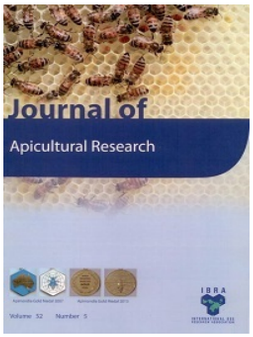

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ORIGINAL RESEARCH ARTICLE

A comparison of the activities of Greek and Turkish propolis against *Paenibacillus larvae*

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Summary

Propolis has a long history of use in traditional medicine dating back to at least 300 BC, and has been reported to have a broad spectrum of biological activities. Since most studies have to date focused on medical uses of propolis, its antimicrobial activity against honey bee diseases has been little studied. One of the aims of this study was therefore to investigate the potential use of propolis in honey bee health, especially against American foulbrood, which causes much damage in the beekeeping industry. The second aim was to reveal the different antimicrobial activities of propolis collected from different geographical areas from the neighbouring countries of Greece and Turkey. Propolis samples collected from several regions of Greece and Turkey were investigated for their *in vitro* antimicrobial activities against *Paenibacillus larvae*. Eighteen ethanol extracts of propolis (EEP), (nine from Greece and nine from Turkey) were tested for antimicrobial activities against ten *P. larvae* isolates. The results showed that all *P. larvae* strains were susceptible to propolis extracts from both Greece and Turkey Furthermore 50 % concentrations of EEP caused significantly wider inhibition zones (P≤0.05) around the discs. Comparisons of the content, and locations and botanical origins of EEPs from Greece and Turkey showed that the kind of vegetation is more important factor than geographic location for their antimicrobial activity. This is the first comprehensive study concerning the antimicrobial activity of propolis samples collected from a wide area around the Aegean Sea.

Comparación de la actividad de propóleos de Turquía y Grecia frente a *Paenibacillus larvae*

Resumen

El propóleos tiene una larga historia de uso en la medicina tradicional que se remonta por lo menos a 300 años antes de Cristo, y se ha descrito que tienen un amplio espectro de actividades biológicas. Como la mayoría de los estudios hasta la fecha se han centrado en los usos médicos de los propóleos, su actividad antimicrobiana frente a enfermedades de las abejas de la miel ha sido poco estudiada. Por lo tanto, uno de los objetivos de este estudio fue investigar el posible uso de los propóleos en la salud de la abeja de la miel, especialmente contra la loque americana, que causa mucho daño en la industria de la apicultura. El segundo objetivo era revelar las diferentes actividades antimicrobianas de propóleos recolectados de diferentes áreas geográficas de los países vecinos de Grecia y Turquía. Se investigó la actividad antimicrobiana *in vitro* de muestras de propóleos recolectados en varias regiones de Grecia y Turquía contra *Paenibacillus larvae*. Se probó la actividad antimicrobiana de dieciocho extractos de propóleos en etanol (EEP), (nueve de Grecia y nueve de Turquía) contra diez aislamientos de *P. larvae*. Los resultados mostraron que todas las cepas de *P. larvae* fueron susceptibles a los extractos de propóleos tanto de Grecia como de Turquía. Además concentraciones al 50% de EEP causaron zonas de inhibición significativamente más amplias (p ≤ 0,05) alrededor de los discos. La comparación de los contenidos, y lugares y orígenes botánicos de los EEP de Grecia y Turquía mostró que el tipo de vegetación es un factor más importante que la ubicación geográfica en relación con la actividad antimicrobiana. Este es el primer estudio exhaustivo en relación con la actividad antimicrobiana de propóleos recolectados de una amplia zona alrededor del Mar Egeo.

Keywords: propolis, *Paenibacillus larvae*, American foulbrood, treatment, infection, natural product

Introduction

Propolis or bee glue is the sticky dark red or brown coloured material that honey bees (*Apis mellifera*) collect from buds and leaves of trees and other plants, mixed with pollen as well as enzymes secreted by bees (Marcucci, 1995). Propolis is considered responsible for the low incidence of bacteria and moulds within hives and bees use it, therefore, as a protective barrier against their enemies (Burdock, 1998) as the origin of the word indicates (in ancient and modern Greek "pro-polis" means "before the city"). Action against microorganisms is an essential characteristic of propolis, and humans have used it for centuries for its pharmacological properties (Bankova *et al*., 2000).

American foulbrood (AFB) is one of the most severe bacterial diseases affecting the larvae of honey bees, and it is responsible for colony loss and reduced honey production in many countries. The causative agent is *Paenibacillus larvae,* a gram positive and spore forming bacterium which is distributed worldwide (Genersch *et al*., 2006), There have only been a few studies relating to the effectiveness of propolis against AFB (Mlagan and Sulimanovic,1982; Antunez *et al.,* 2008; Bastos *et al*., 2008). So far, the main focus of this research was the activity of just a few propolis extracts from one or two only geographical origins against *P. larvae*.

Recent studies have revealed a new type of European propolis called Mediterranean propolis which is distinguished by its high concentration of diterpenoids. This propolis type has been found in southern Greece, Sicily and some Croatian Adriatic islands (Melliou and Chinou, 2004; Popova *et al*., 2009). Greece and Turkey are both Mediterranean countries that are important sources of honey bee propolis, because they are covered by rich plant vegetation. Greek flora has a high biodiversity, with a high percentage of endemic plants (Melliou *et al.,* 2007). Turkey is also a country with diverse geo-morphological characteristics and rich flora and shares a border with Greece, in the north west region, the European part of Turkey. Above this, there is a long coast line in Turkey, in the Aegean Sea, having many similarities with the Greek islands in terms of climatic conditions and floral diversity. A survey of the differences and similarities in chemical characteristics between Greek and Turkish propolis was carried out by Gencay Çelemli *et al.* (2013). As the plant origin of propolis determines its chemical diversity, this brings up the question of the connection between similarities and differences of chemical composition of propolis from these two countries with their antibacterial activity. The antimicrobial activity of Greek and Cypriot propolis ethanolic extracts has been tested against eighteen bacterial strains both pathogenic and non-pathogenic, as well as against two

Table 1. The Sampling provinces for AFB.

Sample	**Province**
01/19	Adana
31/16	Hatay
01/49	Adana
48/27	Muğla
31/18	Hatay
48/35	Muğla
48/07	Muğla
01/29	Adana
31/27	Hatay
48/38	Muğla
ATCC9355	*P. larvae* control strain (ERIC I)

Fig. 1. Sampling locations in Greece and Turkey.

Table 2. The results of GC-MS analysis of propolis samples.

Compounds	Aliphatic acids & their esters	Alcohols	Aldehydes	Carboxylic acids & their esters	Cinnamic acids & their esters	Ethers	Flavonoids	Hydrocarbons	Ketones	Terpenes
Artvin	**17.39**	1.28	3.55	14.94	0.17	-	11.89	3.42	5.49	0.98
Veroia	1.85	3.51	-	0.74	-	0.06	9.83	7.38	2.1	**32.77**
Muğla	0.98	1.58	0.21	1.75	-	-	-	6.44	-	**16.7**
N. Moudania	2.19	**12.25**	-	-	-	0.09	1.94	7.17	1.61	5.48
Alexandroupoli	10.42	1.97	-	1.17	-	-	12.31	**22.47**	-	5.21
Didimoticho	1.98	22.77	0.41	1	-	-	9.46	10.99	1.52	**24.02**
İzmir	1.43	-	-	-	-	-	-	**4.09**	-	-
Tekirdağ	16.7	8.46	0.02	0.11	-	0.07	**31.71**	7.28	0.14	0.1
Tichero	2.8	14.73	-	-	-	-	10.82	**15.56**	1.53	12.9
Chios	4.68	1.9	-	-	-	-	5.38	15.92	1.2	**17.42**
Edirne	4.53	8.3	0.43	-	0.49	-	**37.38**	7.8	0.16	0.57
Elassona	2.77	12.94	-	-	0.81	0.28	**32.76**	1.23	0.53	4.82
Ankara	7.59	8.47	-	0.49	-	-	**35.77**	11.91	1.71	2.61
Çanakkale	**17.47**	2.98	-	0.53	-	0.06	4.24	13.01	-	7.27
Kerkyra	0.51	17.13	-	0.05	3.62	0.03	**18.21**	2.48	0.1	3.38
N. Manolada	12.96	4.83	-	-	-	-	9.08	9.4	0.47	**38.73**
Bartın	12.27	12.18	0.25	0.15	2.31	0.49	**27.74**	9.07	12.18	0.56
Kırklareli	4.45	7.06	0.47	2.07	2.41	0.26	**34.36**	7.47	2.12	0.46

pathogenic fungi, and the results showed that the propolis inhibitory spectrum is broad and its activity strong even at very low concentrations. (Kalogeropoulos *et al.* 2009). Also, Melliou *et al* (2007) observed that the volatiles of all Greek samples tested for their antimicrobial activity against four Gram-negative, two Gram-positive bacterial strains and three human-pathogen fungi showed interesting antimicrobial activity. Previous studies have concerned human health or human usage, but here we study propolis for bee health.

Material and methods

Propolis samples

Propolis samples were collected from the areas indicated in Fig. 1. The samples were chosen so that that similar and different locations were represented in respect of geographic and vegetation variation between the two countries.

Extraction and preparation of EEP

Special propolis traps were used in order to collect clean material. Propolis samples were collected from the apiaries and stored at -4^{o}C until chemical analysis. Each frozen sample was then ground and dissolved in ethanol (96 %) with a ratio of 1/3. This mixture was kept in an incubator at 30°C for two weeks in a tightly closed bottle. After incubation, the supernatant was filtered twice with Watman No. 4 and No. 1 filter papers. The final filtered concentrated solution (1:10, w/v) called ethanol extract 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 analysed 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

Table 3. Inhibition zone diameters (mm) of propolis samples. *The diameter of discs (5mm) are included in the measurements.

Concentration	**100 %(1:1 v/v)**			**50 % (1:2 v/v)**		
	Inhibition Zone*			**Inhibition Zone***		
Propolis	**Min(mm)**	**Max(mm)**	**Mean±SD(mm)**	**Min(mm)**	**Max(mm)**	**Mean±SD(mm)**
Veroia	7	25	17.67±1.6	10	27	18.80±1.7
Elassona	6	13	9.60±0.8	8	24	12.70±1.3
Chios	7	16	9.88±0.8	8	16	10.83±1.2
N. Moudania	6	25	11.10±1.2	6	26	12.87±1.3
Didimoticho	7	14	10.40±1.1	7	17	11.80±1.1
Tichero	5	15	10.03±0.9	7	21	11.47±1.0
Alexandroupoli	6	22	10.53±0.9	9	23	12.13±1.4
Kerkyra	5	12	8.33±0.6	7	18	10.63±1.2
N. Manolada	6	13	8.17±0.6	8	15	10.37±1.1
Bartın	5	14	7.03±0.5	6,5	13	8.95±0.7
Artvin	14	24	18.97±1.4	11	28	15.19±1.3
Tekirdağ	8	13	10.17±1.0	9	13	11.10±0.9
Edirne	6	12	9.77±1.1	8	16,5	11.18±1.1
Kırklareli	5	8	6.07±0.4	6	11	8.42±0.7
İzmir	6	13	10.37±1.1	5	11	8.40±0.6
Muğla	6	17	11.37±1.3	6	16	11.70±1.2
Çanakkale	6	11	8.77±0.8	8	12	9.83±0.9
Ankara	7	11	9.33±0.8	8	14	10.93±1.0

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 280ºC with 20°C/min heating ramp and then kept at 280°C for 30 min.

Organic compounds in the propolis samples were identified using standard Willey and Nist Libraries available in the data acquisition 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 standards were 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%.

P. larvae isolates

Ten *P. larvae* isolates were randomly selected from the collection of the Department of Biology Bee Health Laboratory, Hacettepe University, Turkey. These isolates were obtained from larvae, worker bee and honey from different provinces of Turkey between 2006 and 2011. The samples codes and the provinces are listed in Table 1. Also the ATCC9355 strain of *P. larvae* was used.

Antimicrobial susceptibility test

Susceptibility patterns of *P. larvae* isolates to EEP were assessed by the disc diffusion method following the general guidelines of National Committee for Clinical Laboratory Standards (CLSI, 2013). Discs containing different EEP concentrations (100 % and 50 %) were used. All strains were cultured in Brain-Heart Infusion Broth Medium (BHI-Broth) (Sigma; 42g/l) and overnight bacterial cultures (24 hours) ($1x10^8$ CFU/ml) were transferred to Brain Heart Infusion Agar Medium (BHI) (Sigma; 42g/l). After inoculation of 0.1 ml *P. larvae* bacterial solution, the paper discs were placed the middle of the plates and 10 µl in different concentrations (100 %=1:1 v/v; 50 %= 1:2v/v) of EEP solutions were inoculated to absorber paper discs in 5 mm diameter. All plates were incubated at 37^0C for 24 hours. The discs containing only ethanol (96 %) were used as negative controls. The antibiotic sulbactam ampicillin, known to be effective against *P. larvae*, was used as positive control. All assays were carried out in triplicate.

Determination of the minimum inhibitory concentration of propolis

The minimum inhibitory concentration (MIC) was directly assessed by the observation of turbidity (CLSI, 2013). One ml of the EEP starting solution was added to BHI broth. It was serially diluted and 1 ml of

bacterial suspension (equivalent to 0.5 McFarland) was added to each serial dilution tube with agitation. All sample tubes (as well as positive and negative controls) were incubated at 37^0C for 48 h. The lowest concentration of propolis that prevented bacterial growth, determined by the absence of the turbidity, was defined as the MIC. The turbidity was measured by spectrophotometer at 490 nm.

Statistical analyses

One way ANOVA and Duncan tests were performed in order to determine significant differences in the efficacy of EEPs from different regions of Greece and Turkey against *P. larvae* strains (SPSS22.0 Software programme).

Results

GC-MS analysis

The results according to the GC-MS analysis are summarized in Table 2. The largest difference between Greek and Turkish samples concerned the terpene followed by the flavonoid contents.

Antimicrobial susceptibility test

Ten *P. larvae* isolates from different regions of Turkey and the ATCC9355 strain were found to be susceptible to propolis extracts from Greece and Turkey. Two different concentrations (100 %=1:1 v/v; 50 %= 1:2v/v) of propolis samples were tested for antimicrobial activity and minimum, maximum and mean values with SD (standard deviation) of the inhibition zones are listed in Table 3.

Minimum inhibitory concentration of propolis

All *P. larvae* isolates were highly susceptible to the assessed propolis concentrations, while ethanol did not inhibit bacterial growth. Inhibition diameters around the discs measured when the minimum concentration of propolis (%1.25). The results are summarized in Table 4.

Table 4. MIC (dilution %) values of propolis samples.

Greece		**Turkey**	
propolis	**MIC (dilution %)**	**propolis**	**MIC (dilution %)**
Veroia	1.25	Bartın	2.5
Elassona	2.5	Artvin	1.25
Chios	2.5	Tekirdağ	1.25
N. Moudania	1.25	Edirne	1.25
Didimoticho	1.25	Kırklareli	2.5
Tichero	1.25	İzmir	1.25
Alexandroupoli	1.25	Muğla	1.25
Kerkyra	2.5	Çanakkale	1.25
N. Manolada	2.5	Ankara	1.25

Statistical analyses

Significant differences (P≤0.05) of the antimicrobial activities were determined by ANOVA and Duncan tests (SPSS 22.0 Software programme) There was a significant (ANOVA F=50.873 df=22 Sig=.000 P≤0.001) difference between the antimicrobial activities of 100 % concentrations of propolis extracts from different locations of Greece and Turkey. All propolis samples were classified according to their antimicrobial activities and the groups which have different inhibition zone values (mm) were created by means of Duncan test (Fig. 1; Table 5).

The antimicrobial activities of the different propolis concentrations (100 %, 50 %) and negative control were compared. Significant differences (P≤0.05) were found between concentrations (0 %, 50 %, 100 %) (ANOVA F=57.993 df=2 Sig=.000 P≤0.001). Duncan tests were used for the determination of the differences in the groups (Table 6.)

Discussion

A common strategy for the treatment of honey bee colonies infected with *P. larvae* was the use of antibiotics, particularly oxytetracycline hydrochloride (OTC) in some parts of the world, especially the USA (Hansen and Brødsgaard, 1999). The use of antibiotics in hives is now forbidden in some EU countries because there are no formulations that have obtained the necessary Ministerial registration for manufacture, transport, and sale. Moreover, there are no MRLs (Maximum Residue Limits) for tetracyclines and sulphanomides established for honey according to European Community regulations (Mutinelli, 2003). In other countries such as the USA, Canada and Argentina, preventive treatments with antibiotics are allowed, being considered a routine procedure to prevent outbreaks of AFB (Lindstrom, 2006). Consequently, various strains of *P. larvae* showing resistance to antibiotics, such as oxytetracycline-HCl (OTC), have been discovered in Argentina (Alippi, 2000) as well as in many US regions (Miyagi *et al.*, 2000; De Graaf *et al.*, 2013).

The extensive use of antibiotics can lead to an accumulation of residues in hive products, especially honey, decreasing their quality and making their marketing more difficult (Fuselli *et al.*, 2005). Because of legal and biological issues associated with antibiotic use in hives, bee scientists have been examining natural antimicrobial products for AFB management. The most popular natural product is propolis derived from plant resins and produced by honey bees.

The two aims of this study were: 1. to investigate the *in vitro* antimicrobial activity of Mediterranean or European type of propolis ethanol extracts collected from several regions of the two countries against *P. larvae* as a natural alternative control of AFB, and: 2. to relate the effectiveness of the activity with the differences and

Table 5. The comparative data of the antimicrobial activities of propolis samples by Duncan test. Locations are listed from the highest to the lowest antimicrobial activities of propolis samples against AFB respectively. *The Duncan significance test groups are indicated in parenthesis. Means for groups in homogenous subsets are displayed.

		Inhibition zone diameters (mm) of propolis extracts										
Subgroups	***Subset for alpha = .05***	****a***	****b***	****c***	****d***	****e***	****f***	****g***	****h***	****i***	****j***	****k***
Location	N											
Artvin	30	18.9667										
Veroia	30		17.3667									
Muğla	30			11.3667								
N. Moudania	30				11.1000							
Alexandroupoli	30					10.5333						
Didimiticho	30						10.4000					
İzmir	30						10.3667					
Tekirdağ	30						10.1667					
Tichero	30							10.0333				
Chios	30							9.8833				
Edirne	30							9.7667				
Elassona	30								9.6000			
Ankara	30									9.3333		
Çanakkale	30									8.7667		
Kerkyra	30									8.3333		
N. Manodada	30										8.1667	
Bartın	30											7.0333
Kırklareli	30											6.0667

similarities of their chemical activities and therefore with their geographic/ botanical origins. So, all data obtained from this study are discussed with these perspectives.

The biological activity of propolis on various microorganisms has been demonstrated in both Turkey and Greece (Kartal *et al*., 2003; Kılıç *et al*., 2005; Katırcıoğlu and Mercan, 2006; Melliou *et al*, 2007; Ünlü *et al*., 2008; Kalogeropoulos *et al*., 2009; Popova *et al*., 2010; Arslan *et al*., 2012), but there has been no study of the potential actions of propolis extracts in the treatment of bee diseases such as American foulbrood and few studies have examined antimicrobial properties of propolis against bee pathogens or honey bee immune responses. (Mlagan and Sulimanovic, 1982; Antunez *et al*., 2008; Bastos *et al*., 2008). This study is therefore the first publication about the efficacy of Greek and Turkish propolis in the control of American foulbrood.

Scanning the vegetation and climatic conditions of the sampling areas, the Greek and Turkish sides of the Aegean Sea are almost the same for both vegetation and climatic conditions. Other sampling points, which are far away from each other in the two countries, are mountainous and forested areas including *Abies nordmanniana, Cedrus libani, Fraxinus angustifolia, Juniperus communis, J. excelsa, J. foetidissima, Picea orientalis, Pinus brutia, P. pinea, P. slyvestris, Populus alba, Salix alba* (Fig. 1.)

Statistical analyses revealed that the Artvin and Veroia samples had the highest level of antimicrobial activities respectively against *P. larvae*. Although these places are far apart in the two countries (Fig. 1.), their vegetation related to location properties are very similar (mountainous and forestry area). Considering their chemical contents, the two places contain similar flavonoid ratios (Table 2.).

Considering the first five propolis samples with maximum antimicrobial activities respectively according to Duncan test results (Table 5), on the one hand all places are from different regions of the countries, but on the other hand, all are located on the coast and have similar flora.

Table 6. The comparison of propolis concentrations (%100, %50, negative control) by Duncan Test. Means for groups in homogeneous subsets are displayed. *1,2,3 are significantly different groups.

	N	Subset for α =0.05		
CONS		**1***	**2***	**3***
%50	690	11.5902		
%100	690		10.1862	
negative control	1380			.0000

Propolis samples Didimiticho (Greece) and Tekirdağ (Turkey) were in the same group (f) from their antimicrobial activities according to the Duncan test results. This result confirms the correlation between the vegetation of the locations and the contents of propolis samples and their antimicrobial activities (Salatino *et al*., 2011). These two places are very close and in the same area near the borders.

Chios (Greece) and İzmir (Turkey) are on the opposite sides and very close to the connection point of Greece and Turkey. The results showed that these propolis extracts are in the different groups (İzmir: f ;Chios: g) for their antimicrobial effect. At this point, the difference of the sampling areas (Chios from an island, İzmir from the mainland) may cause the different content of propolis and as a result the different antimicrobial effect. The significance between İzmir and Chios may be caused by their ethyl oleate content; a ratio of 3.04% and 0.90% respectively.

Didimiticho, Tichero, and Alexandroupoli propolis samples were collected from the same area (Alexandroupoli, Greece), but unexpectedly these samples showed different levels of antimicrobial activity against *P. larvae*. When the sampling data were analysed, the different altitudes of these three places were recognized. So, different altitudes may also affect the botanical origin of propolis. The Duncan Test results showed that propolis samples from Greece (Elassona, Kerkyra and N. Manolada) and Turkey (Çanakkale, Ankara and Bartin) have decreasing antimicrobial effect against *P. larvae* respectively. These results showed the effect of vegetation on the contents of the propolis and also the antimicrobial activity. For instance, Ankara which has the least antimicrobial activity was collected from almost desert vegetation in Turkey.

Table 2 shows that it is possible to compare propolis extracts from different locations for their chemical contents by GC-MS. On the other hand, the results revealed that the antimicrobial activities of propolis extracts are not directly related to one or two major components (flavonoids or terpenes) of propolis (Table 5). Considering chemical contents and antimicrobial activities of propolis extracts, major components of propolis samples are different in each extract. So, this study shows that antimicrobial activity might be created by synergism of all compounds of propolis including minor ones.

The MIC values of the propolis extracts showed that vegetative forms of *P. larvae* are susceptible to propolis extracts and have no resistance. Our results confirm previous findings (Kartal *et al*., 2003; Kılıç *et al*., 2005; Katircioğlu and Mercan, 2006; Ünlü *et al.,* 2008; Arslan *et al*., 2012).

During the experiments 100 % and 50 % concentrations of EEPs were tested for their antimicrobial activities. 50 % concentrations were shown to have statistically more effective antimicrobial activities than 100 % concentrations (Table 6). Diluted EEP (50 %=1:2 v/v) may diffuse into the bacterial medium surface more effectively that the 100 % (1:1 v/v) concentration because it contains more ethanol. It is therefore probable that not only the content of propolis but also the diffusion capability of the propolis solution to the surface are relevant to its antimicrobial activity.

The existing literature suggests that the contents of propolis directly depend on the vegetation of the locations where it was collected, and that the antimicrobial activities of propolis extracts directly depend on the content (Banskota *et al.,* 2001; Bankova *et al*., 2008). Our results show that propolis samples collected from closely adjacent sites in Greece and Turkey may not have either similar contents or similar antimicrobial activities. The similarity of vegetation between locations is more important than the distance of between the locations

In conclusion, both Greek and Turkish propolis had effective antimicrobial activities against *P. larvae*. Since *P. larvae*, the causative agent of American foulbrood, is a spore forming bacteria, a natural antimicrobial barrier is always needed in hives to obstruct its sporulation process. Propolis therefore has potential as an alternative natural hive product, perhaps not for treatment, but as a disinfectant solution for the prevention of *P. larvae* infection in honey bee colonies.

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