflavone (0.23–1.92%), while Velikova *et al.* (2000) observed 5,7-dihydroxyflavone and 3,5,7-trihydroxyflavone in contents of 4.9–5.8%, respectively.

Although in previous studies Turkish propolis had been found to contain high amounts of flavonoids (Gencay, 2004; Uzel *et al.*, 2005; Gencay and Salih, 2009), in this study it was found that TR4 and TR5 contained no flavonoids and TR3 had only a very low flavonoid content. However, TR1 and TR2 had quite high flavonoid contents (34.6–31.71%). These two samples had been collected from the European part of Turkey, and they were found to be similar to a temperate poplar propolis type with a high flavonoid content (Salatino *et al.*, 2011), while TR3, TR4, and TR5 originated from the Aegean Sea coast line and were found to be similiar to the Mediterranean propolis type.

Terpenes

Terpenes are among the chemicals responsible for the medicinal, culinary, and fragrant uses of aromatic and medicinal plants (Dorman and Deans, 2000). We found terpenes in high amounts (3.89–39.8%) in all Greek samples (Table I). While Greek samples contained more and various kinds of terpenes and in high amounts, Turkish samples contained less terpenes and in minor amounts. β-Myrcene, selinene, aromadendrene, γ-muurolene, junipene, and totarol are the common terpene compounds in Turkish and Greek samples. In particular, TR3, TR4, and TR5 had relatively higher terpene contents than TR1 and TR2, and thus they are similar to Mediterranean-type propolis, as also shown above for the flavonoids. The same samples had also a high totarol content (7.27–24.47%), which was also found in 10 out of the 12 Greek samples in high amounts (0.33–25.77%). These compounds could be used as a marker for distinguishing propolis collected from areas near the Aegean Sea. The largest difference between Greek and Turkish samples was related to their terpene content.

Totarol was also detected in Greek propolis in previous studies (Kalogeropoulos *et al.*, 2009; Melliou and Chinou, 2004; Melliou *et al.*, 2007; Popova *et al.*, 2009). The first record of totarol in European-type propolis was provided by Melliou *et al.* (2007). This diterpene is present in southern hemisphere conifers, thus characterizing, together with other diterpenes, tropical propolis (Cox *et al.*, 2007). Totarol is a known antimicrobial agent against Gram-positive bacteria (Cowan, 1999), and this compound isolated from Greek propolis was specifically active against *Staphylococcus aureus* and *S. epidemidis* (Melliou and Chinou, 2004). We found totarol in GR2, GR7, GR11, and GR12 in quite high content. Therefore, these samples are also expected to have high antimicrobial activity. Propolis samples containing totarol could be used against methicillin-resistant *Staphylococcus aureus* (MRSA) which is a cause of infections in hospitals.

Among the detected terpenes, 3-carene, β-pinene, limonene, α-pinene, α-terpinene, sabinene, α-humulene, and aromadendrene have also been shown to have antibacterial activities (Dorman and Deans, 2000).

Melliou *et al.* (2007) found 0.5% γ-elemene, but not β-elemene, in Greek propolis samples. We found this compound only in two Greek samples and in none of the Turkish ones. Interestingly, β-elemene was found in high amounts in these two samples, *i.e.* 13.28% in sample GR3 and 13.79% in sample GR11, respectively. This is not the first report of this compound for propolis, since Pino (2006) found it in propolis of both honey bees and stingless bees from Yucatán in minor amounts, but it is the first report for β-elemene in Mediterranean-type propolis, and in high content. The elemenes are an important group of sesquiterpenes widely occurring in nature, and are considered to be produced from germacranes. A recent and higly significant development is the use of β-elemene in the treatment of leukemia and cancer of brain, ovary, prostate, breast, lungs, liver, colon, and other tisues in China (Adio, 2009). The content of β-elemene thus gives a potentially high value for Greek samples.

Discussion

As seen from Table III, flavonoids and terpenes are the major constituents of the examined propolis samples which are thus either high in flavonoids and low in terpenes or *vice versa.*

Based on pollen analysis, Melliou and Chinou (2004) proved that a significant source of propolis from mainland Greece were coniferous trees – especially *Pinus* sp. – the resin of which is rich in terpenic acids like abietic, dehydroabietic, and isopimaric acids, respectively. The resins of certain conifers also accumulate mixtures of terpenes, including the monoterpenes α- and β-pinene and myrcene (Hopkins and Hüner, 2008). The three sites of Turkey from where the samples TR3, TR4, and TR5 originated, are also rich in coniferous species, mainly *Pinu*s sp.

Species of the Cupressaceae possess a terpene composition distinct from that of the Pinaceae. Ferruginol, totarol and derivatives thereof, oxygenated ferruginol, and sempervirol are characteristic for Cupressaceae but not for Pinaceae. Owing to the high totarol content of the investigated samples, we can assume that species of the Cupressaceae are their source. However, microscopic analysis is necessary to support this. Popova *et al.* (2012) compared the diterpenic profiles of Mediterrranean-type propolis samples (from Malta and Greece) with that of the resin from *Cupressus sempervirens* L. They observed higher concentrations of totarol and *epi*-torulosal, as

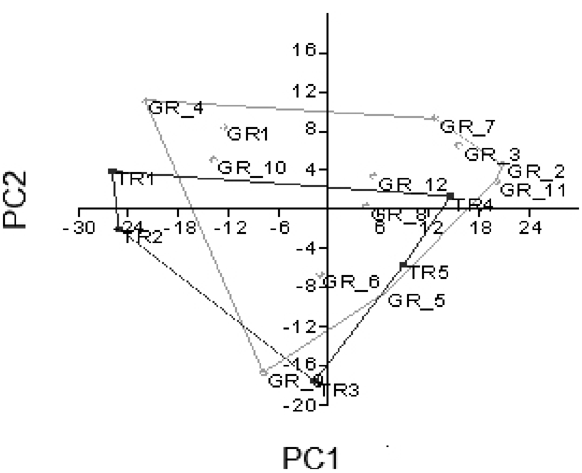

Fig. 2. Principal component analysis: The scores scatter plot on the two first principal components (PC1, PC2) representing the 17 propolis samples.

Table III. Observed classes of compounds and their contents (in % of total ion current) in Turkish and Greek propolis samples.

Class	GR1	GR2	GR3	GR4	GR5	GR6	GR7	GR8	GR9	GR10	GR11	GR12	TR1	TR2	TR3	TR4	TR5
Aldehydes							0.41						0.47	0.02			0.21
Aliphatic acids and their esters	0.56	2.6	1.85	2.77	4.68	2.19	1.98	2.8	10.42	0.51	13.82	2.18	4.45	16.66	17.47	3.04	1.56
Alcohols	9.28	3.36	3.51	12.94	1.9	12.25	22.77	14.73	1.97	17.13	4.83	2.91	7.06	8.46	2.98	4.24	1.58
Ethers			0.06	0.28		0.09				0.03			0.26	0.07	0.06		
Benzoic acids and their derivatives	0.55	0.4	0.74				1.00		1.17	0.05		1.20	3.56	0.15	0.49		1.75
Flavonoids	26.49		9.83	32.76	5.38	1.94	9.46	10.82	12.31	18.21	9.08	12.96	34.36	31.71	4.24		
Hydrocarbons	5.6	0.29	7.38	1.23	15.92	7.17	10.99	15.56	22.47	2.48	9.40	6.69	7.47	7.28	13.01		6.44
Ketones	0.52	2.1	2.1	0.53	1.2	1.61	1.52	1.53		0.1	0.47	0.58	2.12	0.14			
Cinnamic acids and their esters				0.81						3.62		0.19	0.92				
Terpenes	11.35	32.57	33.76	4.82	18.27	6.28	31.94	21.28	5.21	3.89	39.8	23.01	0.46	0.1	7.27	24.47	16.7

well as *epi*-cupressic acid in the resin of *C. sempervirens* and concluded that the diterpene-rich Mediterrranean-type propolis originates mainly from the resin of the common cypress *C. sempervirens.* In our study we found totarol in 10 out of 12 Greek and three out of five Turkish samples. So most of our Greek samples and three Turkish samples (TR3, TR4, TR5) from the Aegean Sea coast can be traced back to the resin of *C. sempervirens*.

With the aim of examining the potential application of GC-MS data in the identification of propolis of the two countries, data listed in Table III were subjected to principal component analysis (PCA). For better interpretation of the data obtained, no individual compounds, but rather the 10 classes of compounds found in the propolis samples were manually integrated, and their percentages were subjected to PCA in order to determine a possible relation between volatile distribution and the geographic origin of the samples. However, as can be seen from Fig. 2, the scores scatter plot on the two first principal components (PC1, PC2), representing the 17 propolis samples, did not reveal a separation of the Greek and Turkish samples into two distinct groups.

According to the cladogram in Fig. 3, GR4, GR10, TR1, and TR2 are in the same clade. Indeed, TR1 and TR2 originated from places close to each other, *i.e.*, they are both from the European part of Turkey, but GR4 and GR10 were collected in places far from each other as well as from the origins of TR1 and TR2. A common characteristic of these samples is their lower terpene content compared to that of the other samples (around 5%), so their origin is likely from Pinaceae species or from *Populus* spp. On the other hand, GR6, TR4, GR2, TR3, TR5, GR3, GR12, GR5, GR7, GR8, GR1, GR9, and GR11 are similiar to each other in having a high terpene content, so one may conclude that the samples originated from Cupressaceae species. Thus, only a general conclusion is possible: 13 of the 17 investigated samples are Mediterranean-type propolis, and four of them are most probably of the European type.

Summarizing our results, we can conclude that Greek propolis and the three Turkish propolis samples collected near the Aegean Sea, TR3, TR4, TR5, show many similarities to each other and are different from the European propolis type.

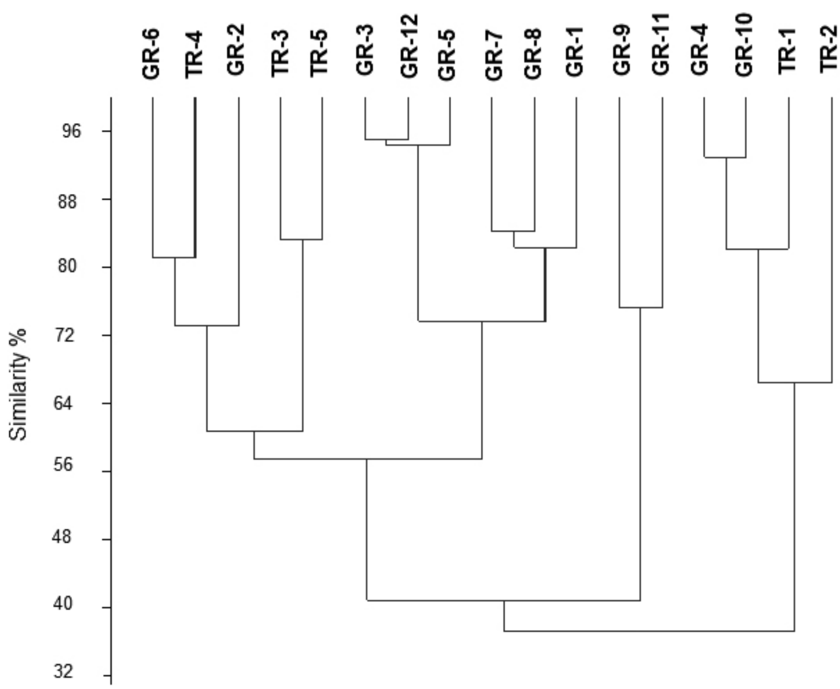

Fig. 3. Principal component analysis: Cladogram of the 17 propolis samples based on general classes of compounds.

The two Turkish samples TR1 and TR2, originating from the European part of Turkey, appeared similar to the European type of propolis with a high flavonoid (31.71 – 34.36%) and low terpenoid (0.1 – 0.46%) content.

The finding of *β*-elemene and totarol in high amounts in the Greek samples highlights the importance of the location and botanical source in the chemical profile and biological properties of propolis.

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