DOI:10.25092/baunfbed. 1060898 *J. BAUN Inst. Sci. Technol., 24(2), 672-689, (2022)*

Antimicrobial activities of natural honeys and royal jellys on some pathogenic bacteria

Seda OĞUR[1*], Yusuf DAYAN[2]

[1]*Bitlis Eren Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, Rahva Yerleşkesi Bitlis*
[2]*Van Yüzüncüyıl Üniversitesi, Fen Bilimleri Enstitüsü, Besin Hijyeni ve Teknolojisi A.B.D., Tuşba, Van*

Geliş Tarihi (Received Date): 20.01.2022
Kabul Tarihi (Accepted Date): 07.06.2022

Abstract

In this study aimed to determine antimicrobial activities of natural honeys (HN) in Bitlis, and natural royal jellys (RJ) in Bitlis & Ağrı on some pathogenic bacteria (Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 29213), Enterococcus faecalis (ATCC 29212), Listeria monocytogenes (ATCC 7644), Streptococcus pyogenes (ATCC 19615), Salmonella Enteritidis (ATCC 13076) and Bacillus cereus (ATCC 11778)). HN and RJ samples at concentrations of 10%, 25%, 50% and 100% were used to identify their antimicrobial activities using the method of hollow agar. 50 µL of each HN and RJ concentrates was inoculated into the wells. The diameters of inhibition zone occurring in petri dishes were measured in millimeters (mm) at the end of the incubation at 37 °C for 24 hours. It was observed that the largest diameters of inhibition zones were in 100% concentrates; whereas, no zones occurred in 10% concentrate. The sample with the most antimicrobial activity was the honey sample (HN1) obtained from the Bitlis Dere region, with an inhibition zone of 39.50±4.93 mm on P. aeruginosa (ATCC 27853). 100% and 50% concentrates of RJ samples had antimicrobial activities on all bacteria strains except S. pyogenes (ATCC 19615). The results of the present study provided preliminary evidence that the examined bee products have potential for use in apitherapy applications.

Keywords: *Honey, royal jelly, antimicrobial activity, pathogenic bacteria, inhibition zone.*

[*]Seda OĞUR, ogursd@gmail.com, http://orcid.org/0000-0002-2041-0790
Yusuf DAYAN, dytyusuf@hotmail.com, http://orcid.org/0000-0003-2026-9018

Doğal balların ve arı sütlerinin bazı patojenik bakteriler üzerindeki antimikrobiyal aktivitesi

Öz

Bu çalışmada Bitlis'teki doğal balların (BL) ve Bitlis ve Ağrı'daki doğal arı sütlerinin (AS) bazı patojenik bakteriler (Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), Staphylococcus aureus (ATCC 29213), Enterococcus faecalis (ATCC 29212), Listeria monocytogenes (ATCC 7644), Streptococcus pyogenes (ATCC 19615), Salmonella Enteritidis (ATCC 13076) and Bacillus cereus (ATCC 11778)) üzerindeki antimikrobiyal aktivitesini belirlemek amaçlanmıştır. Oyuk agar metodu kullanılarak antimikrobiyal aktivitelerini tanımlamak için BL ve AS numuneleri %10'luk, %25'lik, %50'lik ve %100'lük konsantrasyonlarda kullanılmıştır. BL ve AS konsantratlarından her bir kuyucuğa 50 µL inoküle edilmiştir. 37 °C'de 24 saatlik inkübasyondan sonra petrilerde oluşan inhibisyon zonları milimetre (mm) olarak ölçülmüştür. En büyük inhibisyon zonu çapları %100'lük konsantratlarda gözlemlenirken, %10'luk konsantratlarda zon oluşmamıştır. En iyi antimikrobiyal aktiviteye sahip numune P. aeruginosa (ATCC 27853) üzerinde 39,50±4,93 mm inhibisyon zonu oluşturan, Bitlis Dere mevkiinden elde edilen BL1 numunesi olmuştur. AS numunelerinin %100'lük ve %50'lik konsantratları S. pyogenes (ATCC 19615) dışındaki tüm bakteri suşları üzerinde antimikrobiyal aktiviteye sahip olmuştur. Bu çalışmanın sonuçları, incelenen arı ürünlerinin apiterapi uygulamalarında kullanım potansiyeline sahip olduğuna dair ön kanıtlar sağlamıştır.

Anahtar kelimeler: *Bal, arı sütü, antimikrobiyal aktivite, patojenik bakteri, inhibisyon zonu*

1. Introduction

"Apitherapy" is defined as the use of honeybee products, prepared at different rates and with different compositions, as a drug in the treatment of human diseases. There are apitherapy centers treating diseases only using bee products in some Eastern Europe countries, especially in China.

Apitherapy is one of the traditional and complementary medicine practices allowed to be applied formally in Turkey based on the Regulation on Traditional and Complementary Medicine Practices of the Republic of Turkey Ministry of Health [1]. This regulation states that honey, pollen, propolis, and royal jelly may be applied orally and topically and in secondary immunodeficiency to support the immune system [1]. In many research in Turkey, the potential advantage of apitherapy has been mentioned, but it has been observed that the scientific studies on this subject have not been conducted comprehensively and adequately as necessary [2].

Honey is defined as "a sweet and thick substance produced by honeybees (*Apis mellifera*, *Apis mellifica*) collecting nectar in the flowers of plants or sweet materials secreted by the living parts of the plants and some homoptera and transforming their compounds in their organisms, and depositing them in honeycombs to mature" [3]. The contribution of

honey, that has been used medically against current bacterial infections and gastrointestinal diseases for thousands of years [4], to the healing of wounds has been proved many times [5, 6].

The antibacterial effect of honey especially on Gram (+) bacteria has been documented by various studies. Its bacteriostatic and bactericidal effects in many strains, most of which are pathogenic, have been reported [7]. The bacteria isolated from wounds such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Salmonella* enterica ser. Typhimurium are among the bacteria on which honey is effective [4]. In vitro studies have revealed that honey, used in injected wounds, has not only an effect of preventing only the growth of bacteria such as *Proteus mirabilis*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *E. coli*, *Streptococcus faecalis*, *Clostridium perfringens* and *S. aureus* but also a lethal effect on them [8].

Royal jelly is the food of queen bees and its nutritional value is quite high. Royal jelly contains water of about 66%, carbohydrate of 14.5%, lipid of 4.5%, the amino acid of 13%, and all the vitamins of Group B as well as vitamins A, D, C, and E, biologically active substances, some important mineral matters, and some unidentified substances. In addition, it contains 8 of the 10 essential amino acids (methionine, leucine, lysine, valine, phenylalanine, tryptophan, isoleucine) with natural hormones and enzymes. It includes pantheic acid, which is very important for metabolism, acetylcholine, protein, 10-hydroxy-2 decanoic acid which is a fatty acid strengthening the immune system, sepanine acid, and oleic acid which is very ideal to regulate digestion and appetize after a recovery period of diseases [9].

In Europe and America, for the last 30 years, royal jelly has been considered as a special food extending human life and ensuring human beings to be healthy and vigorous due to its vital substances. In the mid-1960s, royal jelly had a wide area of use in diets and cosmetics sectors in France and England due to its therapeutic characteristics. It has been reported that it is pharmacologically used as antibacterial [10]. Fontana et al. [11] purified peptides with 4 antimicrobial characteristics from royal jelly by using HPLC and Q-Tof-MS/MS techniques: Jelleine I-IV. Jelleine I-III exhibits antimicrobial activity especially against yeast, Gram (+) and Gram (–) bacteria [11].

Bitlis bee products obtained from endemic plants and rich flora are considered as being of the high quality products in Turkey. The fact that agricultural spraying is low and there are few industrial enterprises has made Bitlis an important region for beekeeping. There are also production areas for organic honey and bee products in Bitlis. As migratory beekeeping can also be performed in the region, the quality and yield of honey and other bee products are higher compared to the stationary beekeeping due to different flora monitoring [12].

The province of Ağrı, located within the borders of Upper Murat section, has a great beekeeping potential due to its low summer temperatures and rich flora features. However, despite this, it has been determined that there is no intense interest in the evaluation of this potential, especially by local beekeepers. The beekeeping potential of the region is mostly used by wandering beekeepers from other provinces. Honey and beeswax production is mostly carried out by beekeepers in Ağrı [13].

In the literature, there is a study [14] determining the antimicrobial activities of the honey and propolis samples in Muş and Bitlis regions on *Klebsiella pneumoniae* (13883), *E. coli* (ATCC 8739), *P. aeruginosa* (9027), *S. aureus* (6538), *Bacillus megaterium* (DSM 32) and *Enterococcus faecalis* (*E. faecalis*-ATCC 29212) bacteria. However, it was observed that not reporting the province of the samples based on their numbers caused the antimicrobial activity of the samples in Bitlis not to be exactly understood in this study [14]. There was also no study examining the antibacterial activity of royal jelly produced in Bitlis and Ağrı.

In this study was tried to determine the antimicrobial activity of the honey samples in Bitlis only and royal jelly samples in Bitlis & Ağrı on five Gram (+) (*Bacillus cereus* (*B. cereus*-ATCC 11778), *E. faecalis* (ATCC 29212), *Listeria monocytogenes* (*L. monocytogenes*-ATCC 7644), *S. aureus* (ATCC 29213), *Streptococcus pyogenes* (*S. pyogenes*-ATCC 19615)) and three Gram (–) (*E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *Salmonella* Enteritidis (*S.* Enteritidis-ATCC 13076) pure bacteria cultures.

According to the results of the conducted study was aimed to attend that the examined bee products can be used preferably as supportive of the medical treatment regarded as appropriate for the treatment of some infectious diseases and can have a place in apitherapy.

2. Materials and methods

2.1. Procurement of bacteria strains and media

Five Gram (+) (*B. cereus* (ATCC 11778), *E. faecalis* (ATCC 29212), *L. monocytogenes* (ATCC 7644), *S. aureus* (ATCC 29213), *S. pyogenes* (ATCC 19615)) and three Gram (–) (*E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S.* Enteritidis (ATCC 13076)) bacteria strains obtained from American Type Culture Collection (ATCC) were used to reveal the antimicrobial activity of honey and royal jelly samples. The strains and the required media (Nutrient Broth (LabM, LAB068) and Mueller Hinton Agar (LabM, LAB039)) were provided by a medical company.

2.2. Procurement of honey and royal jelly samples

The honey and royal jelly samples were supplied from the registered producers of bee products. One royal jelly sample (RJ1) from Ağrı, one royal jelly sample (RJ2) and three honey samples (HN1, HN6, HN8) from the Merkez district of Bitlis, four honey samples (HN3, HN4, HN5, HN7) from Mutki district of Bitlis, and one honey sample (HN2) from Hizan district of Bitlis were obtained. Thus, a total of 2 royal jelly and 8 honey samples were analyzed. The samples were taken to the laboratory using sterilized jars and kept at 2±2 °C until the antimicrobial activity studies were carried out.

2.3. Reviving and adjusting bacteria strains

The ATCC coded bacteria strains, obtained as a swab, kept at 2±2 °C were inoculated into the tubes containing 5 mL Nutrient Broth (LabM, LAB068) and they were incubated for 24 hours at 37 °C so that they were revived. Revived bacteria strains were taken to the tubes containing 5 mL of 0.85% sterile physiological saline with loop (3 loopful) and inoculated and they were adjusted to 0.5 McFarland turbidity (about 10^8 cfu/g) [15].

2.4. Preparing honey and royal jelly samples

The samples were mixed with distilled water and their concentrates of 10%, 25%, and 50% were prepared. The honey samples were kept in the water bath, adjusted to 37 °C, for 2 hours in order to flow easily. After the royal jelly samples were liquefied more by being kept for 5-10 minutes at room temperature, their concentrates were prepared in the same rates as honey samples.

2.5. Preparation of medium and inoculation of bacteria strains

The Mueller Hinton Agar (LabM, LAB039) medium, sterilized in test tubes and cooled to 45-50 °C were poured into the sterile petri dishes having a 9-cm diameter as 15 mL for each. After it was cooled rather well and hollows of 6 mm were burrowed using sterile Pasteur pipette on the medium, the adjusted bacteria strains were spread the medium using sterile swab based on Kirby Bauer disk diffusion method and the names of the inoculated bacteria were written on the petri dishes as well as the codes and the concentrates of the samples to be placed in the hollows were written on the upper part of the hollows [16].

2.6. Determining antimicrobial activity using hollow agar method

50 µL of honey and royal jelly samples of 100%, 50%, 25%, and 10% were placed into the hollows. Apple vinegar (AV) and sterile distilled water were used as positive control and negative control, respectively. The diameters of inhibition zones occurring after the incubation of the petri dishes at 37 °C for 24 hours were measured in mm using a ruler [17]. The final results were obtained by repeating this method two times and studying in 2 parallels.

2.7. Statistical analysis

The data were assessed in Statistical Package for the Social Sciences (IBM SPSS Statistics, Version 20.0) with one-way analysis of variance [18]. The level of $p<0.05$ was accepted as statistically significant. The results were shown as mean ± standard deviation in mm.

3. Results

The largest inhibition zone diameters showing the antimicrobial activity of the honey and royal jelly samples were determined in 100% concentrates and no zone was observed in 10% concentrates. It was observed that an extra zone of 0.10-0.20 mm formed in some of the honey samples in 25% concentrates (HN2-*E. faecalis* (ATCC 29212)-7.00 mm inhibition zone, HN3-*E. faecalis* (ATCC 29212)-8.00 mm inhibition zone, HN2-*L. monocytogenes* (ATCC 7644)-8.00 mm inhibition zone, HN3-*P. aeruginosa* (ATCC 27853)-8.00 mm inhibition zone).

It was observed that one (RJ2) of the 25% concentrates of the royal jelly samples had a very little effect only on *L. monocytogenes* (ATCC 7644) (9.00 mm inhibition zone) and the other sample (RJ1) had also a very little effect on *L. monocytogenes* (ATCC 7644) (8.00 mm inhibition zone) and *E. faecalis* (ATCC 29212) (10.00 mm inhibition zone). For this reason, the antimicrobial activities of only 100% and 50% concentrates of the samples on the bacteria strains were shown in tables (Table 1 and Table 2) and these concentrates were analyzed statistically.

It was found that the difference between the antimicrobial activities of the bacteria strains of honey and royal jelly samples was significant ($p<0.05$) (Table 1 and Table 2).

3.1. Antimicrobial activities of honey samples

Table 1 shows the antimicrobial activities of 100% and 50% concentrates of the honey samples.

Table 1. Antimicrobial activities of honeys

Sample	Concentration	Bacteria strain- Inhibition zone (Mean ± Std. dev., mm)			
		B. cereus **(ATCC 11778)**	***E. coli*** **(ATCC 25922)**	***E. faecalis*** **(ATCC 29212)**	***L. monocytogenes*** **(ATCC 7644)**
HN1	**100%**	-	10.00 ± 0.00^{ACDab}	14.00 ± 0.00^{DFGd}	10.50 ± 0.55^{Cb}
	50%	-	-	11.50 ± 0.55^{EHIa}	-
HN2	**100%**	-	14.00 ± 1.10^{DEa}	15.00 ± 0.00^{DGac}	38.50 ± 1.64^{Db}
	50%	-	-	13.00 ± 1.10^{GHJa}	34.00 ± 1.10^{Db}
HN3	**100%**	11.50 ± 0.55^{Cac}	-	12.50 ± 0.55^{FIab}	13.50 ± 0.55^{EHb}
	50%	8.00 ± 0.00^{Ba}	-	11.50 ± 0.55^{EIb}	11.00 ± 1.10^{CFHb}
HN4	**100%**	-	13.50 ± 0.55^{EGa}	-	11.00 ± 1.10^{CFHb}
	50%	-	8.00 ± 0.00^{BCa}	-	-
HN5	**100%**	-	7.00 ± 1.00^{CHGa}	12.00 ± 1.00^{DEFGb}	9.00 ± 1.00^{CFac}
	50%	-	-	11.00 ± 1.00^{DEFGab}	-
HN6	**100%**	-	7.50 ± 0.55^{BHa}	23.00 ± 1.10^{Cb}	8.50 ± 0.55^{FIa}
	50%	-	-	-	-
HN7	**100%**	-	15.00 ± 0.00^{Eb}	-	8.00 ± 1.00^{FGIa}
	50%	-	12.00 ± 1.00^{ACEa}	-	-
HN8	**100%**	23.00 ± 1.10^{Da}	9.50 ± 2.73^{ABEFb}	-	12.00 ± 0.00^{ECGb}
	50%	20.00 ± 0.00^{Dad}	8.50 ± 1.64^{ABFGbc}	-	8.50 ± 1.64^{CHIbc}
AV	**100%**	-	8.40 ± 0.70^{Ba}	9.70 ± 1.33^{BEa}	10.60 ± 2.01^{CHIac}
		S. aureus **(ATCC 29213)**	***S.* Enteritidis (ATCC 13076)**	***S. pyogenes*** **(ATCC 19615)**	***P. aureginosa*** **(ATCC 27853)**
HN1	**100%**	15.50 ± 0.55^{Dc}	13.00 ± 1.10^{ABd}	-	39.50 ± 4.93^{CGIe}
	50%	14.00 ± 0.00^{DEb}	-	-	12.50 ± 1.64^{BDab}
HN2	**100%**	9.00 ± 0.00^{CEGd}	14.00 ± 1.10^{AEGac}	32.50 ± 2.74^{Ab}	37.50 ± 2.74^{Cb}
	50%	-	-	15.00 ± 000^{Bc}	15.50 ± 0.55^{DEc}
HN3	**100%**	10.50 ± 0.55^{BCHc}	15.00 ± 0.00^{AEGId}	-	28.00 ± 2.19^{AFHIe}
	50%	-	-	-	22.00 ± 2.19^{EFc}
HN4	**100%**	11.50 ± 0.55^{BHb}	39.00 ± 1.10^{Dc}	-	37.00 ± 2.19^{Cc}
	50%	10.00 ± 1.10^{BEFb}	22.50 ± 2.73^{Ec}	-	27.50 ± 2.74^{AFGHc}
HN5	**100%**	10.00 ± 1.00^{BEFHab}	8.00 ± 1.10^{CFa}	-	21.00 ± 1.10^{FGJd}
	50%	10.00 ± 0.00^{BEFHa}	-	-	14.00 ± 2.19^{BDJb}
HN6	**100%**	8.50 ± 0.55^{FGa}	9.00 ± 1.10^{CFHa}	-	23.50 ± 1.64^{FGHb}
	50%	-	-	-	12.00 ± 1.00^{Ba}
HN7	**100%**	14.50 ± 0.55^{DIb}	9.00 ± 0.00^{BFHIa}	-	31.00 ± 1.10^{CHc}
	50%	10.00 ± 1.00^{BEFHa}	9.00 ± 1.00^{BFHIa}	-	21.00 ± 3.29^{ABDFGb}
HN8	**100%**	11.00 ± 1.10^{BEFHIb}	12.00 ± 1.10^{BGHIb}	-	27.50 ± 2.74^{AFGHa}
	50%	10.00 ± 0.00^{BEFHb}	8.50 ± 0.55^{Fc}	-	19.00 ± 1.10^{EHJd}
AV	**100%**	8.70 ± 0.82^{CFa}	16.10 ± 2.07^{AEb}	-	14.80 ± 3.94^{BDEcb}

*Upper case letters indicate the difference between lines in the same column, lower case letters indicate the difference between columns in the same line ($p<0.05$).
The difference between the mean values indicated by the same letter is insignificant ($p>0.05$).
(-): Inhibition zone was not detected.

The honey sample HN1 (100%) had the best antimicrobial activity by creating an inhibition zone of 39.50 ± 4.93 mm on *P. aeruginosa* (ATCC 27853) (Table 1).

The 50% concentrates of some honey samples had an antimicrobial activity as good as the 100% concentrates. It was determined among 50% concentrates of the honey samples that the HN2 sample formed the best inhibition zone (34.00 ± 1.10 mm) on *L. monocytogenes* (ATCC 7644). The 50% concentrate of the HN5 sample formed an

inhibition zone of 20.00 ± 0.00 mm on *B. cereus* (ATCC 11778). The 50% concentrate of HN4 samples showed a quite good antimicrobial activity by forming an inhibition zone of 22.50 ± 2.73 mm on *S.* Enteritidis (ATCC 13076) and an inhibition zone of 27.50 ± 2.74 mm on *P. aeruginosa* (ATCC 27853) (Table 1).

Figure 1 shows the photos of some inhibition zones formed by some honey samples, which had good antimicrobial activity on bacteria.

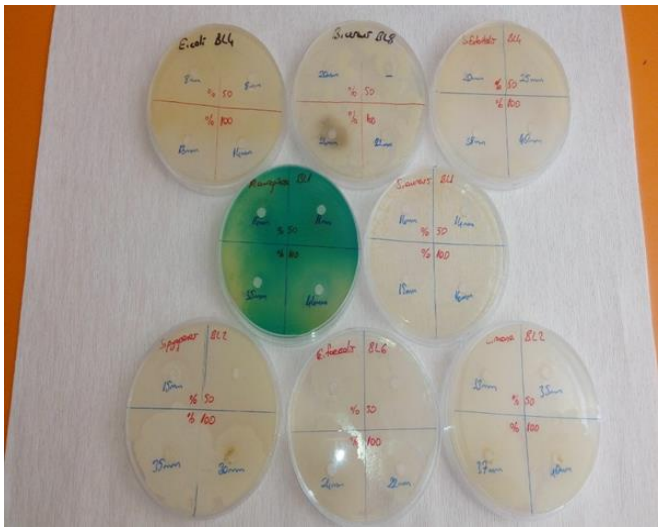

Figure 1. Some inhibition zones created by honey samples (HN1 indicated as BL1, HN2 indicated as BL2, HN4 indicated as BL4, HN6 indicated as BL6, HN8 indicated as BL8)

The samples HN1, HN2, HN4, HN5, HN6, and HN7 had no antimicrobial activity on *B. cereus* (ATCC 11778), the sample HN3 had no antimicrobial activity on *E. coli* (ATCC 27853) and the sample HN4 had no antimicrobial activity on *E. faecalis* (ATCC 29212). The honey samples other than the HN2 sample had no antimicrobial activity on *S. pyogenes* (ATCC 19615) (Table 1).

S. Enteritidis (ATCC 13076) was the strain in which apple vinegar had the best antimicrobial activity. It was determined that apple vinegar had no antimicrobial activity on *B. cereus* (ATCC 11778) and *S. pyogenes* (ATCC 19615). The difference between the antimicrobial activities of apple vinegar on *E. coli* (ATCC 27853), *E. faecalis* (ATCC 29212), *L. monocytogenes* (ATCC 7644), and *S. aureus* (ATCC 29213) was insignificant ($p>0.05$) (Table 1).

It was determined that the difference between the antimicrobial activities of the 100% concentrate of the samples HN1, HN4, and HN6 on *E. coli* (ATCC 27853) was significant ($p<0.05$). The antimicrobial activity of the honey samples other than HN6 on *E. faecalis* (ATCC 29212) was similar ($p>0.05$). The difference between the antimicrobial activities of 50% concentrates of the samples HN4, HN7 and HN8 on *S.* Enteritidis (ATCC 13076) was significant ($p<0.05$) (Table 1).

3.2. Antimicrobial activities of royal jelly samples

Table 2 shows the antimicrobial activities of 100% and 50% concentrates of the royal jelly samples.

Table 2. Antimicrobial activities of royal jellys

Bacteria strain	Inhibition zone (Mean ± Std. dev., mm)			
	RJ1		RJ2	
	100%	50%	100%	50%
***B. cereus* (ATCC 11778)**	13.00 ± 0.00^{Aa}	12.00 ± 0.00^{ACa}	10.00 ± 0.00^{Aac}	8.50 ± 0.55^{Ba}
***E. coli* (ATCC 25922)**	11.50 ± 0.55^{ADb}	10.00 ± 0.00^{ACDa}	8.00 ± 0.00^{BCac}	-
***E. faecalis* (ATCC 29212)**	21.00 ± 1.10^{ACc}	10.50 ± 0.55^{BEHa}	18.00 ± 0.00^{ADb}	9.00 ± 0.00^{BDGab}
***L. monocytogenes* (ATCC 7644)**	26.50 ± 0.55^{Ad}	19.00 ± 1.10^{Bb}	18.50 ± 1.64^{BEb}	10.00 ± 0.00^{CDb}
***S. aureus* (ATCC 29213)**	21.00 ± 1.10^{Ac}	11.50 ± 0.55^{Ba}	$13.00 \pm 3.23^{BCDFabcd}$	9.50 ± 0.55^{CFab}
***S.* Enteritidis (ATCC 13076)**	13.50 ± 0.55^{AEGa}	10.50 ± 0.55^{BCHa}	8.00 ± 1.10^{CFc}	-
***S. pyogenes* (ATCC 19615)**	-	-	-	-
***P. aureginosa* (ATCC 27853)**	32.00 ± 1.10^{ACe}	10.00 ± 1.00^{Ba}	19.50 ± 4.93^{BEFHabd}	-

*Upper case letters indicate the difference between columns in the same line, lower case letters indicate the difference between lines in the same column ($p<0.05$).
The difference between the mean values indicated by the same letter is insignificant ($p>0.05$).
(-): Inhibition zone was not detected.

RJ1 (100%), among the royal jelly samples, had the best antimicrobial activity. The 100% concentrate of the RJ1 sample suppressed the development of *P. aeruginosa* (ATCC 27853) with the inhibition zone of 32.00 ± 1.10 mm. RJ1 (100%) sample formed an inhibition zone of 26.50 ± 0.55 mm on *L. monocytogenes* (ATCC 7644) and RJ2 (100%) sample formed an inhibition zone of 18.50 ± 1.64 mm. RJ2 (100%) sample had the least antimicrobial activity on *E. coli* (ATCC 25922) and *S.* Enteritidis (ATCC 13076) with an inhibition zone of 8.00 ± 0.00 mm and an inhibition zone of 8.00 ± 1.10 mm, respectively (Table 2).

Figure 2 shows the photos of some inhibition zones formed by the RJ1 sample, that had the best antimicrobial activity among royal jelly samples on bacteria.

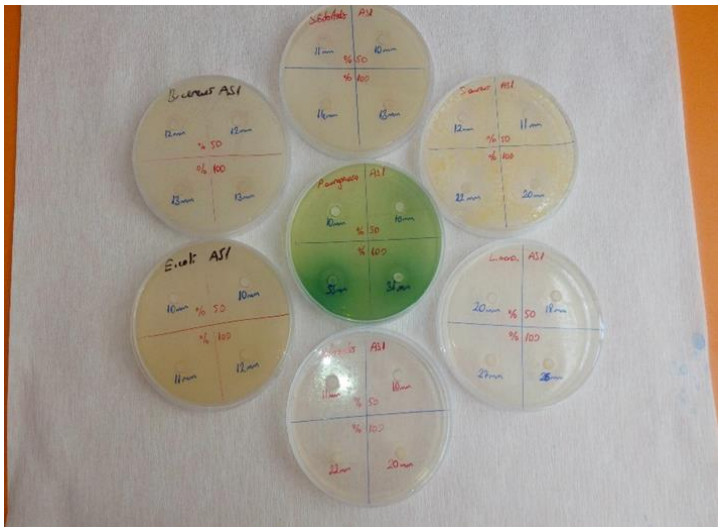

Figure 2. Some inhibition zones created by RJ1 (RJ1 indicated as AS1)

The difference between the antimicrobial activities of the 100% and 50% concentrates of RJ1 sample on *E. faecalis* (ATCC 29212) and *L. monocytogenes* (ATCC 7644) was significant ($p<0.05$) and the difference between their antimicrobial activities on *B. cereus* (ATCC 11778) and *E. coli* (ATCC 29212) was insignificant ($p>0.05$). It was found that the difference between the antimicrobial activity of the 100% and 50% of concentrates of RJ2 sample on *B. cereus* (ATCC 11778), *L. monocytogenes* (ATCC 7644), *S. aureus* (ATCC 29213), *S.* Enteritidis (ATCC 13076), and *P. aeruginosa* (ATCC 27853) was significant ($p<0.05$). It was determined that the difference between the antimicrobial activities of 50% concentrate of RJ1 and 100% concentrate of RJ2 on *L. monocytogenes* (ATCC 7644) was insignificant ($p>0.05$) (Table 2).

4. Discussion

The honey sample HN1 (100%) had the best antimicrobial activity by creating an inhibition zone of 39.50 ± 4.93 mm on *P. aeruginosa* (ATCC 27853). This result is quite higher than the inhibition zone of 12-25 mm determined on *P. aeruginosa* by Alan et al. [14] when they added a honey sample of 0.1 mL into the hollows to determine the antimicrobial activity of the honey samples from the different districts of Muş and Bitlis provinces. Aksoy and Digrak [19] researched the antimicrobial effect of the honey samples collected from the different districts of Bingöl province and the black-hive honey bought from a supermarket. It was found that honeys had 14-21 mm and 34 mm inhibition zone, respectively on *P. aeruginosa*. It was lower than the maximum inhibition zone determined in the present study, although they have put 0.1 mL of honey into the hollows.

P. aeruginosa which is resistant against many antimicrobial compounds [20] is protected from the host immune effectors in scar tissue, also it produces toxins degrading the fibrin and collagen molecules required for the tissue regeneration and therefore delays recovery of chronic injuries [21]. It is very important that all the honey and royal jelly samples in the present study were determined to have antimicrobial activity on this important bacterium (Table 1 and Table 2).

The sample HN5 (100%) that formed an inhibition zone of 7.00 ± 1.00 mm on *E. coli* (ATCC 27853) had the lowest antimicrobial activity (Table 1). It may be asserted that the formation of a smaller inhibition zone on *E. coli*, compared to the other bacteria is normal, as it is known to produce β-lactamase enzyme that is resistant to antibiotics [22].

The honey sample HN8 (100%) that formed an inhibition zone of 23.00 ± 1.10 mm had the best antimicrobial activity on *B. cereus* (ATCC 11778) (Table 1). It is a positive development that a significant antimicrobial effect has been determined to form on *B. cereus* that causes eye infections such as posttraumatic endophthalmitis, keratitis, and panophthalmitis as well as burn, wound and skin infections, meningitis, lower respiratory tract infections, endocarditis, bacteremia and sepsis [23].

The honey sample HN6 (100%) that formed an inhibition zone of 23.00 ± 1.10 mm had the best antimicrobial activity on *E. faecalis* (ATCC 29212) (Table 1). It was determined by Alan et al. [14] that antimicrobial activity on *E. faecalis* was an inhibition zone of 15-33 mm. This result may be considered as similar to the present study. However since honey of 50 µL (0.05 mL), as much as half of the amount used (0.1 mL) by Alan et al. [14], was put in the hollows in applied method by us, it may be asserted that HN6 sample

had a better antimicrobial effect on *E. faecalis* (Table 1). The antibiotic resistance of *E. faecalis* that threatens human life and causes, especially, hospital-acquired infections, is naturally at a high level, which increases its pathogenicity [24]. Both royal jelly and honey samples were determined to have an antimicrobial effect on *E. faecalis* (ATCC 29212) in the present study (Table 1 and Table 2) and this suggests that drugs to be developed from the active substances in these bee products may be used in the treatment of the relevant infections.

The sample HN2 (100%) that created the inhibition zones of 38.50 ± 1.64 mm and 32.50 ± 2.74 mm respectively, was the honey sample with the best antimicrobial activity on *L. monocytogenes* (ATCC 7644) and *S. pyogenes* (ATCC 19615) (Table 1). It is promising that the sample HN2 and the royal jelly samples have an effective antimicrobial activity on *L. monocytogenes* (Table 1 and Table 2) that causes meningitis, septicemia, conjunctivitis (inflammation of the transparent outer layer covering eyelids and eyeball), skin and mucosa localizations and monocytosis in the blood table (the increase of monocytes, included in blood, in number) [25].

S. pyogenes may cause scarlet fever by releasing some toxins related to throat infections [24] and the resistance it develops against penicillin and macrolide used in the treatment of the infections it causes poses an important problem [26]. The reason why the samples other than HN2 did not have an antimicrobial effect on this bacteria (Table 1 and Table 2) may be considered to be this resistance developed by *S. pyogenes* (ATCC 19615).

The honey sample HN6 (100%) that formed an inhibition zone of 39.00 ± 1.10 mm had the best antimicrobial activity on *S.* Enteritidis (ATCC 13076) (Table 1). *S.* Enteritidis is quite important as it is the most frequent (64.89%) serotype isolated from human clinical samples in *Salmonella* infections [27] and causes complications such as endovascular infection, bone and deep-organ apsis [28]. Within this context, it is a very valuable data that the finding of the present study indicated that the samples RJ1, HN1, HN2, HN3, and HN8 formed an inhibition zone of 12-15 mm on *S.* Enteritidis (ATCC 13076) (Table 1 and Table 2).

Ogbu et al. [29] determined based disc diffusion method that the inhibition zone on *E. coli* formed by the 250 mg/mL (25%) concentration of honey extract obtained from a region located in Nigeria was 6.70 ± 0.30 mm. On the other hand, 50 µL amount of the 100% concentrate of the sample HN7 had the best antimicrobial activity on *E. coli* (ATCC 27853) with the inhibition zone of 15.50 ± 0.55 mm in the present study (Table 1). The honey sample which had the best antimicrobial activity on *S. aureus* (ATCC 29213) was the sample HN1 (100%) formed an inhibition zone of 15.05 ± 0.55 (Table 1). This result was quite higher than the antimicrobial activity (inhibition zone of 7.70 ± 0.30 mm on *S. aureus*) of the 250 mg/mL (25%) concentration of honey extract [29]. It was determined by Erdogrul and Erbilir [30] that 9 of 21 honey samples, produced commercially in Kahramanmaraş, had an inhibition zone of 15-25 mm against *S. aureus.* This result may be considered to be similar to the present study (Table 1).

Alan et al. [14] and Aksoy and Digrak [19] observed that the honey samples formed inhibitions zones of 12-25 mm and 16-51 mm on *S. aureus,* respectively, and 12-32 mm and 14-35 mm on *E. coli,* respectively. This may be caused by the difference in the compound of the antimicrobial active substances from the flowers from which the honey samples were obtained and/or the fact that the amount of honey put into the hollows was

high (0.1 mL). The inhibition zone of 28 mm formed by black-hive honey obtained from a supermarket on *S. aureus* and the inhibition zones of 29 mm and 36 mm formed on *E. coli* by black-hive and comb honey [19] were quite higher than the antimicrobial effect seen on the same bacteria in the present study (Table 1). However, the inhibition zone of 14 mm formed on *E. coli* in the same study by the extracted honey [19] was similar to the antimicrobial effect of the honey samples HN2, HN4, and HN7 on the same bacteria in the present study (Table 1). Polat [31] found that the undiluted pure honey samples (100%) formed an inhibition zone of 14-15 mm on *S. aureus* when 100 µL (0.1 mL) of them were placed in the hollows. This was similar to the antimicrobial effect of the 100% concentrates of the samples HN1 and HN7 on the same bacteria in the present study (Table 1). It was observed in the same study [31] that the inhibition zone of 14-16 mm formed on *E. coli* by the pure honey samples was similar to the antimicrobial effect of the 100% concentrates of the samples HN2, HN4, and HN7 in the present study (Table 1).

Polat [31] determined that the undiluted pure honey samples (100%) had an inhibition zone of 14-16 mm on *L. monocytogenes* when they were put into the hollows in the amount of 100 µL (0.1 mL). This was similar to the antimicrobial effect of the 100% concentrate of the sample HN3 in the present study (Table 1). The inhibition zone of 12-16 mm formed on *P. aeruginosa* by the pure honey samples [31] was similar to the antimicrobial effect of the 50% concentrates of the samples HN1, HN2, HN5, and HN6 on the same bacteria in the present study (Table 1).

As in the present study, it has been considered in similar studies investigating the antibacterial activities of honey samples that the antibacterial effect of different honey concentrations may be different and the studies have been designed in this way. The effect of the differences in the types and amounts of the antimicrobial compounds obtained from the flora of the plant from which honey is obtained has an important effect on the antimicrobial effect of the honey. By this means, some of the different kinds of honey have the same activity on the same bacteria, some have a different activity on the same bacteria, and some have no activity.

Polat [31] observed that pine honey and wild strawberry honey have 300 mg/mL (30%) MIC (minimum inhibitory concentration) value and heather honey has 275 mg/mL (27.5%) MIC value against *P. aeruginosa.* This may be asserted to be similar only for the HN3 sample (Table 1). However, there are studies [32, 33] stating that manuka honey, obtained from manuka flowers in New Zealand, has 100 mg/mL (10%) MIC value [32] and 60 mg/mL (6%) MIC value [33] against some species of *P. aeruginosa*.

Obaseiki-Ebor et al. [34] stated in their first study on the antimicrobial activity of distilled honey that most of Gram (+) and Gram (–) bacteria were inhibited by a 40% concentration of honey. They reported in their following study [35] that the honey concentrations of 50% and higher inhibited the development of *E. coli, Vibrio cholerae, Yersinia enterocolitica, Plesiomonas shigelloides, Aeromonas hydrophila, Salmonella* Typhi, *Shigella boydi* and *Clostridium jejuni* efficiently. Malika et al. [36] found that the 25% and 50% (v/v) concentrations of the honey obtained from aromatic and medicinal plants inhibited most of the bacteria tested. It was also reported that the effect of the honey samples on Gram (–) bacteria was more compared to Gram (+) bacteria [36]. Dastouri et al. [37] stated that honey in concentrations of 40% and higher showed antibacterial activity against *Bacillus anthracis, B. cereus, Pasturella multicidae, Proteus vulgaris,*

and *S. aureus* and the best activity was obtained from the honey in the concentration of 80%.

Omafuvbe and Akanbi [38] examined the activity of 10 honey samples collected from different geographical regions of Nigeria on *Klebsiella pneumoniae (K. pneumoniae), E. coli, S. aureus, B. cereus, Proteus vulgaris, P. aeruginosa, Salmonella* spp., *Shigella* spp., and *Clostridium sporogenes (Cl. sporogenes*). The high concentrations (50-100%) of the honey samples tested in the study inhibited *B. cereus, K. pneumoniae* and *Cl. sporogenes.* On the other hand, they reported that the honey samples did not show inhibitory effects against *S. aureus and P. aeruginosa* [38].

Dogan [39] researched the antimicrobial activity of 27 honey samples obtained from Giresun, Kars, Bayburt, Erzurum, and Hakkari provinces. It was determined that the honey obtained from Bayburt province had the best antimicrobial activity with the inhibition zones of 7.50 mm against *E. coli* (BC 1402), 9.00 mm against *B. cereus* (ATCC 33019), and 14.00 mm against *S. aureus* (ATCC 29213) [39]. The antibacterial activities of Bayburt honey [39] and HN6 sample (100%) on *E. coli* and also the antibacterial activities of Bayburt honey [39] and the samples HN7 (100%) and HN1 (50%) on *S. aureus* (ATCC 29213) were similar (Table 1).

Karadal et al. [40] examined the antibacterial activity of the multi-floral kinds of honey obtained from Central Anatolia and Black Sea region and the chestnut and pine honey obtained from supermarkets based on the agar well method. It was observed that the values of the honey samples of the Black Sea region with the antibacterial activity (6.0 ± 1.7 mm and 4.2 ± 0.8 mm inhibition zone respectively) which is the best against *L. monocytogenes* (ATCC 15313) and *S.* Enteritidis (ATCC 13311) are lower [40] than the values of the honey samples in the same bacteria in the present study (Table 1). Also, the activity of the chestnut honey with the best antibacterial activity (inhibition zone of 9.5 ± 3.9 mm) against *S. aureus* (ATCC 29213) was lower [40] than the antibacterial activity of the honey samples in the present study apart from the samples HN2 and HN6 (Table 1).

Khalil et al. [41] assessed that the zone diameters larger than 18 mm were significant, those between 16-18 mm were good, those between 13-15 mm were low, those between 9-12 mm were insignificant and those below 8 mm signified no activity. It was determined in the same study [41] that the honey samples had low activity against *E. coli* (14.40 ± 0.45 mm). Accordingly, it is very important that an inhibition zone of $\geq$18 mm was formed on *B. cereus* (ATCC 11778) by 100% and 50% concentrates of HN8, on *E. faecalis* (ATCC 29212) by 100% concentrate of RJ1 and RJ2 and 100% concentrate of HN6, on *L. monocytogenes* (ATCC 7644) by 100% concentrates of RJ1 and RJ2, 50% concentrate of RJ1 and 100% and 50% concentrates of HN2, on *S. aureus* (ATCC 29213) by 100% concentrates of RJ1, on *S.* Enteritidis (ATCC 13076) by 100% and 50% concentrates of HN4, on *S. pyogenes* (ATCC 19615) by the 100% concentrates of HN2, on *P. aeruginosa* (ATCC 27853) by the 100% concentrates of RJ1 and RJ2, 100% concentrate of RJ2, by 100% concentrates of HN1, HN2, HN5 and HN6, and by 100% and 50% concentrates of HN3, HN4, HN7 and HN8 (Table 1 and Table 2).

The 50% concentrates of the samples HN1, HN2, HN5, and HN6 had no antimicrobial activity on *E. coli* (ATCC 27853), the 50% concentrates of the samples HN1, HN4, HN5, HN6, and HN7 had no antimicrobial activity on *L. monocytogenes* (ATCC 7644), and the

50% concentrates of the samples HN1, HN2, HN3, HN5, and HN6 had no antimicrobial activity on *S.* Enteritidis (ATCC 13076) (Table 1). Some of the honey samples were determined to have no antimicrobial effect on some of the bacteria tested in the studies of Alan et al. [14] and Aksoy and Digrak [19]. This is compatible with the present study.

RJ1 (100%) sample suppressed the development of *S. aureus* (ATCC 29213) with an inhibition zone of 21.00 ± 1.10 mm. This effect had also become the best antimicrobial effect observed on *S. aureus* (ATCC 29213) (Table 2). As *S. aureus* rapidly gains resistance against the antibiotics used [42] the treatment of both the hospital and the community-acquired infections it causes is quite challenging [43]. Thanks to this positive effect of the RJ1 sample, the active substance responsible for the antimicrobial effect in royal jelly may be purified and used in developing new antibiotics for the treatment of *S. aureus* based diseases.

It was determined among the 50% concentrates of the royal jelly that the RJ1 sample formed the best inhibition zone (19.00 ± 1.10 mm) on *L. monocytogenes* (ATCC 7644) (Table 2).

It has been reported that royal jelly has a powerful antibiotic effect against *E. coli, Salmonella, Proteus, Bacillus subtilis* and *S. aureus* bacteria [44]. It is similar to the present study (Table 2) that 1000 mg/mL concentrate of the pure royal jelly created an inhibition zone of 20 mm against *S. aureus* (ATCC 14776) and an inhibition zone of 12 mm against *E. coli* (ATCC 29532) [45].

Ramanathan et al. [46] researched the antibacterial effect of the royal jelly obtained from Kerala city of India against *E. coli* MTCC40, based on the hollow agar method. They prepared 20 mg/mL concentration of the royal jelly with sterile water and inoculated it into the wells of 4 mm at the amounts of 500 µg and 1000 µg. As a result of the inoculation of 1000 µg, the inhibition zone of 5 mm determined on *E. coli* (MTCC40) was quite lower [46] than the value determined in the present study (Table 2).

Garcia et al. [47] examined the antibacterial activity of the royal jelly obtained from two different regions of Argentina, by well diffusion method, against *S. aureus* MS 1 (ATCC 25923), *E. faecalis* 1 (ATCC 29212), *E. coli* isolated from well water, *P. aeruginosa* isolated from a patient with catheter infection, *E. faecalis* 2 isolated from the skin infections in humans, *S. aureus* MS 2, *S. aureus* MR 1 and *S. aureus* MR 2 isolated from cows with mastitis. 1 mL of bacterial culture was added to 30 mL of Mueller Hinton Agar medium and placed into 90 mm of petri dish and then 4 mm of wells were burrowed and pure royal jelly and 50 µL of its concentrations of 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, and 10% were put into these wells. The undiluted 1st royal jelly sample created an inhibition zone of 14.7 ± 0.6 mm on *S. aureus* MR 1, the undiluted 2nd royal jelly sample created an inhibition zone of 5.7 ± 0.6 mm on *P. aeruginosa*, 9.7 ± 0.6 mm on *E. coli*, 10.7 ± 0.6 mm on *E. faecalis* 2, 12.0 ± 0.0 mm on *E. faecalis* 1, 20.7 ± 0.6 mm on *S. aureus* MR 2, 18.7±0.6 mm on *S. aureus* MS 2, 15.7 ± 0.6 mm on *S. aureus* MS 1 [47]. These values were lower than the inhibition zone diameters of the RJ1 sample on the same bacteria (Table 2). It was determined in the same study [47] that the 50% concentration of the 2nd royal jelly sample had an antibacterial effect on *S. aureus* MR 1, *S. aureus* MS 1 and *S. aureus* MS 2. The 50% concentration of the 1st royal jelly sample did not have an antibacterial effect on the other bacteria (*E. coli, P. aeruginosa, E. faecalis* 2, *E. faecalis* 1, *S. aureus* MR 2, *S. aureus* MR 1, *S. aureus* MS 1) apart from *S. aureus*

MS 2 [47] and 100% and 50% concentrates of RJ1 and RJ2 did not have antibacterial effect on *S. pyogenes* (ATCC 19615) and 50% concentrate of RJ2 did not have antibacterial effect on *E. coli* (ATCC 25922), *S.* Enteritidis (ATCC 13076), and *P. aeruginosa* (ATCC 27853) in the present study (Table 2).

As in the present study, different studies have been conducted proving that royal jelly has an antibacterial effect on *B. cereus* [48], *E. faecalis* [49], *E. coli* [47-49, 50, 51], *P. aeruginosa* [47-49, 52, 53] and *S. aureus* [47-51, 53, 54]. It has been reported that RJ has antibacterial activity against both Gram (+) and Gram (–) bacteria mainly due to fatty acids present in RJ, such as trans-10-hydroxydec-2-enoic acid, 3-hydroxydodecanoic acid, 11-oxododecanoic acid, and 11-S-hydroxydodecanoic acid [55, 56]. Furthermore, a series of short peptides (jelleines, royalisin) present in RJ has also been shown to possess strong antibacterial properties against Gram (+) and Gram (–) bacteria [11, 56-58].

The peptide RJ has formerly shown total inhibition of bacterial growth for *S. aureus, L. monocytogenes* at very high concentrations (≥200 μg/mL). An additional constituent, 10 hydroxy delta decanoic acid (10 HDA), the major component of the lipid fraction of RJ occupies 10% of the total weight of RJ. Earlier studies indicating that the antimicrobial effect and minimum dosage of 10 HDA for various pathogenic microbes were *E. coli* 0.625 mg/mL and *S. aureus* 2.5 mg/mL, showed that 10 HDA could effectively inhibit the growth of bacterium [58]. It has been proven that the royalisin isolated from royal jelly has antibacterial effect against *P. aeruginosa* and *S. aureus* [53] and jelleines has antibacterial effect against *E. coli, P. aeruginosa* [11], *L. monocytogenes* [59] and *S. aureus* [11, 59], and 10-hydroxy-2-decanoic acid has antibacterial effect against *S. aureus* [49, 54], *E. coli* [54], and *E. faecalis* [49].

In conclusion, according to the findings obtained in the study, it was revealed that the honeys of Bitlis and royal jellys of Bitlis & Ağrı have a potential to using supportive of the medical treatment of some of the infectious diseases and they may be have a place in apitherapy applications. It may be develop new drugs using the active substances of bee products, obtained from these regions, forming the antimicrobial effect, in order to treat some infectious diseases. Because, since the bee products are natural nutrients that does not contain the harmful chemicals contained in medicines, they have an advantage in medical treatment and health protection.

Acknowledgements

We would like to thank Tubitak BIDEB for supporting this study with the project number 1919B01160321.

References

[1] Republic of Turkey Ministry of Health, The regulation on traditional and complementary medicine practices, (2014). http://www.mevzuat.gov.tr/MevzuatMetin/yonetmelik/7.5.20164-ek.pdf, (18.01.2022)

[2] Ozturk, O. and Selcuk, M. Y., Apitherapy in primary care, **Turkish Journal of Family Medicine and Primary Care, 10**, 3, 124–125, (2016).

[3] Hisil, Y. and Borekcioglu, N., The composition of honey and honey tricks, **Gıda (The Journal of Food), 2**, 79–82, (1986).

[4] Mundo, M. A., Padilla-Zakour, O. I. and Worobo, R. W., Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys, **International Journal of Food Microbiology, 97**, 1, 1–8, (2004).

[5] Molan, P. C., The evidence supporting the use of honey as a wound dressing, **International Journal of Lower Extremity Wounds, 5**, 1, 40–54, (2006).

[6] Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U. and Molan, P. C., Medical honey for wound care-still the 'latest resort'?, **Evidence-based Complementary and Alternative Medicine, 6**, 2, 165–173, (2008).

[7] Molan, P. C., Honey as an antimicrobial agent, **Proceedings, International Conference** on Bee Product: Properties, Applications and Apitherapy Israel, p. 27, (1997).

[8] Jeffrey, A. E. and Echazarreta, C. M., Medicinal uses of honey, **Revista Biomedica, 7**, 1, 43–49, (1996).

[9] Aslan, A. and Bayraktar, A., Chemical composition and importance of royal jellys, **Proceedings, Congress** of Food Engineering Gaziantep, Turkey, p. 339–349, (1996).

[10] Ozturk, O., Health effects of bee products, (2012). http://www.balder.org.tr/sunumlar/AUSUE_Semineri_Prof_Dr_Oguz_Ozturk_sunumu_(No_13).pdf, (05.01.2022).

[11] Fontana, R., Mendes, M. A., de Souza, B. M., Konno, K., César, L. M., Malaspina, O. and Palma, M. S., Jelleines: A family of antimicrobial peptides from the royal jelly of honeybees (*Apis mellifera*), **Peptides, 25**, 6, 919–928, (2004).

[12] Cagliyan, A., Beekeeping activities in Bitlis, **Journal of Geography, 30**, 1–25, (2015).

[13] Kaya, F., Structure of beekeeping in Ağrı and evaluation status, **The Journal of Social Sciences Institute of Ataturk University, 12**, 2, 35–55, (2008).

[14] Alan, Y., Atalan, E., Erbil, N., Bakir, O., Orman, Z. and Panik, P., Investigation of antimicrobial activity of honey and propolis collected in Mus and Bitlis region, **Mus Alparslan University Journal of Science, 2**, 1, 221–229, (2014).

[15] Collins, C. H. and Lyne P. M., Microbiological methods, Butterworth and Co. (Publishers) Ltd., London, UK, (1985).

[16] Anonymous, NCCLS (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial susceptibility testing, The 9th International Supplement; M100-S9, Villanova, PA, United States Commonwealth of Pennsylvania, (1999).

[17] Valgas, C., Souza, S. M. D., Smânia, E. F. and Smânia, Jr. A., Screening methods to determine antibacterial activity of natural products, **Brazilian Journal of Microbiology, 38**, 2, 369–380. (2007). doi.org/10.1590/S1517-83822007000200034

[18] Anonymous, IBM SPSS Statistics for Windows, Version 25.0. New York, USA: IBM Corp, Armonk, (2017).

[19] Aksoy, Z. and Digrak, M., In vitro studies on antimicrobial effects of honey and propolis gathered in Bingol region, **Fırat University Turkish Journal of Science and Technology, 18**, 4, 471–478, (2006).

[20] Olaitan, P. B., Adeleke, O. E. and Ola, I. O., Honey: a reservoir for microorganisms and an inhibitory agent for microbes, **African Health Sciences, 7**, 3, 159–165, (2007).

[21] Scmidtchen, A., Holst, E., Topper, H., Bjorck, L., Elastase–producing *Pseudomonas aeruginosa* degrade plasma proteins and extracellular products of human skin and fibroblasts, and inhibit fibroblast growth, **Microbial Pathogenesis, 34**, 1, 47–55, (2003).

[22] Bilgehan, H., Clinical microbiology (special bacteriology), Baris Publications, Izmir, Turkey, (2000).

[23] Mengeloglu, F. Z., Terzi, H. A. and Bilici, M., A case report with catheter caused *Bacillus cereus* bacteremia and investigating the clonal relatedness between the isolates by PFGE, **Dicle Medical Journal, 38**, 3, 358–360, (2011).

[24] Ryan, K. J. and Ray, C. G., (2004) Sherris medical microbiology (4th ed.), Mc Graw Hill, New York, USA, (2004).

[25] Goulet, V., Jacquet, C., Martin, P., Vaillant, V., Laurent, E. and de Valk, H., Surveillance of human listeriosis in France, 2001-2003, **Euro Surveillance, 11**, 6, 79–81, (2006).

[26] Kara, A., Parlakay, A. O., Gur, D., Cengiz, A. B., Tezer, H., Ciftci, E., Keser, M., Ozen, M., Kantaroglu, O. C., Tutanc, M., Salihoglu, B., Yuksekkaya, S., Celikel, E., Ince, E., Arica, V., Hatipoglu, S., Odabas, D., Altay, F., Karbuz, A. and Ceylan, M., Turkey resistance evaluation pilot study results of group a beta hemolytic *Streptococcus*, **Journal of Pediatric Infection, 5**, 3, 96–99, (2011).

[27] Erdem, B., *Salmonella* serotyped in 1998-2000, **Turkish Journal of Infection, 15**, 137–140, (2001).

[28] Acheson, D. and Hohmann, E. L., Nontyphoidal salmonellosis, **Clinical Infectious Diseases, 32**, 2, 263–269, (2001).

[29] Ogbu, K. I., Ochai, S. O., Olabode, M. P., Olaolu, O.S. and Maimadu, A. A., Comparative study on the antibacterial activities of bee product (propolis, pollen, bee wax and honey), **IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS), 13**, 2-II, 20–27, (2018). doi: 10.9790/3008-1302022027

[30] Erdogrul, O. and Erbilir, F., Investigation of microbial quality and antimicrobial effects of honey samples produced in Kahramanmaras, **Kahramanmaras Sutcu Imam University Journal of Engineering Sciences, 10**, 1, 1–5, (2007).

[31] Polat, I., Investigation of antimicrobial, antioxidant activities, pesticide and antibiotic residues of some honey produced in the south Marmara region, MSc Thesis, Balikesir University, Institute of Science, Balıkesir, Turkey, (2011).

[32] Cooper, R., The use of honey as an antiseptic in managing *Pseudomonas* infections, **Journal of Wound Care, 8**, 4, 161–164, (1999).

[33] Cooper, R. A., Halas, E. and Molan, P. C., The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns, **Journal of Burn Care & Rehabilation, 2**3, 6, 366–370, (2002).

[34] Obaseiki-Ebor, E. E., Afonya, T. C. A. and Onyekweli, A. O., Preliminary report on the antimicrobial activity of honey distillate, **Journal of Pharmacy and Pharmacology, 35**, 11, 748–749, (1983).

[35] Obaseiki-Ebor, E. E. and Afonya, T. C. A., In vitro evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of some antimycotic agents, **Journal of Pharmacy and Pharmacology, 36**, 4, 283–284, (1984).

[36] Malika, N., Mohamed, F. and Chakib, E. A., Antimicrobial activities of natural honey from aromatic and medicinal plants on antibio-resistant strains of bacteria, **International Journal of Agriculture & Biology, 6**, 2, 289–293, (2004).

[37] Dastouri, M. R., Fakhimzadeh, K., Shayeg, J., Dolgari-Sharaf, J., Valilou, M. R., Maheri-Sis, N., Evaluating antibacterial activity of the Iranian honey through MIC

method on some dermal and intestinal pathogenic bacteria, **Journal of Animal and Veterinary Advances, 7**, 4, 409–412, (2008).

[38] Omafuvbe, B. O. and Akanbi, O. O., Microbiological and physico-chemical properties of some commercial Nigerian honey, **African Journal of Microbiology Research, 3**, 12, 891–896, (2009).

[39] Dogan, H., Determination of chemical, physical and antimicrobial properties of flower honeys, MSc Thesis, Ataturk University, Institute of Science, Erzurum, Turkey, (2014).

[40] Karadal, F., Ertas Onmaz, N., Abay, S., Yildirim, Y., Al, S., Tatyuz, I. and Akcay, A., A study of antibacterial and antioxidant activities of bee products: propolis, pollen and honey samples, **The Ethiopian Journal of Health Development, 32**, 2, 116–122, (2018).

[41] Khalil, A. T., Khan, I., Ahmad, K., Khan, Y. A., Khan, M. and Khan, M. J., Synergistic antibacterial effect of honey and Herba Ocimi Bacilici against some bacterial pathogens, **Journal of Traditional Chinese Medicine, 33**, 6, 810–814, (2013).

[42] Pesavento, G., Ducci, B., Comodo, N. and Lo Nostro, A., Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA), **Food Control, 18**, 3, 196–200, (2007).

[43] Oncul, O., Erdemoglu, A., Ozsoy, M. F., Altunay, H., Ertem, Z. and Cavusoglu, S., Nasal *Staphylococcus aureus* carriage in healthcare staff, **Klimik Journal, 15**, 3, 74–77, (2002).

[44] Garcia-Amoedo, L. H. and de Almeida-Muradian, L. B., Determination of trans-10-hydroxy-2-decanoic acid (10-HDA) in royal jelly from São Paulo State, Brazil, **Food Science and Technology (Campinas), 23**, 62–65, (2003).

[45] Eshraghi, S. and Seifollahi, F., Antibacterial effects of royal jelly on different strains of bacteria, **Iranian Journal of Public Health, 32**, 1, 25–30, (2003).

[46] Ramanathan, A. N. K. G., Krishna, A., Nair, A. J. and Sugunan, V. S., Antimicrobial activity of royal jelly from Indian honeybee, *Apis cerana*, **International Journal of Allied Practice, Research and Review, V**, II, 07–12, (2018).

[47] Garcia, M. C., Finola, M. S. and Marioli, J. M., Antibacterial activity of royal jelly against bacteria capable of infecting cutaneous wounds, **Journal of ApiProduct and ApiMedical Science, 2**, 3, 93–99, (2010). doi: 10.3896/IBRA.4.02.3.02

[48] Ratanavalachai, T. and Wongchai, V., Antibacterial activity of intact royal jelly, its lipid extract and its defatted extract, **Thammasat International Journal of Science and Technology, 7**, 1, 5–12, (2002).

[49] Garcia, M. C., Finola, M. S. and Marioli, J. M., Bioassay directed identification of royal jelly's active compounds against the growth of bacteria capable of infecting cutaneous wounds, **Advances in Microbiology, 3**, 138–144, (2013). http://dx.doi.org/10.4236/aim.2013.32022

[50] Boukraa, L., Meslem, A., Benhanifia, M., Hammoudi, S. M., Synergistic effect of starch and royal jelly against *Staphylococcus aureus* and *Escherichia coli*, **The Journal Alternative and Complementary Medicine, 15**, 755–757, (2009). http://dx.doi.org/10.1089/acm.2008.0483

[51] Moselhy, W. A., Fawzy. A. M. and Kamel, A. A., An evaluation of the potent antimicrobial effects and unsaponifiable matter analysis of the royal jelly, **Life Science Journal, 2**, 10, 290–296, (2013).

[52] Boukraa, L., Additive activity of royal jelly and honey against *Pseudomonas aeruginosa*, **Alternative Medicine Review, 13**, 4, 330–333, (2008).

[53] Bilikova, K., Huang, S. C., Lin, I. P., Simuth, J. and Peng, C. C., Structure and antimicrobial activity relationship of royalisin, an antimicrobial peptide from royal jelly of *Apis mellifera*, **Peptides, 68**, 190–196, (2015). http://dx.doi.org/10.1016/j.peptides.2015.03.001

[54] Eshraghi, S., An evaluation of the potent inhibitory effects of royal jelly fractions against *Streptomyces* bacteria, **Pakistan Journal of Medical Sciences, 21**, 1, 63–68, (2005).

[55] Melliou, E. and Chinou, I., Chemistry and bioactivity of royal jelly from Greece, **Journal of Agricultural and Food Chemistry, 53**, 23, 8987–8992, (2005). doi: 10.1021/jf051550p

[56] Alreshoodi, F. M. and Sultanbawa, Y., Antimicrobial activity of royal jelly, **Anti-Infective Agents, 13**, 1, 50–59, (2015). doi: 10.2174/22113525136661503182344 30

[57] Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T. and Kobayashi, K., A potent antibacterial protein in royal jelly purification and determination of the primary structure of royalisin, **The Journal of Biological Chemistry, 265**, 19, 11333–11337, (1990).

[58] Biliková, K., Hanes, J., Nordhoff, E., Saenger, W., Klaudiny, J. and Šimúth, J., Apisimin, a new serine–valine-rich peptide from honeybee (*Apis mellifera* L.) royal jelly: purification and molecular characterization, **FEBS Letters, 528**, 1–3, 125–129, (2002).

[59] Romanelli, A., Moggio, L., Montella, R. C., Campiglia, P., Iannaccone, M., Capuano, F., Pedone, C. and Capparelli, R., Peptides from royal jelly: studies on the antimicrobial activity of jelleins, jelleins analogs and synergy with temporins. **Journal of Peptide Science, 17**, 5, 348–352, (2011). http://dx.doi.org/10.1002/psc.1316