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Pollen Analyses and Antimicrobial Properties of the Natural Honey from the East Mediterranean Part of Anatolia

Menderes Cenet

Department of Biology, Osmaniye Korkut Ata University, Karacaoğlan Campus, Osmaniye, 80000, Turkey

ABSTRACT

The pollen analysis of eight floral honey samples from Kahramanmaraş region were analyzed and seven samples were the unifloral and the one was the multifloral origin. The microscopical analyses indicated that 61 taxa (41 at the genus level and 20 at the species level) belonging to 34 families were identified. *Astragalus* sp., *Trifolium* sp., *Myrtus communis* and *Castanea sativa* were the predominant taxa in the unifloral honey samples. Antimicrobial effects of eight honey samples proved the most effective inhibitors against *Bacillus megaterium* and *Staphylococcus aureus*. Honey samples (H07 and H08) showed significant antifungal effects on *Candida albicans*.

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INTRODUCTION

Honey production, is very common in most countries of the world, which has been estimated that annual production is about 1170000 ton in the world (Mendes *et al*., 1998; Nanda *et al*., 2003). In Turkey, annual production of honey is about 80000 ton and constitute 5.7% of the worldwide production. Turkey, is the second country in honey production rankings of the world, which has convenient environments for honey bee products (Kahraman *et al*., 2010; Tornuk *et al*., 2013).

Honey composition is very variable and their diversity is related to various parameters such as content of nectar and pollen, color, taste, ash, protein, sugar and viscosity. Type of production as well as handling steps are also important parameters for determining the quality of honey (Azeredo *et al*., 2003; Küçük *et al*., 2007). Botanical identification of honey, is essential in the EU countries, which is not only identifying the quality, but also indicating botanical and geographical origins. Therefore, information on the botanical origin of honey must be written on their label (The Council of European Union, 2002; Terrab *et al*., 2004).

In Anatolia, melitopalinology studies date back to the 1980s (Sorkun and Inceoglu, 1984; Sorkun and Yulug, 1985). The pollens of the flowering plants were identified in honey samples, *e.g.,* 8 samples from Erzurum (Sorkun and Yuluğ, 1984), 7 samples from Elazığ (Gür, 1993), 24 samples from Konya (Kaplan, 1993), 12 samples from Gümüşhane (Türker, 1993) 20 samples from various regions in Turkey (Sorkun and Doğan, 1995), 25 samples from Antalya (Silici and Gökçeoglu, 2007), 17 samples from İzmit (Yılmaz, 1969), 6 samples from Marmaris (Kemancı, 1999) and 74 samples from various regions of Turkey (Doğan and Sorkun, 2001).

Kahramanmaras province is located between 37-38 North latitude and East longitude 36-37. Kahramanmaras landforms consist of mountainous areas and generally consists of Southeast Taurus mountains with extensions and remaining depression areas between them. Height of wide plains is 350 meters up to 3000 meters (Kahramanmaras.gov.tr). Turkey has three distinct phytogeographical regions (the Mediterranean region, Eastern Anatolia, Southeastern Anatolia Region), which is located in the area where most approach each other. Close to the impaired Mediterranean climate, Kahramanmaras shows a climatic feature. The season is hot and dry in summer and tepid and rainy in winter. Depending on the elevation, towards the north of Kahramanmaras, characteristics of terrestrial climate is seen (Kahramanmaras.gov.tr). Kahramanmaras located in the transition zone of the Mediterranean and Irano-Turanian phytogeographic regions. The plants in relic style in some parts of Kahramanmaras belonging to the Euro-Siberian floristic region could be also observable.

To the best of our knowledge, there has been no previous study on pollen analysis and antimicrobial activities of Kahramanmaras honey samples. Therefore, the aim of this study is to analyze the botanical origin of honey and antimicrobial activities of the honey specimens from different regions of Kahramanmaras (Eastern Mediterranean region), which provides significant information for consumers and the references on the quality as well as bioactivities of East Mediterrenean honey.

* Corresponding author: mcenet@osmaniye.edu.tr
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Table I.- Pollen analysis of honey samples taken from Kahramanmaraş.

Honey sample No.	Regional name	Pollen spectrum and percentage
H01	Ahır Dağı (Kahramanmaraş)	**Astragalus 66* ** ****Pyracantha coccinea 7, Centaurea 6, Ajuga 4, Cousinia 4* *****Trifolium, Crataegus monogyna, Salvia, Brassica rapa, Salix Scabiosa, Castanea sativa,* Umbelliferae, Compositae
H02	Bulgurkaya (Andırın)	**Myrtus communis 62* ***Salix 26* ***Umbelliferae 6 *****Astragalus, Robunia pseudoacacia,* Compositae, *Salvia, Scabiosa Olea europeae,* Oleaceae, *Veronica, Quercus, Pinus*
H03	Yeşiltepe (Andırın)	**Castanea sativa 80* ** ****Anagallis arvensis 12, Salvia 4* *****Senecio, Ajuga, Myrtus communis, Quercus cocciferae, Iris, Betula Ornithogalum*
H04	Çağlayancerit	**Trifolium 55* ** ****Taraxacum officinalis 11, Triticum vulgare 9, Carduus 5, Centaurea 4* *****Astragalus, Vicia, Cousinia, Salvia, Crataegus, Prunus, Linaria Styrax officinalis, Quercus, Pinus, Dianthus, Geranium*
H05	Keklikoluk (Göksun)	**Astragalus 84* ** ***Fagaceae 4 ***** Vicia, Cousinia, Carduus, Ferula, Olea europeae, Castanea sativa Pinus, Lysimachia, Betula, Ankyropetalum,* Caryophyllaceae, *Onosma, Salvia*
H06	Gücüksu (Göksun)	**Astragalus 72* ***Ajuga 18* ****Carduus 6* *****Vicia cracca, Taraxacum officinalis, Echinops, Crataegus,* Rosaceae *Heracleum, Olea europeae, Castanea sativa, Pinus, Ornithogalum Agrostemma githago,* Caryophyllaceae, *Helianthemum,* Malvaceae *Helleborus, Galium*
H07	Mehmetbey (Göksun)	* ***Vicia cracca 37, Cistus 20* ****Centaurea 12, Cousinia 8, Astragalus 6, Geranium 5* *****Scorzonera, Ajuga, Salvia,* Lamiaceae, *Brassica rapa, Conium maculatum, Olea europeae,* Fagaceae, *Pinus, Onosma Solanum, Populus, Tilia, Vitis, Luzula*
H08	*Zeytinılıcası* (Kahramanmaraş)	**Astragalus 48* ** ****Lathyrus 12, Carduus 8, Vicia 6, Gossypium hirsitum 6, Cistus 4, Conium maculatum 4* *****Centaurea, Cousinia, Chiccorium inthybus, Salvia Alyssum, Brassica rapa,* Oleaceae, *Phleum, Triticum vulgare, Quercus, Ornithogalum, Luzula, Eleagnus angustifolia, Convolvulus arvensis*

*Dominant pollen (45%), ** Secondary pollen (16–45%), ***Minor pollen (3–15%), ****Rare pollen (3%).

MATERIALS AND METHODS

Honey samples (n=8) were collected from Kahramanmaras region (Eastern Mediterranean region). The collecting sites were coded with H01: Ahır Dağı (Kahramanmaraş-Merkez), H08: *Zeytinılıcası* (Kahramanmaraş-Merkez); H02: Andırın (Bulgurkaya); H03: Andırın (Yeşiltepe); H04: Çağlayancerit; H05: Göksun (Keklikoluk), H06: Göksun (Gücüksu), H07: Göksun (Mehmetbey). The samples after collection were retained at room temperature before analyses.

Pollen analyses, identification and enumeration

The melissopalynological technique was employed for the recognition of the botanical origin of honey samples (Maurizio, 1951; Louveaux *et al*., 1978). To prepare pollen slides, honey in distilled water (10g/20ml), was initially kept for 15 min at 45 °C to obtain a clear supension, and then spin for 20 min at 6500 rpm. The sediment was mixed with a solution of glycerine and gelatine (1:1.5) for slide preparation. This was followed by smearing the precipitate on a slide, keeping the slide on a hot plate and then fixing the samples (Ulukanli *et al*., 2012; Çenet *et al*., 2015).

Enumeration, identification and photographs of pollen grains from each slide were carried out using a light microscope (Olympus CX21) under 10x40 and 10x100 magnifications and a Euromex PB 4161, respectively. The following scala of ≥45% and more: dominant; 16-44%: seconder; 3-15: minor and <3%: trace were used for the determination of the pollens (%) in honey samples, as described by Louveaux *et al*. (1978). For identification, a comparative study was made between the present pollen grains in honey samples and those of earlier literature (Kapp, 1969; Aytuğ *et al*., 1971; Faegri and Iversen, 1975; Pehlivan, 1995; Erdtman, 1996; Sorkun, 2008).

Agar well diffusion assay

Antimicrobial activity was tested using the agar well diffusion assay (Collins *et al*., 1995). *Klebsiella pneumoniae* 13883, *Bacillus megaterium* DSM 32, *Pseudomonas aeroginosa* 9027, *Bacillus subtilis* IMG 22**,** *Enterobacter cloaca* ATCC 13047, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Candida albicans* 30114 and *Rhodotorula rubra* 116 were used for antimicrobial assay. Each bacterial and fungal culture was inoculated in Nutrient Broth (NB) (Difco) at 37±0.1°C/ 24 h and in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1°C/ 24 h, respectively. In the agar well diffusion assay, turbidity of the freshly grown microorganism in broth medium was adjusted in the saline solution according to the Mac Farland Unit (0.5). An aliquot (0.1 ml) from microbial suspension was spread onto solidified agar medium (15 ml) in petri dish (Muller-Hinton Agar (MHA, Oxoid) for bacterial cultures and Saboraud Dextrose Agar (SDA) for fungal cultures. Each plate was punched for generating four wells (10 mm in diameter) using with a sterile cork borer. A 0.1 ml from 65% honey solution was aseptically loaded into each agar-well. After 24 h incubation at 35°C, diameter of inhibition zones was measured for each microorganism under assay. Each assay was carried out as triplicate.

RESULTS AND DISCUSSION

In the present study, eight natural honey samples, collected from the province of Kahramanmaraş (Kahramanmaraş Merkez, Andırın, Göksun, Çağlayancerit and Ahırdağı) in the East Mediterranean region of Anatolia, were analyzed for their botanical origin as well as their antimicrobial potential (Figs. 1, 2). According to the microscobic analysis of the pollen composition, sixty-one pollen taxa belongs to 34 families were recognized in all samples (Table I). Forty-one and twenty of the taxa were at the genus and species level, respectively. The number of taxa (pollen types) varied from 10 to 21 in each honey sample. The pollen analysis revealed that the botanical origins of seven honey samples were the unifloral and one was with the multifloral origin with regard to pollen taxa.

As indicated in Table I, the predominant taxa in the honey samples were observed as follows; *Astragalus sp.* in two honey samples from Kahramanmaraş-Merkez (H01 and H08), and two samples from Göksun (H05 and H06); *Myrtus communis* and *Castanea sativa* in two honey samples from Andırın (H02 and H03); *Trifolium* sp. in a honey sample from Çağlayancerit (H04). The seconder group of pollens were the following taxa in three honey samples; *Ajuga* sp., *Cistus* sp. (H07), *Vicia cracca* (H07), and *Salix* sp. (H02). The minor group of taxa observed in honey samples were *Pyracantha coccinea, Cousinia* sp*, Centaurea* sp*., Ajuga* sp*., Anagallis arvensis, Salvia* sp*., Taraxacum officinalis, Triticum vulgare, Carduus* sp., *Astragalus* sp*., Geranium* sp., *Lathyrus* sp*., Vicia* sp*., Gossypium hirsitum, Cistus* sp*.,* and *Conium maculatum*.

In all honey samples, the most frequent pollens belonged to five taxa as follows; *Astragalus, Salvia* sp*., Castanea sativa, Olea* sp*.,* and *Quercus* sp. In a previous study of Andrada *et al*. (1998), species such as *Trifolium* sp. and *Astragalus* sp., which have a long flowering period and are used as the sources of pollen and nectar by bees, were also frequently observed in honey samples. In the previous studies in Turkey as well as in other countries, the predominance of the pollens belonging to *Trifolium* sp. were recorded in honey samples taken from İzmir (Mercan *et al*., 2007) and Yozgat (Kaya *et al*., 2005),

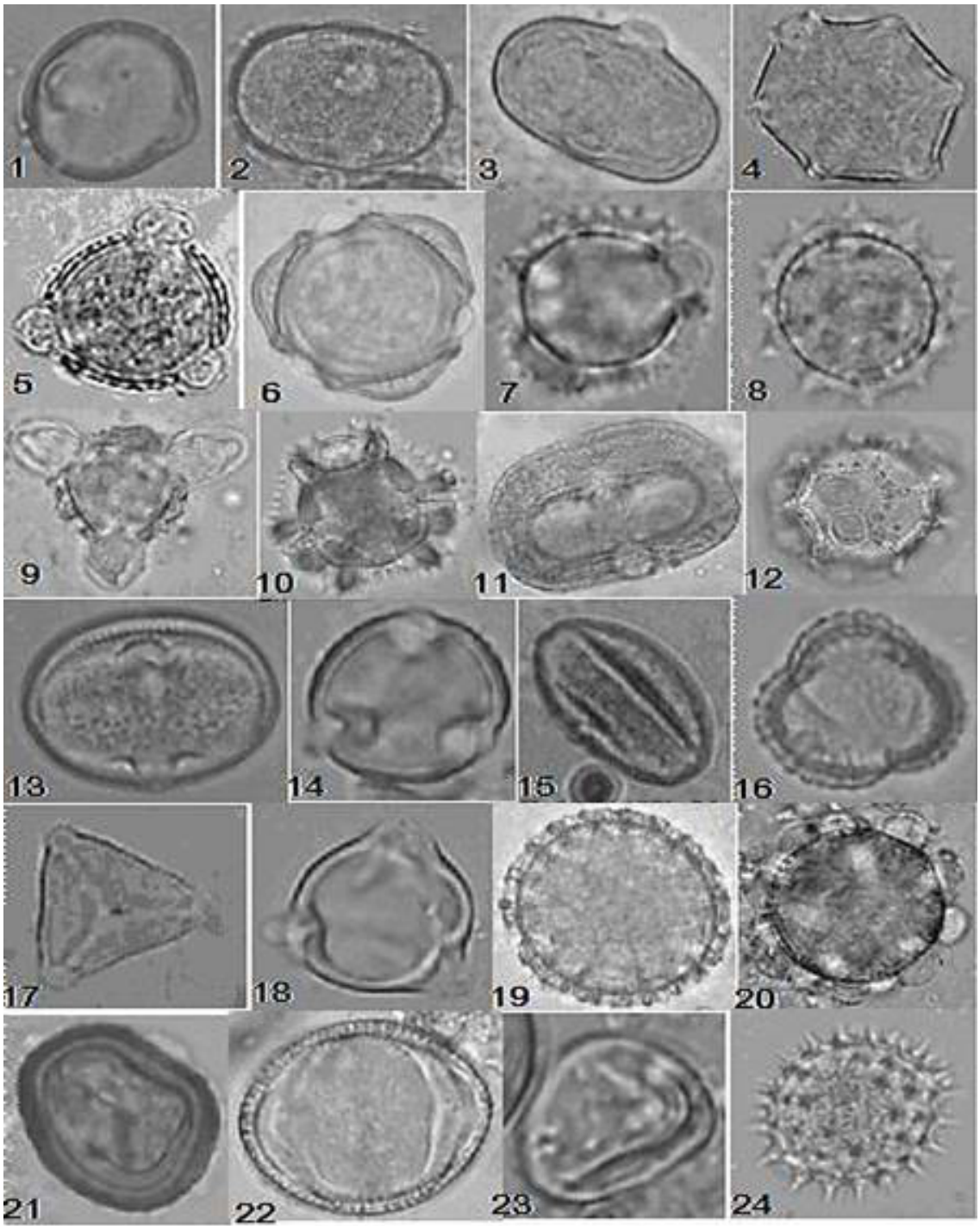

Fig. 1. Pollen grains in eight honey samples. **1**, Fabaceae *Astragalus* sp. 20 µm; **2**, *Trifolium* sp. 55 µm; **3**, *Vicia cracca* 26µm; **4**, Lamiaceae *Salvia* sp. 35 µm; **5**, *Ajuga* sp. 27 µm; **6**, Compositae *Cousinia* sp. 46 µm; **7**, *Scorzonera* sp. 13 µm; **8**, *Senecio* sp. 18 µm; **9**, *Carduus tenuifloris* 43µm-59 µm; **10**, *Taraxacum officinale* 24-27 µm; **11**, *Echinops* sp. 120 µm; **12**, *Chiccorium inthybus* 40µm; **13**, *Centaurea* sp. 46µm; **14**, Fagaceae *Castanea sativa* 12 µm; **15**, *Quercus cocciferae* 18 µm-25 µm; **16**, Cruciferae *Brassica rapa* 20µm; **17**, Myrtaceae *Myrtus communis* 17µm; **18**, Betulaceae *Betula* sp. 16 µm; **19**, Geraniaceae *Geranium* sp. 48 µm; **20**, Caryophyllaceae *Dianthus* sp. 42 µm; **21**, *Ankyropetalum* sp. 25µm; **22**, Ranunculaceae *Helleborus* sp. 33 µm; **23**, Boraginaceae *Onosma* sp. 18 µm; **24**, Malvaceae *Gossypium hirsitum* 48 µm.

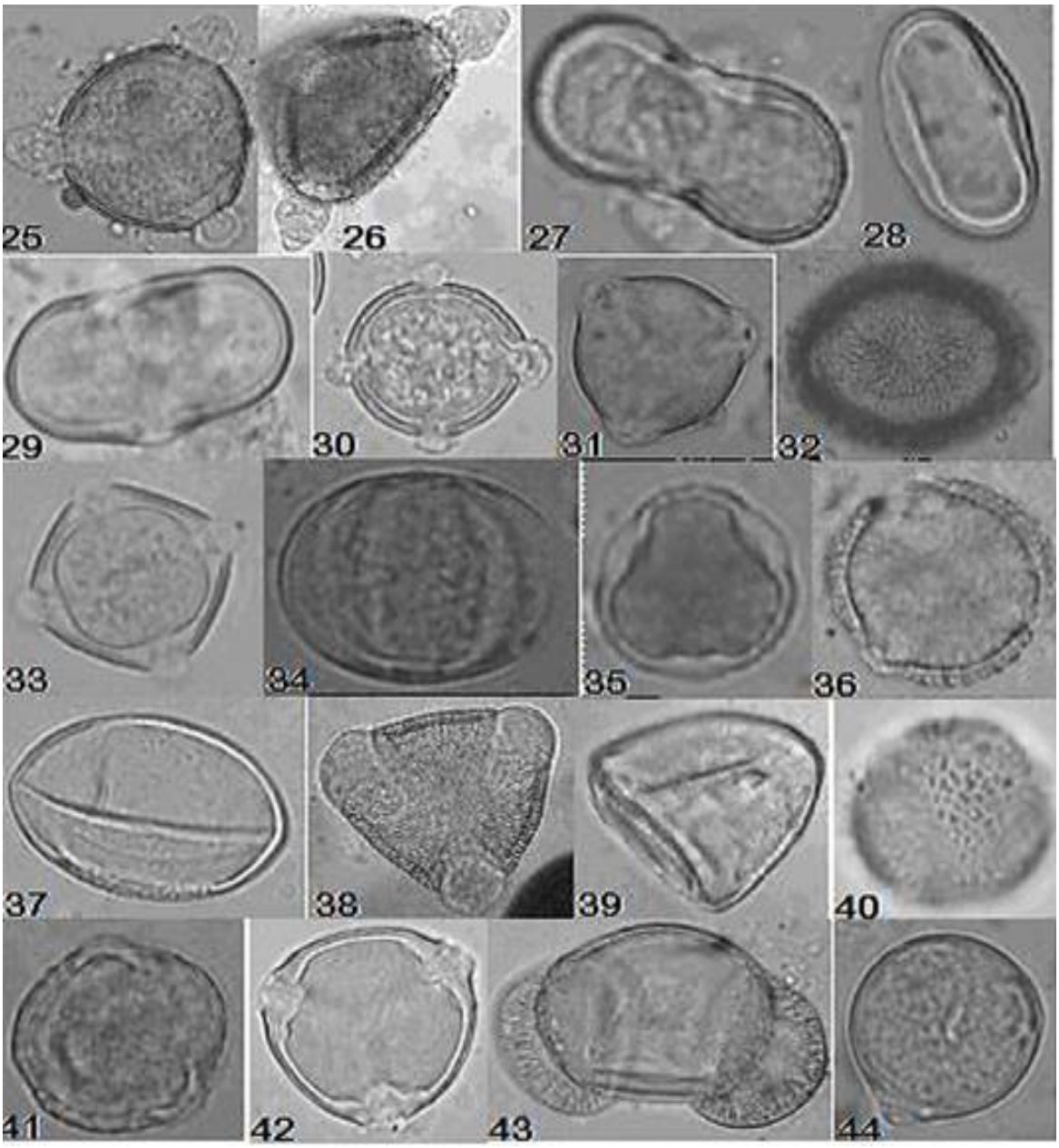

Fig. 2. Pollen grains in eight honey samples. **25**, Cistaceae *Cistus salviifolius* 44 µm; **26**, *Helianthemum* sp. 80 µm; **27**, Umbelliferae *Ferula* sp. 38 µm; **28**, *Conium maculatum* 23 µm; **29**, *Heracleum* sp. 36 µm; **30**, Vitaceae *Vitis* sp. 23 µm; **31**, Styracaceae *Styrax officinalis* 34 µm; **32**, Dipsacaceae *Scabiosa* sp. 50 µm; **33**, Eleagnaceae *Eleagnus angustifolia* 27 µm; **34**, Scrophulariacae *Veronica* sp. 10 µm; **35**, Scrophulariaceae *Linaria* sp. 14 µm; **36**, Juncaceae *Luzula* sp. 26 µm; **37**, Hyacinthaceae *Ornithogalum* sp. 48 µm; **38**, Convolvulaceae *Convolvulus arvensis* 52 µm; **39**, Boraginaceae *Onosma* sp. 40 µm; **40**, Oleaceae *Olea europeae* 27 µm; **41**, Primulaceae *Anagallis arvensis* 33 µm; **42**, Solanaceae *Solanum* sp. 50 µm; **43**, Pinaceae *Pinus* sp. 71 µm; **44**, Poaceae *Holcus lanatus*.

Ireland (Downey *et al*., 2005), Sicily-Italy (Longhitano *et al*., 1986). The pollens of *Castanea sativa* being the predominant pollen were also reported from honey samples obtained from Adapazarı, Rize, Bursa in Turkey (Sorkun *et al*., 1989; Doğan and Sorkun, 2001; Erdoğan *et al*., 2006). Like *Trifolium* sp. and *Castanea sativa*, the predominant pollens of *Myrtus communis* were determined in honey samples from Algeria and Argentina (Costa *et al*.,

1995; Ouchemoukh *et al*., 2007).

The predominant pollens of *Astragalus* sp. in honey samples collected from Kahramanmaras region differed from some studies reported from Elazığ and Antalya (Gür, 1993; Silici and Gökçeoglu, 2007) as the pollen of *Astragalus* sp. was classified as the seconder group of pollen.

The seconder group of pollens belonging to *Cistus* sp. in Kahramanmaraş honey samples, were also recorded as the dominant pollen in honey samples from İzmir, Çanakkale, and Balıkesir and seconder in honey samples from Konya and İstanbul (Gemici, 1991; Çakır and Tümen, 1992; Kaplan, 1993; Dalgıç *et al*., 1995; Demircan, 2005). The seconder type of pollens of *Salix* sp. observed in the present study, were also similar to those honey samples from Manisa and Antalya (Kaya *et al*., 2005; Silici and Gökçeoglu, 2007).

It has been reported that some kinds of honey contain accessory antibacterial and antifungal components and aromatic acids (Floris and Prota, 1989). The origin and composition of honey has been reported to be significant influences on the antimicrobial activities of honey. The floral composition of the region has significant influences on the honey composition (Mercan *et al*., 2007).

In the present study, antimicrobial activity was tested on ten microorganisms with agar well diffusion assay (Table II). Of the honey samples, H07 was found to be most effective inhibitor on *B. megaterium* and *S. aureus* with inhibition zones of 26 mm, and on *P. aeruginosa* and *B. subtilis* with inhibition zones of 25 mm. In addition, H07 and H08 samples showed antifungal activity on *C. albicans* with the imhibition zone of 23 mm (Table II). The best antimicrobial activity of honey samples were found on *E. coli* and *C. albicans* with 21 mm in H01 sample, *C. albicans* with 20 mm in H02 sample, *E. coli* and *C. albicans* with 22 mm in H03 sample, *S. aureus* with 24 mm in H04 sample, *P. aeruginosa* with 22 mm in H05 sample, *B. megaterium* with 22 mm in H06 sample, *B. megaterium* and *S. aureus* with 26 mm in H07 sample, *C. albicans* with 23 mm in H08 sample (Table II). In previous studies, some scientists reported that different floral honey samples showed good antimicrobial activities on different pathogenic bacteria and fungi (Khalil *et al*., 2001; Mercan *et al*., 2007; Küçük *et al*. 2007; Ifra and Sheikh, 2009). It appeared that the present results were agreement with the other previous findings.

CONCLUSION

The findings of this study indicated that the pollens of *Astragalus* sp. were detemined as the dominant pollen. To the best of our knowledge, this is the first report not only in Turkey but also in other countries. Honey samples from Göksun county showed quite rich pollen distribution with regard to *Astragalus* sp. Honey from Keven (*Astragalus* sp.) are produced and offered commercially for sale on the markets of Göksun county. The use of *Astragalus* sp. as a medicinal source has goes back to the traditional Chinese system and the West countries where they focused intensively to explore their pharmacological possibilities and clinical applications (Sinclair, 1998).

The floral source or geographical origin of honey samples is known to be an important contributor on its biological activities and pollen composition. In this study, honey sample H07 (Göksun İlçesi Mehmetbey Köyü) proved more effective as inhibitors against *B. megaterium* and *S. aureus*. Also, this honey sample showed antibacterial activity against *P. aeruginosa* and *B. subtilis* and showed antifungal activity to *C. albicans*. It could be suggested that honey sample coded with H07 (Göksun İlçesi Mehmetbey Köyü) contains antimicrobial compounds related to pollen spectrum.

Table II.- Antimicrobial results of the honey from Kahramanmaraş region.

Microorganisms	Agar well diffusion assay (10 mm in diameter)*							
	Inhibition zone (mm)							
	H01	H02	H03	H04	H05	H06	H07	H08
K. pneumoniae 13883	14	15	16	16	14	19	13	19
B. megaterium DSM 32	20	12	21	18	21	22	26	22
P. aeruginosa 9027	19	18	16	14	22	12	25	21
B. subtilis IMG 22	15	15	19	19	20	21	25	18
E. coli ATCC 8739	21	14	22	20	21	20	15	20
E. cloaca ATCC 13047	14	16	15	13	14	20	18	17
S. aureus 6538	19	18	20	24	17	19	26	21
C. albicans 30114	21	20	22	21	20	20	23	23
R. rubra 116	18	12	17	18	19	20	15	20

The results are the mean value of three independent assays.

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Statement of conflict of interest

The authors declare no conflict of interest.

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