Investigation of pollen analysis and antimicrobial effects of honey from Posof (Ardahan) district

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Abstract
This study aimed to determine the pollen composition and antimicrobial activity of honey samples from Posof District, Ardahan Province, located in the Eastern Anatolia region of Türkiye. A total of 29 honey samples were collected from various villages in Posof. As a result of melissopalynological analysis in these honey samples, 19 different plant families were identified. A total of 18 honey samples were identified as monofloral, while the remaining 11 were classified as multifloral. One sample was dominated by pollen from the Cistaceae family, while the dominant pollen in other monofloral honey samples belonged to the Fabaceae family. The most abundant pollen types in the honey samples were Fabaceae (40.5%), Rosaceae (11.7%), Lamiaceae (9.3%), Boraginaceae (7.5%), and Cistaceae (7.2%). Antimicrobial activity of the honey samples was tested at different concentrations (0.50%, 0.25%, 0.125%) against eight bacterial strains, including Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (ATCC 25925), and *Enterococcus faecalis* (ATCC 29219), and Gram-negative bacteria *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (O157:H7 RSSK 09007), *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 35218), and *Pseudomonas aeruginosa* (ATCC 27853) using the disc diffusion method.

Keywords: *Antibacterial activity, honey, pollen analysis, Posof*

INTRODUCTION

Türkiye, with approximately 12,165 plant species, is one of the richest countries in the world in terms of flora diversity, owing to its varied ecosystems, geographical location, and climate diversity. This richness is a result of the country being at the intersection of three floristic regions, along with its diverse climatic conditions (Dülgeroğlu and Aksoy, 2018; Savci et al., 2018; Şenkul and Kaya, 2017). Türkiye's rich vegetation and geographical diversity have positioned it as a globally significant player in honey production. This richness enhances the pollen diversity in Turkish honey, thereby increasing its nutritional value (Apan et al., 2021).

Throughout history, honey has been used both as a food product and as a natural remedy. Its rich content of nutrients and bioactive components reinforces the positive health effects of honey (Alvarez-Suarez et al., 2009; Pranskuniene et al., 2022; Zubair and Aziz, 2015). Türkiye, with its vast flora diversity, holds a significant position in global honey production. The country's rich variety of plant species creates distinct differences in the pollen profiles of regionally produced honeys (Külekçi and Bulut, 2016; Tel et al., 2019). In this context, pollen analysis is a crucial tool for determining the origin of honey and ensuring quality control. Pollen analyses reveal not only the botanical and geographical origin of honey but also its purity and quality. The high pollen diversity in Turkish honey contributes to its unique aromatic and nutritional properties (Keskin et al., 2021; Mısır et al., 2020).

Honeys obtained from various regions of Türkiye exhibit diverse pollen profiles, and this diversity can also influence the antimicrobial effects of the honey (Keskin et al., 2020; Özkök and Bayram, 2021; Şenkul and Kaya, 2017). The pollen content of honey is a significant factor that affects its antimicrobial properties (Kösoğlu et al., 2019; Onbaşlı, 2019). Pollen derived from different plant species diversify the antimicrobial characteristics of honey, thereby enhancing its positive health effects (Kunat-Budzyńska et al., 2023). Particularly, honeys produced in regions with rich plant diversity, such as Türkiye, stand out not only for their nutritional value but also for their pollen diversity and antimicrobial effects (Mercan et al., 2007). The antimicrobial effects of honey are closely related to pollen diversity, as pollen from various plant species contain different bioactive components that influence the biological activity of the honey (Acar, 2021; Özkök and Bayram, 2021).

The Posof region, a district of Ardahan Province, is notable for its rich vegetation, diverse climatic conditions, and high altitude. In the floristic study of Posof, Damal, and Hanak districts of Ardahan Province, a total of 1,225 taxa belonging to 411 genera and 95 families were identified, with the highest numbers of taxa found in the families Asteraceae (190), Fabaceae (78), Lamiaceae (70) Rosaceae (70), and Caryophyllaceae (65) (Esen, 2010). The Caucasian honey bee subspecies (*Apis mellifera caucasica*) is commonly found in northeastern Türkiye, particularly in the Ardahan region and its surroundings, with Posof recognized as a gene center for this subspecies. Research conducted in the Ardahan region on the genetic diversity and adaptability of *Apis mellifera caucasica* reveals the contribution and importance of these bees to local ecosystems (Kambur and Kekeçoğlu, 2020). These characteristics make Posof honey comparable to other regional honeys in terms of both pollen diversity and antimicrobial activities. Particularly, pollen analyses of Posof honeys reveal the presence of pollen from endemic and rare plant species, highlighting their significant role in geographical indication and quality assessments (Şık et al., 2017).

Previous studies on Posof honeys have focused on pollen analysis, but this study is the first to determine both pollen composition and antimicrobial activity at a local

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level (Sorkun et al., 2014; Şık et al., 2017). In this study, the pollen profiles of honeys produced in the Posof region were examined, and the obtained data were compared with honeys from other regions of Türkiye. Additionally, the antimicrobial effects of these honeys were evaluated.

MATERIALS AND METHODS

The 29 honey samples used in this study were collected from local beekeepers in 22 villages of the Posof District in Ardahan Province during August and September 2020. The list of villages from which the samples were collected, along with the altitude and coordinates of these villages, is provided in Table 1. The samples were placed in 500-gram glass jars, with the region's name, the date of collection from the hive, and the producer's name recorded on each jar. All honey samples were stored at room temperature in a dry, dark room throughout the research period.

Palynological analysis

To determine the pollen diversity in 10 grams of honey, the samples were prepared using a standard method accepted and employed by international beekeeping institutes (Lieux, 1972; Louveaux et al., 1978; Maurizio and Hodges, 1951). Honey samples for pollen analysis were prepared as follows: Initially, crystallized or solidified honey samples were softened in a water bath at 40-45°C. The honey was homogenized using a sterile glass rod. A 10-gram portion of this homogenized honey was transferred to a test tube, and 20 ml of distilled water was added. The tubes were placed in a water bath at 45°C for 10-15 minutes to dissolve the honey. The solution was then centrifuged at 3500-4000 rpm for 45 minutes. After the supernatant was discarded, the tubes were inverted and allowed to dry. The pollen residues at the bottom of the tubes were transferred onto a microscope slide using a basic fuchsin glycerin-gelatin mixture, and the mixture was covered with a cover slip while still warm at 30-40°C. The preparations were left to dry, inverted for 12 hours. The place of honey collection and the sample number were written on the label, making the samples ready for microscopic examination. Pollen preparations were examined using an Olympus CX21 microscope with a 40X objective. Pollen grains were identified by scanning the 18x18 mm slide area. Relevant literature and the pollen preparation collection of the Department of Biology at Kafkas University were utilized during this process (Erdtman, 1969; Faegri and Iversen, 1989; Sorkun, 2008). For each honey sample, two tubes were prepared, with two preparations from each tube, resulting in a total of four pollen preparations. In each preparation, 200 pollen grains were counted using the 40X objective. The percentages of the counting results were calculated, and the pollen was classified according to the dominant (≥45%), secondary (16-44%), minor (3-15%), and trace (<3%) proportions found in Posof honeys (Barbattini et al., 1991; Warakomska and Jaroszynska, 1992). In the tables, dominant pollen are shown in red, secondary pollen in green, minor pollen in blue, and trace pollen in yellow (Table 2 and Table 3)

Determination of antimicrobial effect

For the antimicrobial efficacy test, strains of *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*, obtained from the Microbiology Division of the Department of Biology, Faculty of Arts and Sciences, Kafkas University, were used. The antimicrobial properties of the honey samples were tested using the disk diffusion method, which was modified from the method of Anand et al. (2019). The test bacteria were incubated overnight in Nutrient Broth and then homogenized using a vortex. Colonies were transferred to 3-5 ml of Mueller-Hinton Broth (MHB) and adjusted to the 0.5 McFarland standard. Bacterial suspensions were spread onto Mueller-Hinton Agar plates in 100 µl volumes and allowed to dry at room temperature for 10 minutes. Sterile 6 mm disks were impregnated with 15 µl of honey dilutions (0.5 ml/ml, 0.25 ml/ml, and 0.125 ml/ml) and placed onto the dried plates. DMSO was used as the negative control, and netilmicin, ofloxacin, and cefoperazone/sulbactam antibiotics were used as positive controls. The plates were incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones were measured, and honey samples showing inhibition zones of 5 mm or greater were considered effective.

Table 1. *Coordinates and altitudes of the villages where honey samples were taken*

Village	Number of samples	Coordinate	Altitude (m)
Alabalık	2	41°25'17"N 42°37'01"E	2044
Balgözü	1	41°26'52"N 42°54'24"E	2044
Baykent	2	41°25'14"N 42°38'50"E	1820
Binbaşıeminbey	1	41°32'55"N 42°47'25"E	1532
Çambeli	1	41°29'33"N 42°47'02"E	1361
Çamyazı	1	41°28'34"N 42°44'24"E	2052
Derindere	1	41°26'11"N 42°55'54"E	2300
Gönülaçan	1	41°34'01"N 42°43'24"E	1788
Günbatan	2	41°28'20"N 42°36'38"E	2044
Günlüce	1	41°31'18"N 42°39'57"E	1802
Gürarmut	1	41°30'29"N 42°39'18"E	1596
Kaleönü	2	41°26'14"N 42°38'04"E	1788
Kolköy	2	41°26'39"N 42°36'44"E	1815
Kopuzlu	1	41°30'13"N 42°38'35"E	1570
Kumlukoz	1	41°34'12"N 42°47'10"E	1532
Kurşunçavuş	1	41°31'28"N 42°37'33"E	1788
Özbaşı	1	41°29'53"N 42°41'33"E	1532
Söğütlükaya	1	41°28'30"N 42°41'20"E	2044
Süngülü	1	41°28'22"N 42°52'13"E	1802
Taşkıran	2	41°33'43"N 42°45'39"E	1587
Türkgözü	1	41°34'49"N 42°49'18"E	1276
Yeniköy	2	41°28'40"N 42°49'10"E	1625

Table 2. *Distribution of pollen seen in monofloral honeys in Posof district (%)*

Family/Samples Number	3	10	11	12	14	17	18	19	20	27	29
Amaranthaceae					2						
Apiaceae		2		0.5	2	2	3		2	4	5.5
Asteraceae	3.5	9	1		8	10	4	1	3	6.5	4.5
Boraginaceae	12.5	8	6.5	31.5		9.5	8	1		8	4
Brassicaceae	1.5	1	1		2	2.5		0.5	9	3	3
Campanulaceae	1				2	3	3	1.5	2	2	
Caryophyllaceae						0.5					
Cistaceae	0.5	5	3.5	2.5	7	4.5	5	47	6	3.5	2.5
Dipsacaceae			1	1		2	2.5	0.5	4	2	1.5
Ericaceae					1			10		0.5	
Euphorbiaceae	1	2	1	2		0.5			2		
Fabaceae	70	53	65	46.5	56	45	45.5	29.5	61	46	58
Hypericaceae	0.5		1.5	1	1						
Lamiaceae	3.5	8	2.5	9.5	11	8	9	1.5	3	8.5	8.5
Onagraceae	1			1		1.5		0.5		1	
Pinaceae								0.5			
Plantaginaceae		1		0.5				1			
Poaceae	1.5	6				0.5	3.5				2
Poligonaceae		1				1	4		1	2	
Rosaceae	1.5	4	9.5	2	8	8	7.5	5.5	7	11	9.5
Salicaceae	1.5			0.5		1.5				1	1
Scrophulariaceae											
Solanaceae											
Urticaceae	0.5		7.5	1.5			5			1	

Statistical analysis

The antimicrobial effects of Posof honey samples were determined by repeating all measurements three times, and the zone diameters were presented as mean ± SD. Tukey's Multiple Comparison Test was used for the statistical evaluation of the results ($p < 0.05$). The IBM SPSS Statistics 20 statistical package program was used to perform statistical analyses. Different letters displayed on the columns indicate that the differences between the antimicrobial effect values of the honey samples are statistically significant.

RESULTS

Pollen analysis of the 29 honey samples was conducted, and the results are presented in Tables 2 and 3. According to microscopic analyses, pollen from a total of 19 families, including Apiaceae, Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Dipsacaceae, Ericaceae, Euphorbiaceae, Fabaceae, Hypericaceae, Lamiaceae, Onagraceae, Pinaceae, Plantaginaceae, Poaceae, Polygonaceae, Rosaceae, Salicaceae, Scrophulariaceae, Solanaceae, and Urticaceae, was identified in the honey samples collected from Posof. The samples with the lowest number of families (11 families) were Samples 11, 13, 14, 20, and 29, while the highest number of families (19 families) was found in Sample 2. Among the 29 samples, 11 were identified as monofloral (Samples 3, 10, 11, 12, 14, 17, 18, 19, 20, 27, and 29), and 18 were identified as multifloral. In Sample 18, pollen from the Cistaceae was predominant, whereas in the other monofloral honey samples, pollen from the Fabaceae was dominant (Tables 2 and 3). According to the data in the tables, pollen from various families is present in varying proportions in Posof honey samples. The distribution percentages of the families identified in Posof honey samples are shown in Figure 1. Upon evaluation, it was determined that the most abundant pollen in the analyzed honey samples belonged to Fabaceae (40.5%), Rosaceae (11.7%), Lamiaceae (9.3%), Boraginaceae (7.5%), and Cistaceae (7.2%). Pollen from Fabaceae, Rosaceae, and Lamiaceae was found in all samples, while pollen from Amaranthaceae, Solanaceae, and Scrophulariaceae was detected in only three samples (Figure 1).

The antimicrobial effects of the honey samples were determined using eight bacterial strains, including Gram-positive bacteria *S. aureus* (ATCC 29213), *S. aureus* (ATCC 25925), and *E. faecalis* (ATCC 29219), as well as Gram-negative bacteria *K. pneumoniae* (ATCC 700603), *E. coli* (O157:H7 RSKK 09007), *E. coli* (ATCC 25922), *E. coli* (ATCC 35218), and *P. aeruginosa* (ATCC 27853). The inhibition zone diameters (measured in millimeters) for different concentrations of honey (0.50%, 0.25%, 0.125%) were measured for each bacterial strain, and the results are presented in Tables 4 and 5.

The antimicrobial effects of the honey samples against Gram-positive bacteria are shown in Table 3. According to these results, antimicrobial effects were observed in honey samples 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 14, 17, 18, 26, 27, 28, and 29 against *S aureus* (ATCC 29213), while no effect was observed in the other samples. The strongest effect was detected in honey sample 27. Against *S.*

Table 3. *Distribution of pollen seen in multifloral honeys in Posof district (%)*

Family/Samples Number	1	2	4	5	6	7	8	9	13	15	16	21	22	23	24	25	26	28
Amaranthaceae		1			0.5													
Apiaceae		4.5	7	2.5	4.5	1	6			1		2.5			3	4.5	1.5	1.5
Asteraceae	9	6.5	5.5	5.5	6	1	4.5	5	9.5		1.5	1	2	6	4.5	7.5	9.5	4.5
Boraginaceae	1.5	8	9	3.5	9.5	4.5	3.5	0.5	7	10	8	10.5	7	11	6	7	12	9
Brassicaceae		1	6.5	1	2		2.5	2	3	4		6			4	2.5	2.5	2
Campanulaceae	7.5	7.5	2.5	7	1.5		1	4.5		1	1	1	4	4.5	3.5	3	1	1.5
Caryophyllaceae		1.5	1							1								10
Cistaceae	2.5	0.5	0.5		13.5	18	14	7	6.5	2	8.5	3	14	1.5	10	10.5		9
Dipsacaceae	3.5	5	4	4.5	2		1	2	4				1	1	4	4.5	2.5	2
Ericaceae		0.5				0.5		0.5		1	1	0.5		5.5				
Euphorbiaceae	7.5	1	3	0.5	0.5	0.5		1	0.5	4	5	1.5	1		1.5			
Fabaceae	12	37.5	16.5	36.5	38.5	44	33	29	40	34	40	14.5	36	39	41	36	39	32.5
Hypericaceae	2.5					2.5				2	2	4	1		1			
Lamiaceae	1.5	11	4	33.5	9.5	3.5	8	31.5	7	12	10.5	3	14	14	5.5	5.5	15.5	6
Onagraceae	1	1	3.5	0.5	1	0.5	1.5								2.5	1	2	
Pinaceae			0.5		0.5											0.5		
Plantaginaceae		0.5													0.5	1		
Poaceae	0.5		0.5		0.5	1		1		1	7	1	1	2		1.5	1	1
Poligonaceae		0.5	3		1.5		0.5	2.5	1.5	4	2	0.5	4	0.5	1	0.5	2	1.5
Rosaceae	36	11.5	28.5	3	6	4.5	22	12.5	20	15	10	28.5	11	11.5	11	9.5	9.5	15.5
Salicaceae	10	0.5	4	2	1.5	5	2.5	1	1	4	1	20		2.5	0.5	2.5	1	4
Scrophulariaceae			0.5			0.5				1								
Solanaceae		0.5			1	1.5												
Urticaceae	5					11.5				3	2.5	2.5	4	1	0.5	2.5	1	

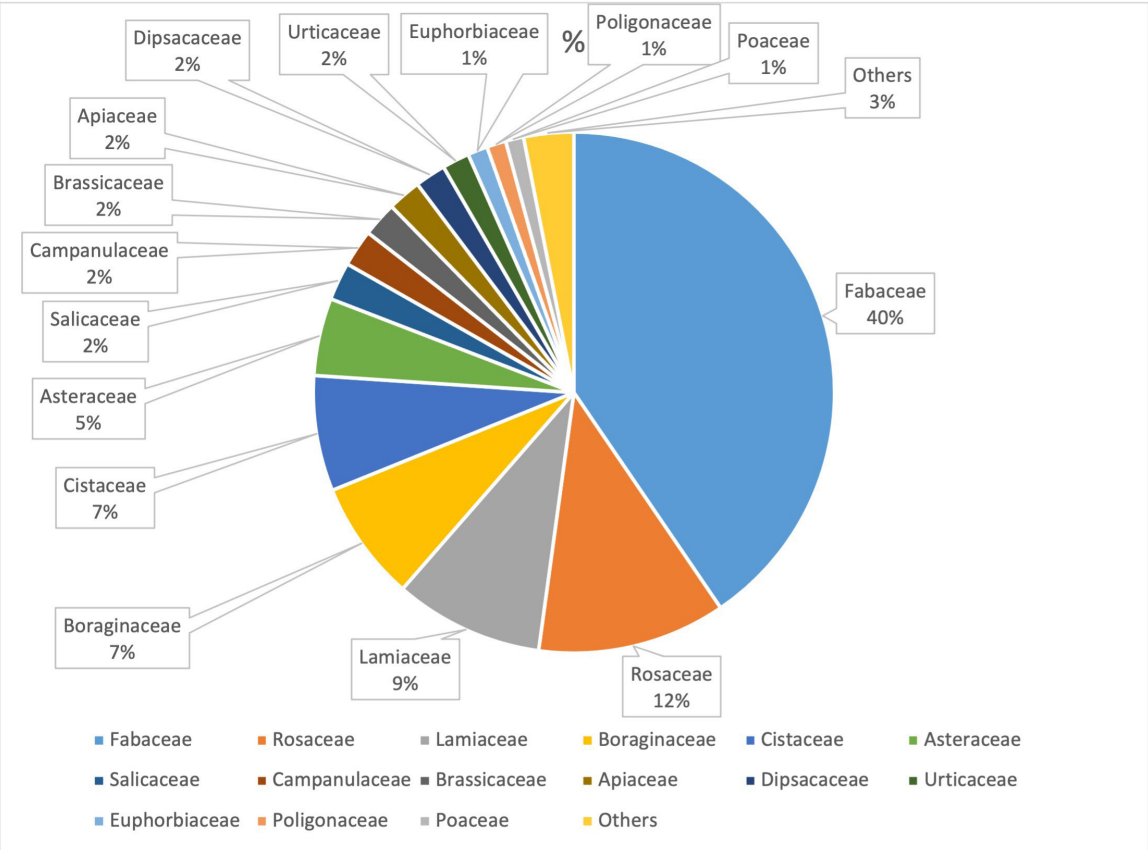

Figure 1. *Distribution percentages of plant taxa seen in Posof honeys (%)*

aureus (ATCC 25925), antimicrobial activity was observed only in honey samples 2, 27, and 28. For *E. faecalis* (ATCC 29219), antimicrobial effects were found in 10 honey samples (7, 8, 11, 13, 14, 22, 24, 25, 26, and 27), with the most significant effects observed in samples 14, 25, and 27 (Table 4).

The antimicrobial effects of the honey samples against Gram-negative bacteria are presented in Table 4. According to these results, antimicrobial effects were observed in honey samples 1, 2, 3, 12, 13, 14, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27, and 28 against *K. pneumoniae* (ATCC 700603), while no effect was found in the other samples. The most effective result was seen in honey sample 17. Against *E. coli* (O157:H7 RSKK 09007), antimicrobial activity was observed in honey samples 1, 2, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 22, 24, 25, 26, and 27. For *E. coli* (ATCC 25922), only samples 6, 8, and 17 showed activity. Antimicrobial effects against *E. coli* (ATCC 35218) were observed in honey samples 1, 3, 8, 9, 10, 12, 16, 22, 24, 25, 26, and 27. Finally, against *P. aeruginosa* (ATCC 27853), honey samples 1, 2, 6, 7, 11, 13, 14, 15, 16, 17, 18, 20, 22, 24, 26, 27, 28, and 29 were found to be effective (Table 5).

The data in Tables 4 and 5 reveal that the antimicrobial efficacy of different honey samples against various bacteria varies depending on both the concentration and the honey sample. For *K. pneumoniae* (ATCC 700603), honey sample 17 exhibited the highest inhibition zone diameters across all concentrations. For *E. coli* (O157:H7 RSKK 09007), the highest antimicrobial activity at all concentrations was observed in honey sample 18. In the case of *E. coli* (ATCC 25922), the highest inhibition zone diameter was recorded at a 0.25% concentration, with sample 26 showing the strongest effect. For *E. coli* (ATCC 35218), sample 13 demonstrated the highest inhibition zone diameters, particularly at 0.25% and 0.125% concentrations. For *P. aeruginosa* (ATCC 27853), sample 18 stood out as the most effective honey sample, showing the highest inhibition zone diameters at all concentrations. These findings suggest that certain honey samples possess stronger antimicrobial properties against specific bacteria (Tables 4 and 5).

Table 4. *Antimicrobial effects of honey samples against gram positive bacteria*

	***S.aereus* (ATCC 29213)**			***S.aereus* (ATCC 25925)**			***E.faecalis* (ATCC 29219)**		
Samples Number	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**
1	8.27 ± 0.24^{cd}	6.70 ± 0.18^{bcd}	6.68 ± 0.16^{d}						
2	8.65 ± 0.13^{bc}	6.65 ± 0.13^{d}	6.65 ± 0.15^{d}	8.27 ± 0.24^{a}					
3	8.22 ± 0.20^{cd}	7.68 ± 0.16^{a}	6.62 ± 0.10^{d}						
4									
5	8.23 ± 0.23^{cd}	7.67 ± 0.21^{a}	6.75 ± 0.22^{cd}						
6									
7	9.20 ± 0.18^{ab}	7.67 ± 0.15^{a}	6.62 ± 0.13^{d}				9.27 ± 0.24^{a}	6.70 ± 0.18^{cd}	6.75 ± 0.22^{b}
8	9.23 ± 0.23^{ab}	7.23 ± 0.23^{ab}	6.70 ± 0.20^{cd}				7.70 ± 0.18^{cd}	6.68 ± 0.16^{cd}	6.75 ± 0.22^{b}
9	7.70 ± 0.18^{de}	7.20 ± 0.18^{abcd}	6.68 ± 0.16^{d}						
10	8.27 ± 0.24^{cd}	7.25 ± 0.23^{ab}	6.60 ± 0.13^{d}						
11	7.20 ± 0.18^{e}	6.67 ± 0.15^{cd}	6.75 ± 0.22^{cd}				8.67 ± 0.14^{ab}	7.70 ± 0.18^{a}	7.25 ± 0.23^{ab}
12	7.22 ± 0.23^{e}								
13							7.72 ± 0.20^{cd}	6.65 ± 0.13^{d}	6.72 ± 0.19^{b}
14	7.17 ± 0.18^{e}	6.70 ± 0.18^{bcd}	6.58 ± 0.10^{d}				8.27 ± 0.24^{bc}	7.70 ± 0.18^{a}	7.27 ± 0.24^{ab}
15									
16									
17	9.27 ± 0.24^{ab}	7.63 ± 0.13^{a}	6.68 ± 0.18^{d}						
18	8.70 ± 0.18^{abc}	7.22 ± 0.2^{abc}	7.20 ± 0.18^{bc}						
19									
20									
21									
22							7.25 ± 0.23^{d}	7.23 ± 0.23^{ab}	6.70 ± 0.18^{b}
23									
24							8.22 ± 0.2^{bc}	7.70 ± 0.18^{a}	7.23 ± 0.23^{ab}
25							8.20 ± 0.23^{bc}	7.72 ± 0.20^{a}	7.70 ± 0.18^{a}
26	7.70 ± 0.18^{de}	7.70 ± 0.18^{a}	7.70 ± 0.18^{ab}				7.20 ± 0.2^{d}	7.18 ± 0.16^{bc}	6.68 ± 0.18^{b}
27	9.28 ± 0.25^{a}	7.72 ± 0.23^{a}	7.72 ± 0.23^{a}	6.70 ± 0.18^{c}	6.70 ± 0.18^{a}	6.61 ± 0.72^{a}	8.22 ± 0.2^{bc}	7.68 ± 0.16^{ab}	7.75 ± 0.23^{a}
28	7.23 ± 0.23^{e}	7.20 ± 0.18^{abcd}	6.68 ± 0.18^{d}	7.70 ± 0.18^{b}	6.72 ± 0.19^{a}	6.69 ± 0.10^{a}			
29	9.23 ± 0.23^{ab}								

Table 5. Antimicrobial effects of honey samples against gram negative bacteria

	K.pneumoniae **(ATCC 00603)**			***E.coli*** **(O157:H7 RSKK 09007)**			***E.coli*** **(ATCC 2522)**			***E.coli*** **(ATCC 3521)**			***P.auriginosa*** **(ATCC 253)**		
Samples Number	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**	**0.50%**	**0.25%**	**0.125%**
1	7.10±0.10^{ab}	6.62±0.1^{b}	6.52±0.76^{b}	7.12±0.13^{efg}	6.70±0.18^{e}	6.65±0.13^{d}	-	-	-	6.73±0.23^{c}	6.70±0.18^{c}	6.65±0.13^{c}	10.27±0.24^{b}	7.70±0.18^{de}	7.70±0.18^{c}
2	6.53±0.15^{c}	6.53±0.1^{b}	6.47±0.06^{b}	6.63±0.13^{g}	6.60±0.10^{e}	6.52±0.08^{d}	-	-	-	-	-	-	7.78±0.25^{fg}	-	-
3	6.62±0.13^{bc}	6.53±0.15^{b}	6.52±0.08^{b}	-	-	-		-	-	7.75±0.22^{ab}	7.70±0.18^{ab}	6.70±0.18^{c}	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	6.70±0.18^{b}	6.70±0.18^{b}	6.62±0.06^{a}	-	-	-	7.28±0.25^{gh}	6.70±0.18^{fg}	6.68±0.18^{d}
7	-	-	-	-	-	-	-	-	-	-	-	-	6.77±0.24^{h}	6.77±0.24^{fg}	6.73±0.21^{d}
8	-	-	-	8.68±0.18^{b}	8.23±0.23^{b}	6.70±0.18^{d}	6.72±0.2^{b}	6.68±0.16^{b}	6.67±0.88^{a}	6.77±0.24^{c}	6.73±0.21^{c}	-	-	-	-
9	-	-	-	6.72±0.19^{fg}	6.67±0.14^{e}	6.65±0.13^{d}	-	-	-	6.75±0.22^{c}	6.72±0.19^{c}	6.68±0.16^{c}	-	-	-
10	-	-	-	8.05±0.13^{cd}	7.70±0.18^{cd}	7.20±0.18^{c}	-	-	-	6.75±0.22^{c}	6.70±0.17^{c}	6.68±0.16^{c}	-	-	-
11	-	-	-	6.70±0.18^{g}	6.67±0.14^{e}	6.62±0.1^{d}	-	-	-	-	-	-	6.72±0.19^{h}	6.70±0.17^{fg}	6.67±0.14^{d}
12	7.13±0.15^{ab}	6.57±0.76^{b}	6.52±0.08^{b}	-	-	-	-	-	-	6.73±0.20^{c}	6.72±0.19^{c}	6.72±0.19^{bc}	-	-	-
13	7.05±0.18^{abc}	6.60±0.13^{b}	6.50±0.1^{b}	6.70±0.18^{g}	6.65±0.15^{e}	6.60±0.1^{d}	-	-	-	-	-	-	8.27±0.24^{ef}	7.30±0.26^{ef}	-
14	6.65±0.18^{bc}	6.57±0.16^{b}	6.52±0.13^{b}	8.23±0.23^{bc}	8.17±0.18^{bc}	7.27±0.24^{c}	-	-	-	-	-	-	8.73±0.23^{de}	6.73±0.2^{fg}	6.70±0.17^{d}
15	-	-	-	6.62±0.16^{g}	6.58±0.14^{e}	6.52±0.08^{d}	-	-	-	-	-	-	7.27±0.24^{gh}	6.73±0.2^{fg}	6.70±0.17^{d}
16	7.25±0.22^{a}	6.57±0.76^{b}	6.52±0.08^{b}	7.63±0.13^{de}	6.65±0.13^{e}	6.52±0.08^{d}	-	-	-	6.73±0.20^{c}	6.70±0.20^{c}	6.68±0.16^{c}	8.73±0.2^{de}	6.73±0.2^{fg}	6.67±0.14^{d}
17	7.23±0.23^{a}	7.13±0.13^{a}	7.07±0.06^{a}	6.65±0.18^{g}	6.60±0.13^{e}	6.55±0.09^{d}	9.33±0.29^{a}	7.27±0.28^{a}	6.70±0.10^{a}	-	-	-	9.77±0.24^{bc}	9.27±0.24^{b}	8.70±0.18^{b}
18	-	-	-	9.23±0.23^{a}	9.15±0.18^{a}	9.08±0.1^{a}	-	-	-	-	-	-	12.27±0.24^{a}	10.23±0.23^{a}	9.3±0.28^{a}
19	6.67±0.18^{bc}	6.57±0.08^{b}	-	8.53±0.15^{bc}	8.30±0.28^{b}	8.27±0.28^{b}	-	-	-	-	-	-	-	-	-
20	6.62±0.13^{bc}	-	-	6.63±0.15^{g}	6.58±0.1^{e}	6.55±0.05^{d}	-	-	-	-	-	-	9.27±0.24^{cd}	8.27±0.24^{cd}	7.72±0.19^{c}
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	6.68±0.18^{bc}	6.60±0.10^{b}	6.58±0.08^{b}	7.23±0.23^{ef}	7.23±0.23^{d}	6.63±0.19^{d}	-	-	-	6.68±0.16^{c}	6.63±0.12^{c}	6.60±0.1^{c}	6.67±0.15^{h}	6.62±0.1^{g}	6.58±0.08^{d}
23	6.77±0.24^{abc}	6.70±0.17^{b}	6.55±0.05^{b}	-	-	-	-	-	-	-	-	-	-	-	-
24	6.57±0.08^{c}	6.57±0.16^{b}	6.52±0.03^{b}	6.67±0.18^{g}	6.63±0.15^{e}	-	-	-	-	8.27±0.24^{a}	8.20±0.17^{a}	7.28±0.25^{a}	6.63^{h}±0.13	6.60±0.1^{g}	6.60±0.10^{d}
25	6.73±0.20^{abc}	6.65±0.13^{b}	6.58±0.08^{b}	7.57±0.16^{de}	7.55±0.13^{d}	7.30±0.28^{c}	-	-	-	6.67±0.14^{c}	6.58±0.08^{c}	6.53±0.06^{c}	-	-	-
26	7.13±0.13^{ab}	6.52±0.03^{b}	6.47±0.03^{b}	6.67±0.18^{g}	6.65±0.15^{e}	6.63±0.13^{d}	-	-	-	7.27±0.24^{bc}	7.27±0.24^{b}	7.17±0.14^{ab}	8.30±0.26^{ef}	7.67±0.14^{de}	7.67±0.14^{c}
27	6.73±0.20^{abc}	6.70±0.17^{b}	6.65±0.15^{b}	7.55±0.13^{de}	6.68±0.20^{e}	6.67±0.18^{d}	-	-	-	6.70±0.18^{c}	6.63±0.12^{c}	6.60±0.09^{c}	6.72±0.19^{h}	6.68±0.16^{fg}	6.67±0.14^{d}
28	6.67±0.18^{bc}	6.65±0.13^{b}	6.53±0.06^{b}	-	-	-	-	-	-	-	-	-	6.73±0.21^{h}	6.73±0.21^{fg}	6.68±0.16^{d}
29	-	-	-	-	-	-	-	-	-	-	-	-	8.65±0.13^{de}	8.43±0.38^{c}	7.70±0.18^{c}

DISCUSSION

The primary purpose of bees visiting plant flowers is to collect nectar and pollen, which serve as essential food sources that fulfill their protein, vitamin, and mineral needs (Burgut et al., 2023; Cengiz, 2018; Genç and Dodoloğlu, 2017; Özbakir and Alişiroğlu, 2019). The pollen-collecting behavior of bees varies depending on factors such as the flowering periods of plants, nectar production, and pollen quantities. In Türkiye, the plant families most preferred by bees include Asteraceae, Lamiaceae, and Rosaceae (Cengiz, 2018; Öztürk and Görhan, 2021).

Pollen analyses provide significant insights into the pollen diversity of Posof honeys from Ardahan province. The analyses revealed that the most prevalent pollen types in these honeys are from the Fabaceae (40.5%), Rosaceae (11.7%), Lamiaceae (9.3%), Boraginaceae (7.5%), Cistaceae (7.2%), and Asteraceae (4.8%). Eleven honey samples were identified as monofloral honeys. The pollen content varies depending on the source of the collected pollen and nectar, and this variation is influenced by the plant flora, geographical features, and climatic conditions of the region (Anklam, 1998). Pollen diversity in honey and the flora of the region were generally similar, except for the Asteraceae family. Pollen belonging to the families Fabaceae, Rosaceae and Lamiaceae were observed in high amounts in honey samples. Also Among the plant families most preferred by bees for pollen, Fabaceae, Asteraceae, Brassicaceae, and Rosaceae are prominent. Fabaceae family, particularly the flowers of legumes, serves as a rich pollen source for bees, providing essential proteins, vitamins, and minerals necessary for their nutrition (Mısır et al., 2023). Plants that bloom in the spring months increase pollen collection activities among bees. During this period, plants from the Fabaceae and Asteraceae families are among the most frequently visited species by bees (Şimşek, 2023).

In the pollen analysis studies of honeys conducted in the Ardahan region by Sorkun et al. (2014) and Şık et al. (2017), various plant families were identified. Sorkun et al. (2014) detected pollen from Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Dipsacaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Pinaceae, Poaceae, Polygonaceae, Rosaceae, and Salicaceae. Şık et al. (2017) identified pollen from Amaranthaceae, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Fabaceae, Hypericaceae, Lamiaceae, Salicaceae, and Scrophulariaceae. These results show similarities with the plant families found in our study.

Türkiye is notable for its rich plant diversity, which is attributed to several key factors, including its geographical location, climate diversity, and phytogeographic characteristics. Positioned at the intersection of the Irano-Turanian, Mediterranean, and Euro-Siberian phytogeographic regions, Türkiye hosts a wide range of plant species with varying climatic and soil requirements (Karaköse et al., 2018; Savcı et al., 2018; Tel et al., 2019). This makes Türkiye a significant location globally in terms of plant diversity.

Regional similarities and differences in the plant species preferred by bees have been observed. For instance, in Şırnak honey, Fabaceae, Lamiaceae, and Rosaceae are secondary, while Boraginaceae is dominant (Gürbüz et al., 2019). In the Adapazarı districts of Hendek, Akyazı, and Kocaali, Asteraceae, Fabaceae, Lamiaceae, Rosaceae, and Cistaceae are prominently found (Erdoğan et al., 2008). In Sinop, Fabaceae is predominant (Özler, 2015). In Kars, which has a similar climate, the most common pollen types are from Fabaceae, Boraginaceae, and Asteraceae (Gençay et al., 2018). In Anzer honey, Fabaceae, Asteraceae, Boraginaceae, and Rosaceae are prominently present (Sorkun and Doğan, 1995). In Posof honey, while Fabaceae and Asteraceae pollen overlap with Anzer honey, Boraginaceae pollen is less prevalent in Posof. This variation reflects the diversity of regional vegetation and the different pollen compositions resulting from regional flora.

The antimicrobial effects of the honey samples examined in this study against Gram-positive and Gram-negative bacteria are consistent with other research on the antibacterial properties of honey. The literature indicates that the antibacterial effects of honey are attributed to several factors, including low pH, high sugar concentration, and hydrogen peroxide production through the glucose oxidase enzyme (Bhushanam et al., 2021; Saxena et al., 2010). In our study, specific honey samples were found to exhibit a stronger antibacterial effect, particularly against the Gram-positive bacteria *S. aureus* and *E. faecalis*.

The observation of the best antimicrobial effect against *S. aureus* (ATCC 29213) in honey sample 27 suggests that this sample may contain potentially more potent bioactive compounds. The literature indicates that honey is effective against Gram-positive bacteria, with hydrogen peroxide production playing a significant role in this effect (Almasaudi, 2021; Bhushanam et al., 2021). However, the antimicrobial activity observed only in honey samples 2, 27, and 28 against *S. aureus* (ATCC 25925) suggests that these samples may have different biochemical profiles.

In addition to honey samples effective against Gram-positive bacteria, significant antimicrobial effects have also been observed against Gram-negative bacteria. Specifically, the effectiveness of many honey samples against *K. pneumoniae* (ATCC 700603) and *E. coli* strains suggests that the phenolic compounds and defensins in these honeys may have the potential to disrupt the cell membranes of these bacteria (Oliveira et al., 2018; Stavropoulou et al., 2022). The honey sample 17, which demonstrated the best effect, is particularly effective against *K. pneumoniae*, highlighting its potential for clinical applications.

It is known that the antibacterial effect of honey is not limited to hydrogen peroxide production alone, but also involves various phytochemicals and antimicrobial peptides (Almasaudi, 2021; Kwakman et al., 2011). This study demonstrates that different honey samples exhibit varying levels of antibacterial activity against various bacterial strains, and this activity may be influenced by the botanical source, geographical origin, and the biologically active compounds present in the honey.

In conclusion other studies conducted throughout Türkiye also show parallels with our findings (Bayram et al., 2019; Güneş, 2021; Karagözoğlu and Kıran, 2022; Mercan et al., 2007; Yalazi and Zorba, 2020). Also the

findings of our study suggest that honey's antibacterial potential spans a wide spectrum, indicating its potential use as an alternative therapeutic agent against bacteria. Future research should involve a more detailed analysis of the chemical components of these honey samples and testing their clinical efficacy, which will help us better understand the medicinal potential of honey.

CONCLUSION

Twenty-nine honey samples were collected from local beekeepers during the 2020 honey harvest season in the Posof District of Ardahan Province. These samples underwent palynological and microbiological analyses. According to the pollen analysis results, 11 honey samples were identified as monofloral, while 18 were identified as multifloral. The predominant pollen types in the honey samples were from Fabaceae, Rosaceae, Lamiaceae, Boraginaceae, and Cistaceae. Considering the region's geographic structure and rich vegetation, it can be concluded that the honey produced in this area has a highly diverse botanical content. Posof honey is derived from various plant species, highlighting the richness of the region's flora in terms of honey plants. Additionally, the antimicrobial effects of the honey were assessed. To accurately identify the precise botanical sources of honey produced in Posof, further pollen analysis at the genus level with additional samples is required. Furthermore, beyond pollen analysis and antimicrobial activity, it is important to examine physical and chemical properties such as proline, water-insoluble solids, moisture (Brix), free acidity, electrical conductivity, pH, HMF, fructose, glucose, sucrose, and maltose, as these are critical in determining honey quality.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Statement

The authors declare that the approval of an ethics committee is not required for the scope of this study.

Author Contributions

The research idea, material acquisition, various laboratory processes, and result evaluation were carried out by SA and AGU.

Availability of data and materials

All data and materials of the study are available in contact with the corresponsible author.

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