MELLIFERA RESEARCH ARTICLE

Determination of Antimicrobial Activity, Palynological Characteristics and Chemical Composition of Some Honey Samples from Turkey

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A B S T R A C T

Honey has been used in traditional medicine since ancient times. Many parameters such as botanical, geological, climatic features constitute the characteristic features of honey. For this reason, it is important to reveal the chemical, palynological and antibacterial properties of honey in regional studies with honey. In this study, antimicrobial, palynological and chemical properties of some honey samples produced in Turkey were determined. The effects of honey samples of Rize (Anzer), Gümüşhane and Sivas (Zara) provinces on some pathogens were investigated via antimicrobial test. According to this test, all of the honey samples was effective *Staphylococcus aureus and Saccharomyces cerevisiae*, only honey from Anzer region was effective on *Escherichia coli*, and none of the honey samples showed activity on *Listeria monocytogenes* and *Candida albicans*. In the palynological analysis, 36 pollen taxa were determined. Chemical compositions of honey samples were determined by GC-MS analysis. GC-MS results showed all of the honey samples had antibacterial and antioxidant properties. The structure types of honey samples were determined by FTIR analysis and chemical bond types in FTIR correlated with the chemical compositions of GC-MS results.

Keywords: Palynological characteristics; chemical composition; honey; antimicrobial activity

Introduction

Honey is a natural sweet substance that is collected by the honey bees (*Apis mellifera*) from nectars of plants, living parts of plants secretions or excretions of insects on the

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living parts of plants and mixed with their own substances and dehydrated and matured [1]. Honey is a natural product that varied structure affected by like botanical and geographical source factors, intensity of nectar flow, climatological situations beekeepers management, packing process, storage time and the storage conditions [2]. Although it varies depending on these factors, honey is mainly composed of around 81% carbohydrates (31% glucose, 38% fructose and 12% from other carbohydrates like maltose and sucrose), 17% water and 1–2% of other enzymes/compounds. These 1-2% other substances are very significant contributors to the bioactive characteristics of the honey such as antimicrobial activity, antioxidant activity and determines variability of honey [3, 4]. Honey is widely known for its wound-healing properties and has been used since ancient times. One of the most widely known positive effects is that it has antibacterial properties [3, 5]. As a result of the spread of antibiotics, the clinical use of honey has almost been abandoned in modern medicine, and therefore the use of large amounts of antibiotics recently has resulted in the formation of common resistant bacteria. However, the increase in the spread of antibiotic-resistant bacteria, the antimicrobial activity of honey is increasingly valued and is still used as a clinical application in many cultures [5]. Factors of the comprehensive spectrum antimicrobial activity of honey are very high in nature. The most well-known causes of honey antimicrobial activity are high levels of osmolarity - ~ 80% (w / v) of solids, naturally low pH, hydrogen peroxide and the presence of phenolic acids, flavonoids and other substances from some flower sources. [4, 6, 7]. The strong antimicrobial effect of honey against bacteria that are resistant to antibiotics and its positive results in the treatment of wound infections that do not heal with long-term antibiotic treatment have attracted considerable attention [5, 8]. Honey acts in a broad spectrum against antibiotic-resistant and biofilm-forming bacteria in different environments [3, 9]. Abd-El Aal et al. stated that honey exhibited a more appeared inhibition effect (approximate 85%) on bacteria and especially on Gram negative bacteria compared to commonly used antimicrobial agents. Moreover, they stated that honey shows a synergistic effect in combination with other antimicrobial agents in both Gram negative and Gram

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positive bacteria. For example, it has been reported that 100% inhibition against Gram positive bacteria such as methicillin-resistant *S. aureus*, compared to the use of honey with other antimicrobial agents and antibiotic alone [7]. Al Somal et al. stated that Manuka Honey has a preventive effect on *Helicobacter pylori* growth [10]. In the study by some researchers it was found that honey is very effective against various clinical bacterial isolates and increases the effect of existing antibiotics as a result of synergistic effect when applied with antibiotic discs [7, 11]. Honey has also been reported by Molan, to have antifungal activity and so can have possible as a local antifungal agent [12]. In Turkey, Kahramanmaraş honey samples proved the most effective inhibitors against *Bacillus megaterium* and *S. aureus*, also honey samples showed significant antifungal effects on *C. albicans* [13]. The honey samples from Western Turkey were more effective antimicrobial *against Baccilus megaterium*, *Baccilus subtilis*, *C. albicans* [14].

Pollen analysis in honey have a great importance in controlling the quality of honey. Honey is a natural product that contains the pollen grains of the plant species, nectar and secretions collected by honey bees, and honeydew elements such as algae and fungal spores. Therefore, pollen analysis is the most effective method used to determine the botanical source of honey [15].

The aim of the present study was to determine and compare the antimicrobial activity, chemical composition (by GC/MS), structure types/vibrations (by FTIR) and palynological properties of honey samples from different localities in Turkey.

Materials and Methods

Materials

Honey samples was procured through a beekeeper were from Anzer-Rize (H1), Gümüşhane (H2), Zara-Sivas (H3) Regions, Turkey. Ethyl alcohol (~96% v/v), which was used as a solvent in extraction and was produced from agricultural products was purchased from Alkomed Kimya Ltd. Sti., Turkey. Sarthorius BP210S branded analytical balance was used for the weighing of honey samples in

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the preparation of the extraction. The extraction was mixed with the help of magnetic fish on the Heidolph MR Hei-Standard branded magnetic stirrer.

Preparation of Honey Extractions

Honey samples was planned to be taken in solution at the rate of 10% (m / m). In other words, 5 g honey sample was added to 50 mL ethanol-water solution (50% v/v) sample and was extracted by mixing during 4 hours with magnetic stirrer at room temperature. In this extraction method, most of the ingredients in honey samples were provided to pass into alcohol. The mixture was filtered on Whatman cellulose filter paper (no:2, diameter: 125 mm) and then the remaining mass on the filter was discarded.

Palynological analysis method

Sorkun method was used for qualitative pollen analysis of honey samples [17]. 20 mL pure water was added on 10 g honey, after the obtained solution was homogenized and centrifuged at 3500 rpm for 45 minutes. Supernatant solution is spilled and the remaining solution was transferred onto the slide by 1-2 mm^3 glycerin-gelatin with basic fucsin. Microscope slides were examined under Labomed light microscope. For determining the importance degree of the botanical origin of honey samples, we used the classification given by the count of 200 pollen grains per sample [17]. Determined pollen types were classified in four classes of frequency [18, 19], (i) predominant pollen types (>45% of the total pollen content); (ii) secondary pollen types (16-45%); (iii) important minor pollen types (3-15%); and (iv) minor pollen types. Samples with pollen grain frequencies of a given plant, above 45%, were called as monofloral.

Characterization methods

For GC-MS measurement, Agilent brand 7890A model GC, 5975C model MS and FID detector were used synchronically. The column brand was BPx90 and the column dimensions were 100 mm x 0.25 mm x 0.25 μm. The flow rate was adjusted to 1 mL/min using column carrier with helium gas. Under chromatographic conditions, the samples increased 5 °C per minute and reached from 120 °C to 254 °C and were kept at this temperature for about 16 minutes. MS results were determined by comparing with WHILEY and NIST

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libraries in the memory of the device. A part of the filtered clear solution was used for GC-MS measurement and the following procedures were carried out at this stage, respectively:

1. 100 µL of sample was mixed with 10 ml of hexane and vortexed.
2. 100 µL of 2 N KOH was added to the resulting solution and vortexed.
3. The solution was centrifuged for 10 minutes at 4500 rpm.
4. It was placed on the GC-MS device using the clear part in the centrifuged tubes and made ready for measurement.

Perkin Elmer Spectrum 100 branded device was preferred for FT-IR measurement and provided to detect the organic bond structures and properties of different chemical molecules in the sample.

Antimicrobial activity

All chemicals and reagents were procured from Merck (Darmstadt, Germany) and prepared in HPLC grade. The water extract of H1, H2 and H3 was tested for their antimicrobial activity against *E. coli* ATCC 25922, *S. cerevisiae* ATCC 76521, *S. aureus* ATCC 29213, *L. monocytogenes* NCTC 5348, and *C. albicans* ATCC 90028.

Antimicrobial activity tests were performed using the agar disc diffusion method described in [16] with some modifications. The water extract of honey samples was adjusted to different concentrations as 125, 250, and 500 mg/mL via sterile pure water. The tested microorganism suspensions (10^6 CFU) were seeded onto Mueller-Hinton agar plates. 40 µL of different concentrations (125, 250 and 500 mg/mL) of honey extract were impregnated to the sterile paper discs (6 mm diameter, 3mm thickness). After that, the discs were put onto the surface of inoculated agar plates. The ampicillin sulbactam 10/10 µg (SAM) disc was also used as positive control. Petri plates were incubated at 37 °C for 24 hours after that, at 4 °C for 1 hour. The determination of antimicrobial activity was determined with measuring the inhibition zone.

Statistical analysis

All measurements were repeated three times, and values are the average of triplicate and expressed as mean ± SD.

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Results and Discussion

Characterization results

GC-MS graphs of H1, H2 and H3 samples were given in Figure 1, the compounds and their properties of H1, H2, H3 samples were given in Table 1. According to the GC-MS results, the groups of alkanes (docosane, tetratriacontane), cycloalkenes (1,5,9-cyclododecatriene, 1,5,9-cyclododecatriene,(E,Z,Z), 5,6-divinyl-1-cyclooctene), ketones (2-t-butyl-6-[2-hydroxy-2-(4-methoxyphenyl)ethyl]-[1,3]dioxin-4-one, N-allylmaleimide, trans-3-ethylidene-1-vinyl-2-pyrrolidone), aromatic acid (ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate), amide (oleamide), aldehydes (9-octadecenal), phenolic compounds (3-hydroxycarbofuranphenol, cis-3-ethyl-2-(4-methoxyphenyl)-4-methyleneoxolane), esters of saturated acid (4-[[4-(4-bromo-phenyl)-thiazol-2-yl]-methyl-amino]-butyric acid), heterocyclic compound (8-azahypoxanthine) were seen over of area as 1%. Especially, oleamide (bioactive fatty acid ester) were found in all samples as the common compound, and this compound had antibacterial and antioxidant properties [20,21]. Different phenolic compounds were seen in the samples and it was known that these compounds and aromatic acids had antibacterial and antioxidant properties in the view of literature data [22, 23, 24]. Also ketone groups and fatty acids methyl esters were obtained antibacterial activity and useful for antibiotics [25, 26]. There is also a concordance with the literature values and the differences in compound names were found in honey species, but they were similar as compound groups. Generally, the compounds of H1, H2 and H3 samples had antibacterial activity and this result was compatible with antibacterial activity tests.

FTIR plots which had range as 4000-650 cm^{-1} and resolution as 4 cm^{-1} of H1, H2 and H3 samples are given in Figure 2. Overall, looking at the FTIR results, three samples were found to show similar peak values. The peaks at 3700-2970 cm^{-1} have the feature of O-H stretching vibration [27]. The features of CH3 asymmetric stretching vibration, C-O stretching and C-N stretching (amide group), aliphatic CH2, CH3 bending, O-H bending in –COOH or CH3 bending were showed the peak values at 2948, 1640, 1425, 1380 cm-1,

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respectively [1]. The peak at 1240 cm-1 was related to C=O, C-O stretching in RC(=O)-OH and P+O asymetric stretching in RO-P(-O2)-OR [27, 28].

Table 1. Chemical composition of honey samples via GC-MS analysis

Region	RT	Area %	Library/ID	Ref#	CAS#	Qual	Chemical Group
H1	25.213	0.88	Docosane	69204	000629-97-0	83	Alkane
	30.912	1.2	Tetratriacontane	75175	014167-59-0	83	Alkane
	51.752	0.17	Benzyl Methyl Ether	64536	000000-00-0	89	Ether
	57.096	0.19	1-Octadecene	26418	000112-88-9	90	Alkene
	62.898	0.37	3-Hydroxycarbofuranphenol	287816	017781-15-6	83	Phenolic compounds
	75.486	5.45	2-t-Butyl-6-[2-hydroxy-2-(4-methoxyphenyl)ethyl]-[1,3]dioxin-4-one	288224	999288-22-7	87	Ketone
	76.551	11.23	Oleamide	83151	000301-02-0	91	Amide group
H2	16.373	1.87	6-aza-5,7,12,14-tetrathiapentacene	507397	000000-00-0	86	Polycyclic aromatic hydrocarbon
	50.201	1.1	Ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate	507399	066564-08-7	94	Aromatic acid-Phenolic compound
	55.122	1.06		507399	066564-08-7	90	
	58.435	0.57	1,5,9-cyclododecatriene	52147	002765-29-9	90	cycloalkene
	63.196	0.23	Cyclooctacosane	70123	000297-24-5	90	cycloalkane
	75.675	3.23	1,5,9-cyclododecatriene,(E,Z,Z)-	52526	002765-29-9	86	cycloalkene
	76.562	7.4	Oleamide	83149	000301-02-0	92	Amide group

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	77.678	0.79	5,6-divinyl-1-cyclooctene	52519	046045-35-6	83	cycloalkene
	78.067	0.42	1,2,4-Triazolo[4,3-B][1,2,4]-triazin-7(8H)-one	286124	000874-40-8	83	Carbocyclic compound
H3	32.411	4.23	Ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate	507399	066564-08-7	83	Aromatic acid-Phenolic compound
	44.628	2.99	4-[[4-(4-bromo-phenyl)-thiazol-2-yl]-methyl-amino]-butyric acid	472704	999472-71-6	91	Esters of saturated acids
	50.195	2.59	Ethyl-(2E)-3-[2-(diethoxyphosphoryl)-4-(dimethylamino) phenyl]-2-propenoate	507399	066564-08-7	95	Aromatic acid-Phenolic Compound
	59.562	2.65		507399	066564-08-7	90	
	67.258	1.43	8-Azahypoxanthine	287663	002683-90-1	80	Heterocyclic compound
	73.672	1.57	9-octadecenal	11106	005090-41-5	83	Aldehyde
	74.17	0.75	cis-3-ethyl-2-(4-methoxyphenyl)-4-methyleneoxolane	286688	000000-00-0	83	Phenolic compounds
	75.629	1.08	N-allylmaleimide	52476	002973-17-3	81	Ketone
	75.858	0.76	5,6-divinyl-1-cyclooctene	52519	046045-35-6	92	cycloalkene
	76.551	3.05	Oleamide	83152	000301-02-0	92	Amide group
	76.997	1.53	trans-3-ethylidene-1-vinyl-2-pyrrolidone	52475	999052-47-7	80	Ketone

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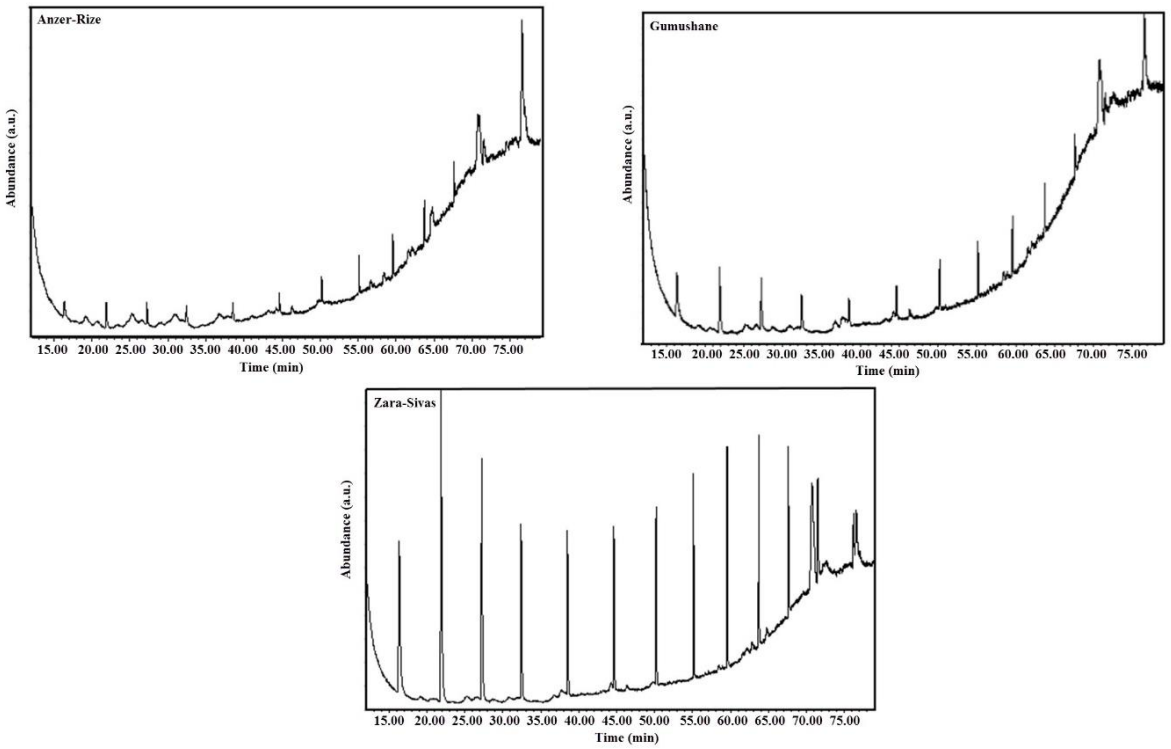

Figure 1. GC-MS graph of honey samples

The peak values of 1030 and 1060 cm-1 showed the property of C-O stretch in -C-O-C-, -C-OH compounds, C-S stretching aryl-S-aryl compounds and C=S stretching [1,2]. These values were compatible with GC-MS results which had the compounds such as amide groups, ketones, ethers, fatty acids, phosphoric compounds, sulfonic compounds etc. Any degradation or different bond types were not observed.

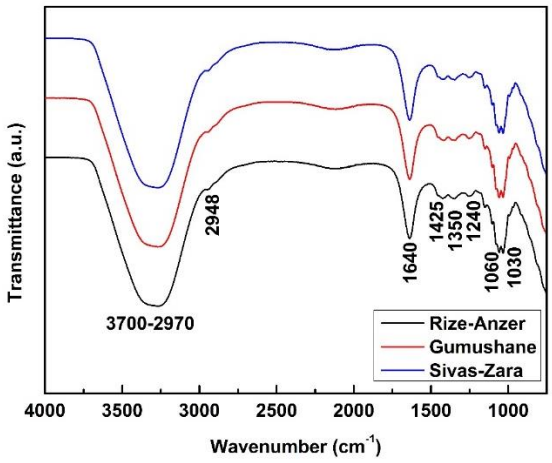

Figure 2. FTIR graph of honey samples

Antimicrobial analysis

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Table 2 indicates the results of the antimicrobial activity of honey samples present at different concentrations (125, 250, 500 mg/mL) against *E. coli, S. cerevisiae, S. aureus, L. monocytogenes,* and *C. albicans* by disc diffusion assay. Also, SAM (ampicillin/sulbactam 20 µg) used as a control and it showed different antimicrobial activities on these microorganisms.

In this study, the antimicrobial activity values of H1, H2 and H3 for 500 mg/mL extract concentration on *S. aureus* were found as 10.0-8.5-8.0 mm the zones of inhibition, respectively. The antimicrobial activity values of H1 and H2 for 250 mg/mL extract concentration on *S. aureus* were found as 6.0-6.5 mm the zones of inhibition, respectively. The antimicrobial activity values of H3 for 250 mg/mL extract concentration on *S. aureus* were not in detectable levels the zones of inhibition. The antimicrobial activity values of H1, H2, and H3 for 125 mg/mL extract concentration on *S. aureus* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2, and H3 were found as 11.5-13.0-12.0 mm zones of inhibition on *S. aureus*, respectively (Table 2.). The antimicrobial activity values determined for *S. aureus* in this study were lower than the antimicrobial activity values reported for *S. aureus* by Aksoy and Dığrak, Alan et al, Silici et al, Mercan et al, Ekici et al, Sheikh et al, Merces et al [29-35]. The antimicrobial activity values determined for *S. aureus* in this study were higher than the antimicrobial activity values reported for *S. aureus* by Silici et al [31]. The antimicrobial activity values determined for *S. aureus* in this study were similar to the antimicrobial activity values reported for *S. aureus* by Sagdic et al [36]. The antimicrobial activity values of H1, H2, and H3 for 125-250 and 500 mg/mL extract concentrations on *L. monocytogenes* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2 and H3 honey samples were found as 34.5-34.0-37.0 mm zones of inhibition on *L. monocytogenes*, respectively (Table 2.). The antimicrobial activity values determined for *L. monocytogenes* in this study were lower than the antimicrobial activity values reported for *L. monocytogenes* by Silici et al, Ekici, Sagdic et al [31, 33, 36].

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The antimicrobial activity values of H1 for 500 mg/mL extract concentration on *E. coli* were found as 6.0 mm the zones of inhibition. The antimicrobial activity values of H2 and H3 for 500 mg/mL extract concentration on *E. coli* were not in detectable levels the zones of inhibition. The antimicrobial activity values of H1, H2, and H3 for 125-250 mg/mL extract concentration on *E. coli* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM was found as 11.0-9.5-8.0 mm zones of inhibition on *E. coli* (Table 2.). The antimicrobial activity values determined for *E. coli* in this study were lower than the antimicrobial activity values reported for *E. coli* by Aksoy and Dığrak, Alan et al, Silici et al, Mercan et al, Ekici,et al, Sheikh et al, Merces et al [29- 35]. The antimicrobial activity values determined for *E. coli* in this study were similar to the antimicrobial activity values reported for *E. coli* by Sagdic et al [36].

The antimicrobial activity values of H1, H2 and H3 honey samples for 500 mg/mL extract concentration on *Saccharomyces cerevisae* were found as 9.0-7.5-6.0 mm the zones of inhibition, respectively. The antimicrobial activity values of H1, H2 and H3 honey samples for 125-250 mg/mL extract concentrations on *Saccharomyces cerevisae* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2 and H3 honey samples were found as 10.5-11.5-7.5 mm zones of inhibition on *Saccharomyces cerevisae*, respectively (Table 2.). The antimicrobial activity values determined for *Saccharomyces cerevisae* in this study were higher than the antimicrobial activity values reported for *Saccharomyces cerevisae* by Alan et al, Silici et al , Ekici et al, Sagdic et al [29, 31, 33, 36].

The antimicrobial activity values of H1, H2 and H3 honey samples for 125-250-500 mg/mL extract concentrations on *Candida albicans* were not in detectable levels the zones of inhibition. The antimicrobial activity of SAM for H1, H2 and H3 honey samples were found as 31.5-27.5-28.5 mm zones of inhibition on *Candida albicans*, respectively (Table 2.). The antimicrobial activity values determined for *Candida albicans* in this study were lower than the antimicrobial activity values reported for *Candida albicans* by Aksoy and Dığrak, Mercan et al [26, 29]. The antimicrobial

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activity values determined for *Candida albicans* in this study were similar to the antimicrobial activity values reported for *Candida albicans* by Alan et al, Silici et al, Ekici et al, Sagdic et al, Sheikh et al, Merces et al [30- 35].

Table 2. Antimicrobial activity of H1, H2, and H3 samples

Tested microorganism	H1				H2				H3			
	Zone of inhibition (mm)[a]											
	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM	500[b]	250[b]	125[b]	SAM
Gram (+)												
S. aureus	10.0 ± 1.4	6.0 ± 0.0	-	11.5 ± 0.7	8.5 ± 0.7	6.5 ± 0.7	-	13.0 ± 1.4	8.0 ± 0.0	-	-	12.0 ± 1.4
L. monocytogenes	-	-	-	34.5 ± 0.7	-	-	-	34.0 ± 1.4	-	-	-	37.0 ± 1.4
Gram (-)												
E. coli	6.0 ± 0.0	-	-	11.0 ± 1.4	-	-	-	9.5 ± 0.7	-	-	-	8.0 ± 1.4
Yeast												
Saccharomyces cerevisae	9.0 ± 0.0	-	-	10.5 ± 0.7	7.5 ± 0.7	-	-	11.5 ± 0.7	6.0 ± 0.0	-	-	7.5 ± 0.7
Fungi												
C. albicans	-	-	-	31.5 ± 2.1	-	-	-	27.5 ± 0.7	-	-	-	28.5 ± 2.1

–: No activity.
a Values are the average of triplicate and expressed as mean ± SD.
b Honey extract concentration (mg/mL); SAM, Ampicillin/Sulbactam (20 µg/disc).

Melissopalynological analysis

Melissopalynology was used to determine the botanical origin of honey, which is important for its traceability [37]. The pollen analysis results are presented in Table 3, Table 4 and Figure 3. In this study, a total of 36 pollen types belonging to 18 families were identified. The pollen grains of the honey samples belonging to the family Apiaceae, Asteraceae, Betulaceae, Boraginaceae, Brassicaceae, Campanulcaeeae, Caprifoliaceae, Caryoplyllaceae, Cistaceae, Ericaceae, Fabaceae, Lamiaceae, Moraceae, Onagraceae, Plantaginaceae, Ranunculaceae, Rosaceae, Salicaceae were identified at different rates. As a result of the pollen analysis, 18 plants were identified at the family level, 18 of them are genus and 1of them is species. The families with the highest numbers of pollen types situated in the honey samples were Asteraceae, Cistaceae, Fabaceae,

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Rosaceae and Salicaceae families. All of the samples were classified as multifloral because they contains the pollen of many plant taxa.

Table 3. Pollen frequency percentages and taxa recovered from the honey samples

Pollen Taxa	Pollen Frequency (%) of Honey Samples From Turkey		
	H1 %	***H2 %***	***H3%***
Apiaceae	0.8		1.9
Type I			0.6
Type II			0.6
Asteraceae		0.7	19.6
Centaurea			17.7
Betulaceae		0.7	
Boraginaceae	3.1	0.7	
Myosotis	9.3		
Shymphytum			0.6
Brassicaeae	3.9	4.48	1.2
Campanulaceae			
Campanula	0.8		0.6
Caprifoliaceae	2.3		
Scabiosa			0.6
Caryophyllaceae		0.7	
Cistaceae			0.6
Helianthemum	18.6	17.9	
Ericaceae			0.6
Rhododendron	1.6		
Fabaceae	11.7	5.9	13.9
Acacia			0.6
Astragalus	1.6	5.2	0.6
Lathyrus	0.8		
Lotus	3.9		
Onobrychis	24.3	6.7	20.9
Trifolium	1.6	8.9	
Lamiaceae	1		0.6
Type I		2.2	
Type II		4.48	
Lamium	0.8	2.2	5.1
Teucrium			0.6
Moraceae			
Morus	0.8		
Onagraceaea	0.8		5.7
Plantaginaceae			
Plantago lanceolata	0.8		
Ranunculaceae			
Ranunculus	5.4		
Rosaceae	1.6	13.4	3.8
Salicaceae			
Salix	4.7	26.12	3.8

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The secondary group of pollens were the following taxa in three honey samples; *Helianthemum* sp., *Salix* sp. for H2 sample, *Onobrychis* sp *Helianthemum* sp. for H1 sample and *Centaurea* sp., *Onobrychis* sp. for H3 sample. Şık et al. (2017) were examined pollen content of honey samples from Ardahan, (Northeast Anatolia). They found 23 different taxa from 13 families. *Astragalus* spp., Apiacaee, Brassicaceae, Fabaceae pollen grains were the most abundant in honey samples. All of the samples were multifloral [38].

Table 4. Polen taxa in honey in percentages

Samples	Honey Type	Predominant pollen (> 45%)	Secondary pollen (16-45%)	İmportant minor pollen (3-15%)	Minor pollen (<3%)
H2	Multifloral	-	*Helianthemum*, *Salix*	Brassicaceae, Fabaceae, *Astragalus*, *Onobrychis*, *Trifolium*, Lamiaceae, Rosaceae	Asteraceae, Betulaceae, Boraginaceae, Caryophyllaceae, *Lamium*
H1	Multifloral	-	*Onobrychis*, *Helianthemum*	Boraginaceae, Brassicaceae, *Myosotis*, Fabaceae, *Ranunculus*, *Lotus*,*Salix*	Apiaceae, *Campanula*, Caprifoliceae, *Rhododendron*, *Astragalus*, *Lathyrus*, *Trifolium*, Lamiaceae, *Lamium*, *Morus*, Onagraceae, *Plantago*, Rosaceae
H3	Multifloral	-	Asteraceae, *Centaurea*, *Onobrychis*	Fabaceae, *Lamium*, Onagraceae, Rosaceae, *Salix*	Apiaceae, Brassicaceae, Campanulaceae, *Scabiosa*, Cistaceae, Ericaceae, *Acacia*, *Astragalus*, Lamiaceae, *Teucrium*

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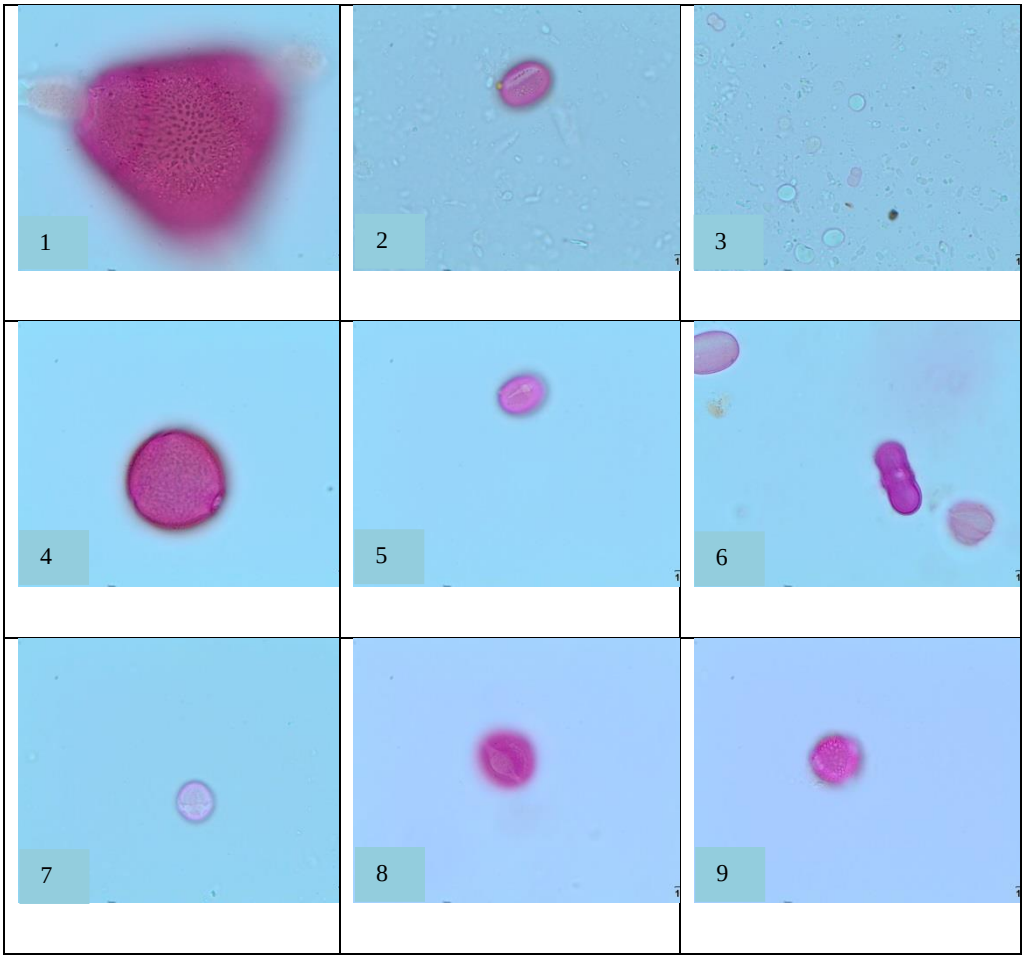

Figure 3. Light photomicrographs of characteristic pollen types from honey samples (*1.Scabiosa (x100)* 2. *Onobrychis (x100)* 3. *Myosotis (x100)* 4. Cistaceae *(x100)* 5. *Astragalus (x100)* 6. Apiaceae *(x100)*7. *Shymphytum (x100)* 8. *Trifolium (x100)* 9. *Salix (x100))*

Pollen spectrum profile determined in the study indicated predominance of the families Fabaceae, Asteraceae presenting a great diversity of botanical taxons. Tümerdem et al. (2016) also observed the predominance of the Fabaceae in honey samples from Ankara province in Turkey [39].

In another study, the melissopalynologic 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 [40]. Özler et al. (2018) observed in the analyzed honey samples from South

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Anatolia that the findings of this study demonstrated that pollen grains of the family of Fabaceae, Rosaceae, the genera of Eucalyptus and Centaurea were in nectar and pollen sources for honey production [41].

According to the our pollen analysis results of H2, *Helianthemum* sp., *Salix* sp., *Astragalus* sp., *Onobrychis* sp., *Trifolium* sp. are the more preferred plant taxa by honeybees likewise in another study 12 honey samples collected from Gümüşhane region, *Astragalus* sp., *Trifolium* sp. taxa were determined dominant by melissopalynologic analysis [42]. Sorkun and Doğan (1995), were made pollen analysis of Anzer honey sample, they were determined that 35 different plant pollen types. Fabaceae, Asteraceae, Boraginaceae are some families that they were found. In our study, Fabaceae appears as the most common pollen family in 32,2% of the H1 honey sample and also Fabaceae family members were traced as being the dominant pollen group according to Sorkun and Doğan (1995). They were found 9% *Myosotis* pollens in 11 honey samples similar to our study [43]. In contrast to our study, *Papaver* sp. pollens were found to be dominant in H3 sample [44]. Fabaceae and Asteraceae were in a large quantity pollen grains in our honey sample from H3.

Conclusion

GC-MS results showed that alkanes, cycloalkenes, ketones, aromatic acid, amide, aldehydes, phenolic compounds, saturated acid esters, heterocyclic compound have over of area as 1%. By and large, the compounds of H1, H2 and H3 samples had antibacterial activity and this result was compatible with antibacterial activity tests. Oleamid (bioactive fatty acid ester) having antibacterial and antioxidant properties appeared as a common component in all samples. In addition, FTIR values were compatible with GC-MS results which had the compounds such as amide groups, ketones, ethers, fatty acids, phosphoric compounds, sulfonic compounds.

H1, H2 and H3, at 125 mg/mL concentration, showed no antimicrobial activity against *E. coli, S. cerevisiae, S. aureus, L. monocytogenes*, and *C. albicans*.

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Among honey samples, H1 and H2 honey samples at 250 mg/mL concentration showed antimicrobial activity only against *S. aureus*. H3 sample at 250 mg/mL concentration did not exhibit any antimicrobial activity on *E. coli*, *S. cerevisiae*, *S. aureus*, *L. monocytogenes*, and *C. albicans*. H1, H2 and H3 honey samples, at 500 mg/mL concentration, exhibited inhibitions on *S. aureus* and *S. cerevisiae*, while they did not show any antimicrobial activity against *L. monocytogenes* and *C. albicans*. Also, H1 honey, at 500 mg/mL concentration, exhibited low antimicrobial activity on *E. coli*, while H2 and H3 honey samples did not show antimicrobial activity on *E. coli*.

Botanical, geographical origin and pollen composition of honey samples are an important on its biological activities.

According to pollen analyses a total of 36 pollen types belonging to 18 families were identified. Additionally, 18 genera and 1 species from 18 plant families were identified. Asteraceae, Cistaceae, Fabaceae, Rosaceae and Salicaceae families were determined to have the most pollen type. All honey samples are classified as multifloral, because they have more than one type of pollen.

Türkiye'deki Bazı Bal Örneklerinin Antimikrobiyal Aktivitesinin, Palinolojik Özelliklerinin ve Kimyasal Bileşiminin Belirlenmesi

Öz: Bal eski zamanlardan beri geleneksel tıpta kullanılmaktadır. Botanik, jeolojik, iklimsel özellikler gibi birçok parametre, balın karakteristik özelliklerini belirlemektedir. Bu nedenle balla yapılan bölgesel çalışmalarda balın kimyasal, palinolojik ve antimikrobiyal özelliklerini ortaya koymak önemlidir. Bu çalışmada, Türkiye'de üretilen bazı bal örneklerinin antimikrobiyal aktivitesi, palinolojik ve kimyasal özellikleri belirlenmiştir. Antimikrobiyal test yoluyla, Rize (Anzer), Gümüşhane ve Sivas (Zara) illerindeki balların bazı patojenler üzerindeki etkileri araştırılmıştır. Bu teste göre, bal örneklerinin tümü *S. aureus* ve *S. cerevisae* üzerinde etkili olmuş, sadece Anzer bölgesinden gelen bal *E. coli* üzerinde etkili olmuş ve bal örneklerinin hiçbiri *L. monocytogenes* ve *C. albicans* üzerinde aktivite göstermemiştir. Palinolojik analizlerde 36 polen taksonu belirlenmiştir.

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Bal örneklerinin kimyasal bileşimleri GC-MS analizi ile belirlenmiştir. GC-MS sonuçları, tüm bal örneklerinin antibakteriyel ve antioksidan özelliklere sahip olduğunu göstermiştir. Bal örneklerinin kimyasal yapı türleri FTIR analizi ile belirlenmiş olup, FTIR'daki kimyasal bağ türleri GC-MS sonuçlarının kimyasal bileşimleri ile bağlantılı olmuştur.

Anahtar Kelimeler: Palinolojik özellikler; kimyasal bileşim; bal; antimikrobiyal aktivite

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