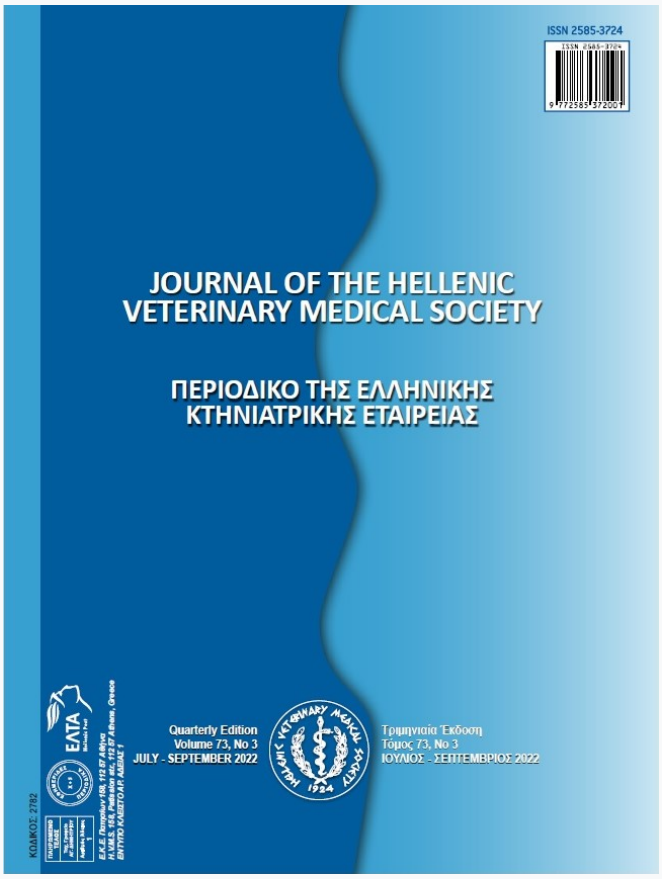
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



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doi: 10.12681/jhvms.27497

To cite this article:

Erkan, C., Kara, Y., Keskin, M., Ertürk, Ö, Gösterit, A., & Kolaylı, S. (2022). Determination of Bioactivity and Antimicrobial Activity of Bumblebee (Bombus terrestris L.) Brood Cover Wax Material. *Journal of the Hellenic Veterinary Medical Society*, *73*(3), 4485–4492. https://doi.org/10.12681/jhvms.27497

J HELLENIC VET MED SOC 2022, 73 (3): 4485-4492
ΠΕΚΕ 2022, 73 (3): 4485-4492

Research article
Ερευνητικό άρθρο

Determination of Bioactivity and Antimicrobial Activity of Bumblebee (*Bombus terrestris L.*) Brood Cover Wax Material

C. Erkan [1], Y. Kara[2], M. Keskin[3], Ö.Ertürk [4], A.Gösterit [5], S. Kolaylı [2]

[1]*Department of Animal Science, Faculty of Agriculture, Yuzuncu Yil University, Van, Turkey*

[2]*Department of Chemistry, Faculty of Science, Karadeniz Technical University, Trabzon, Turkey*

[3]*Vocational School of Health Services, Bilecik Seyh Edebali University, Bilecik, Turkey*

[4]*Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Ordu University, Ordu, Turkey*

[5]*Department of Animal Science, Faculty of Agriculture, Isparta Unıversıty of Applied Sciences, Isparta, Turkey*

ABSTRACT: Bumblebee (*Bombus terrestris L.*) is a bee species that take part in pollination. Although Bumblebee (*Bombus terrestris L.*) is thought to be used only in pollination but, it is not only take part in pollination. It could be used to produce different products. Brood cover wax material is a kind of bumblebee product that produced by Bumblebee (*Bombus terrestris L.*) in order to cover their nest. Although it is considered to be waste for human health, brood cover wax material is an important hive product with its antioxidant and antimicrobial activities. In this study, Bumblebee brood cover wax material was produced under laboratory conditions in a controlled manner. Biochemical characterization of brood cover wax material obtained from five different colonies was performed and antimicrobial activities were determined. Accordingly, it was determined that the total phenolic content of the samples ranged between 3.778±0.165 and 9.504±0.353 mg GAE/g. In addition, it was observed that the samples were rich in p-coumaric acid, luteolin, quercetin, t-sinnamic acid, chrysin and pinocembrin components. Obtained results showed that brood cover wax material, which also possessed antimicrobial activity, had nearly equivalent activity to *Apis mellifera* L. beehive products such as honey, pollen and bee bread. The data obtained could be concluded that brood cover wax material could be used an alternative product for human health applications.

Keywords: Bumblebee; brood cover; total phenolic; antibacterial activity; phenolics

Corresponding Author:
M. Keskin, Vocational School of Health Services, Bilecik Seyh Edebali University, Bilecik, Turkey
E-mail address: merveozdemirkeskin@gmail.com

Date of initial submission: 14-07-2021
Date of acceptance: 04-04-2022

INTRODUCTION

Taxonomically, bumblebees, which are in the Bombinae subfamily of the Apidae family from the Hymenoptera order, are important pollinators for both agricultural products and wild flowering plants in the natural flora (Demirsoy, 2001). Due to their long tongues, dense feathers, large body structures and vibratory pollination behaviors, these bees provide more effective pollination than honey bees, especially in tomatoes and flowers belonging to the Solanaceae family and with deep corolla (de Luca and Vallejo-Marin, 2013; Wahengbam et al., 2019). For this reason, the number of countries and colonies where bumblebees are used increased year by year.

While honey bees (*Apis mellifera* L.) produce products such as honey, pollen, propolis, beeswax, royal jelly and bee venom in addition to their significant contribution through pollination, the only economically known contribution of bumblebees is pollination. In order to get more benefits from bumblebees, bumblebee breeding techniques have been developed under controlled conditions (Gosterit and Gurel, 2018). As a result of the studies carried out for this purpose, the behavior of covering the nest area with a thin wax cover is frequently observed in the colonies, especially when the temperature of the rearing environment decreases.

It is known that some wasp species use similar nesting material in their natural habitats and this material may contain materials such as mud, sand, plant parts and resin, depending on the species (Williams and Goodell, 2000; Cane et al., 2009). Bumblebee (*Bombus terrestris L.*) brood cover wax material is produced nearly in a week by bumblebee and when a bumblebee colony was ends its life for any reason brood cover wax material becomes waste.

Apitherapy is an important treatment method in traditional and complementary medicine practices. Natural hive products such as honey, propolis, pollen, bee bread and royal jelly produced by honey bees are used in Apitherapy. Recently, interest in bee products has increased due to diseases such as Covid 19, cancer, ulcer, etc. Because bee products have different biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer (Münstedt and Bogdanov, 2009; Alvarez-Suarez, 2017; Pasupuleti, et al., 2017; Keskin et al., 2021; Narimaneet al., 2021). Brood cover wax material is a natural bee product that produced by bumblebees. However, this cover material is also produced in the colonies in the breeding of *Bombus terrestris L.*in completely closed laboratory conditions where there is no foraging activity of the colony individuals. Therefore, it is important to assess the biochemical characterization of this cover material, which is produced entirely by in-colonial individuals, to determine its usability in apitherapy applications and to raise awareness about evaluating this material as an alternative product. Thus, in this study, biochemical characterization of brood cover wax material that becomes waste after the death of a bumblebee colony was determined. This study is the first study in which the biochemical characterization of the material has been performed. Antioxidant and antimicrobial activities of the brood cover wax material produced in *B. terrestris* colonies were determined. Results were compared with literature data of *Apis mellifera* L. beehive products such as honey, pollen, bee bread (perga) and propolis that used in Apitherapy applications.

MATERIAL AND METHOD

Production of Brood Cover Wax Material

Cover material was obtained from *Bombus terrestris* colonies reared in a completely closed environment and under laboratory conditions where there is no foraging activity of individuals (Figure 1). According to our personal observations for many years, the decrease in the ambient temperature, the colonies covered the nest area with a thin wax cover. For this reason, in the study, four *B. terrestris* colonies were transferred to a lower temperature (temperature: 15-16 °C, relative humidity: 50-55%) from a rearing cabinet with standard rearing conditions (temperature: 27-28 °C, relative humidity: 50-55%) (Yoon et al., 2002; Gosterit and Gurel, 2016). For this purpose, it was paid attention to have one healthy queen, approximately 90-100 worker bees and a large hatching area in the selected colonies. Colonies were fed ad libitum with fresh frozen pollen and sugar syrup (50 Brix). One week after their transfer to the cold environment, the cover material knitted over the brood area in the colonies was taken and analyzed. Five different brood cover wax material were analyzed (Figure 2).

Extraction of Brood Cover Wax Material

Frozen raw brood cover wax material sample was grinded, and 5g of powdered raw sample was dissolved in 50 ml 70 % ethanol in a glass flask (500 ml), stirred on a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 48 hours and after filtration, the extract was evaporated with a Ro-

Figure 1. Bumblebee nest box

Figure 2. Brood cover wax materials

tary evaporator (Heidolph Hei-VAP Value Digital G3) and stored at -20 °C (Keskin and Kolaylı, 2018).

Determination of Total Phenolic Content

Total phenolic content of nest cover samples was determined according to Slinkardand Singleton (1977). The basis of the determination of the total phenolic content by this method is based on the redox reaction in which phenolic compounds reduce the Folin-Ciocalteu reagent in basic medium and transform themselves into the oxidized form. The Folin-Ciocalteu reagent acts as the oxidizing compound here. By measuring the absorbance of the blue color formed by the reduced reagent as a result of the reaction, it is possible to calculate the total amount of phenolic compounds in the sample. The color intensity of the complex formed is directly proportional to the concentration of phenolic content and gives maximum absorbance at 760 nm. The mixture of the reaction (20 µLsample, 680 µL distilled water, 400 µL 0.5 N Folin reagent, after vortex 400 µL of 10% sodium carbonate added) incubated in a dark place for 30 min then the absorbances at 760 nm were recorded. Gallic acid at different concentrations (1.0; 0.5; 0.25; 0.125; 0.0625 and 0.025 mg/ ml) was used as standard for calibration curve. Results were expressed as mg GAE/g. Analyses were performed in triplicate.

Determination of Total Flavanoid Content

Total flavonoid content was determined according to Fukumoto and Mazza (2000). Quercetin (QE) at different concentrations (0.25; 0.125; 0.0625; 0.03125; 0.015625 and 0.0078125 mg/ml) was used. The absorbance of the tubes against distilled water at

415 nm was recorded 40 minutes after the pipetting was finished. The calibration curve was drawn with the recorded absorbance values versus the concentration (y=4.4349x+0.0325, R^2: 0.9991). The total flavonoid content of brood cover wax material extracts was calculated according to the drawn standard graph, and the total amount of flavonoids was expressed as mg QE/ml nest cover extract. Analyses were performed in triplicate.

Ferric Reducing Power (FRAP)

The FRAP method is the most commonly used method for the determination of the antioxidant capacity of natural products, and it is a method based on the reduction of iron (III) ion in the Fe(III)-TPTZ complex of antioxidant substances and hydrogen transfer (Benzie and Strain, 1999). Fe (III) reduced by the antioxidant substances in the solution gives maximum absorbance at 593 nm. The FRAP reagent consists of mixture of 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ and 300 mM acetate buffer (pH3.6), in 1:1:10 ratio respectively. The calibration curve (y=0.0005x-0.0229, R2: 0.9944) was created using the Trolox standard in different volumes (1000-500-250-125-62.5 µM). 50 µl of brood cover wax material extract and standard Trolox solutions were vortexed with FRAP reagent (1.5ml) and were kept at room temperature for 20 minutes. The absorbance of the tubes were recorded at 593 nm. Results were expressed in terms of mmol $FeSO_4.7H_2O$/g brood cover wax material. Analyses were performed in triplicate.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•) Antioxidant Assay

The DPPH radical (2,2-diphenyl-1-picrylhydrazil) is a commercially available radical and a 100 µM methanol solution of this radical is used in trials. Samples in different concentrations were prepared by diluting the stock solution with methanol. An equal volume (750 µl) of DPPH solution and sample solutions were mixed and left at room temperature for 50 minutes. At the end of the period, absorbance was recorded at 517 nm, where DPPH gives maximum absorbance. Absorbance of the samples were plotted against corresponding concentrations and SC_{50} value was calculated and expressed against the trolox standard (0.000625 to 0.02 mg/ml) (Cuendent et al., 1997). The antioxidant capacity was expressed

Table 1. Validation parameters of HPLC-UV

Standards	R^2	Limit of Detection (LOD) (µg/ml)	Limit of Quantification (LOQ) (µg/ml)
Gallic acid	0.9984	0.0099	0.0331
Protocatechuic acid	0.9986	0.0042	0.0139
Chlorogenic acid	0.9975	0.0199	0.0662
P-OH benzoic acid	0.9988	0.0309	0.1031
Epicatechin	0.9991	0.0569	0.1896
Caffeic acid	0.9991	0.0859	0.2865
Syringic acid	0.9986	0.0203	0.0676
M-OH benzoic acid	0.9997	0.0074	0.0247
Routine	0.9991	0.0838	0.2793
Ellagic acid	0.9998	0.0896	0.2988
P-coumaric acid	0.9981	0.0333	0.1108
Ferulic acid	0.9982	0.0196	0.0653
Myricetin	0.9980	0.0868	0.2895
Resveratrol	0.9999	0.0336	0.1120
Daidzein	0.9995	0.0230	0.0768
Luteolin	0.9999	0.0254	0.0847
Quercetin	0.9999	0.0022	0.0074
t-Cinnamic acid	0.9982	0.0286	0.0954
Apigenin	0.9997	0.0439	0.1463
Hesperidin	0.9997	0.0035	0.0117
Rhamnetin	0.9978	0.0165	0.0546
Chrysin	0.9997	0.0206	0,0687
Pinocembrin	0.9999	0.0852	0.2841
CAPE	0.9998	0.0037	0.0124
Curcumin	0.9952	0.0908	0.3027

as SC_{50} mg/ml Trolox equivalents, by making use of the calibration curve of Trolox ($y=0.7354e^{(-0.409x)}$, R^2: 0.9925). Analyses were performed in triplicate.

Determination of Phenolic Profile by HPLC-UV

Analyzes were made using a UV lamp in reverse phase HPLC. UV analyzes were performed on a UV-Hitachi HPLC (Elite LaChrom, Hitachi, Japan) system equipped with a UV detector that can respond simultaneously at two wavelengths (280 and 315 nm). Analyzes were performed using Fortis phenyl (150x4.6mm 5µ) and applying a gradient program with acetonitrile, water and acetic acid. The injection volume was set to 25 **µl**, the flow rate was set to 1.2 ml/min, and the column temperature was set to 30°C in the column furnace (Can et al., 2015).Validation parameters were given in Table 1. Analyses were performed in triplicate.

Bacterial Strains and Growth Conditions

Antimicrobial activity of samples were studied using ten bacteria (four gram-positive: *Listeria monocytogenes* ATCC®7677, *Bacillus subtilis* B209,, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC®10876), four gram-negative *Pseudomonas aeruginosa* ATCC®27853, *Citrobacter freundii* ATCC®43864 (-), *Escherichia coli,* ATCC®25922, *Klebsiella pneumoniae* ATCC®13883, Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck), yeast *Saccharomyces cerevisiae* ATCC 976, and fungi, *Candida albicans* ATCC®10231) and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and yeast or fungal cells, respectively.

Disc Diffusion Assay

Antimicrobial activity was measured according to Ronald's (1990) method. Bacterial strains were grown in MHA for 24 h at 37°C, and fungal strains were grown in SDA at 27°C for 48 h. Overnight cultures were diluted with 0.9% w/v saline solution and turbidities of bacterial and fungal cell solutions were adjusted to 0.5 McFarland respectively. 100 µL of each diluted suspension was placed over agar in petri dishes and dispersed. Then, sterile discs with a diameter of 6 mm were placed on agar to load stock solution prepared at 30 µg/ml concentrations. As a positive control, nystatin for fungi and amoxicillin and cephazolin for bacteria were used. Alcohol was also used as a negative control. Inhibition zones which formed on the medium were measured in millimeter (mm) after incubation for 24 h at 37°C and 27°C for antibacterial and antifungal activities, respectively. All tests were made in triplicate.

RESULTS

As a result of the study, it was determined that the total amount of phenolic content as a result of the extraction of different bee nests ranged from 3.778 to 9.504 mg GAE/g. It was determined that the total amount of flavonoid substance ranged between 0.9 and 2.97 mg QE/g, the FRAP values of the bee nest samples varied between 21.93 and 52.28 mmol $FeSO_4.7H_2O$/g, and the DPPH• SC_{50} values between 0.84 and 1.92 mg/ml (Table 2). As a result of our study, it was determined that all bee nests contain p-coumaric acid, quercetin, t-cinnamic acid, chrysin and pinocembrin. It was determined that bee nest extracts contained quercetin as a major component (Table 3).

A total of five ethanol extracts from different sample of the brood cover wax material were investigated. The determination of the inhibition zones by diffusion disc plates on agar method showed that allsamples extracts tested exhibited an antimicrobial effect against some of the ten microorganisms tested. The results proved that especially the extract from 1, 2, 3, 4 and 5 showed weakly antibacterial and antifungal activity against *B. subtilis* strains tested. However, the extract of 5 showed only antifungal activity against S. *cerevisiae*, C.*albicans* but did not show antimicrobial activity against the bacteria tested. The extracts from samples 1, 2, 3, 4 and 5 showed too weak antibacterial and antifungal activities against all the strains tested.

Table 2. Antioxidant activity ofbrood cover wax materials

Colony Number	Total Phenolic Content mgGAE/ g	Total Flavanoid Content mgQE/g	FRAP (mmol$FeSO_4.7H_2O$/g)	DPPH SC_{50} (mg/ml)
1	7.005±0.648	1.765±0.070	29.034±0.947	1.638±0.049
2	3.778±0.165	0.900±0.012	21.933±0.638	1.344±0.041
3	5.471±0.177	1.522±0.013	23.879±0.564	1.922±0.044
4	9.504±0.353	2.977±0.038	52.283±0.662	0.847±0.022
5	7.768±0.091	2.195±0.113	32.111±0.925	1.388±0.023

Table 3. Phenolic profile of brood cover wax materials

Phenolics (mg/g)	1	2	3	4	5
Gallic acid	-	-	-	-	-
Protocatechuic Acid	0.986±0.01	-	4.422±0.01	2.673±0.01	5.225±0.01
Chlorogenic Acid	-	-	-	-	-
p-OH Benzoic Acid	-	5.119±0.02	13.713±0.02	12.368±0.01	12.068±0.01
Epicatechin	-	-	-	-	-
Caffeic Acid	-	-	-	-	-
Syringic Acid	-	-	-	-	-
m-OH Benzoic Acid	-	-	-	-	-
Rutin	10.318±0.01	N.D.	16.148±0.02	31.802±0.01	25.060±0.01
Ellagic Acid	-	-	-	-	-
p-Coumaric Acid	11.365±0.01	6.322±0.01	6.648±0.01	11.588±0.01	12.530±0.01
Ferulic Acid	-	-	-	-	-
Myricetin	-	-	-	-	-
Resveratrol	-	-	-	-	-
Daidzein	-	-	-	-	-
Luteolin	32.258±0.02	12.525±0.02	21.809±0.02	33.377±0.02	32.828±0.02
Quercetin	185.567±1.2	87.200±0.9	147.381±1.3	324.808±1.5	185.408±0.9
t-Sinnamic Acid	3.869±0.01	2.915±0.01	2.539±0.01	2.283±0.01	2.374±0.01
Apigenin	28.935±0.01	-	-	24.360±0.01	-.
Hesperetin	-	-	-	-	-
Ramnetin	-	-	-	-	-
Krisin	8.254±0.01	4.864±0.01	2.794±0.01	3.338±0.01	4.547±0.01
Pinocembrin	4.355±0.02	2.525±0.02	0.792±0.02	2.369±0.01	4.377±0.01
Caffeic Acid Phenethyl Ester (CAPE)	-	-	-	-	-
Curcumin	-	-	-	-	-

N.D: Not determined

Table 4. Zone diameters [mm] of inhibition showing the antimicrobial activity of samples

Microorganism	1	2	3	4	5	Ampicillin	Cephazolin	Nystatin
B.subtilis	12.76±0.87	12.70±0.63	12.39±0.56	12.79±0.44	11.77±0.05	32.56±0.65	33.67±0.98	N.T.
B.cereus	7.08±0.67	6.00±0.00	7.20±0.14	7.62±0.65	8.25±0.62	23.58±0.054	26.43±0.053	N.T.
S.aureus	8.78±0.43	6.00±0.00	8.34±0.66	7.58±0.75	8.77±0.34	11.76±0.54	6.00±0.00	N.T.
L.monocytogenes	6.00±0.00	6.00±0.00	8.68±0.10	7.57±0.35	8.73±0.83	26.34 ±0.54	30.45±0.73	N.T.
C.freundii	9.86±0.73	9.45±0.87	9.99±0.87	7.86±0.73	8.42±0.43	14.89±0.12	16.86±0.67	N.T.
K.pneumoniae	9.63±0.12	12.79±0.45	10.86±.73	7.36±0.67	8.91±0.63	14.74±0.84	16.17±0.56	N.T.
P.aeruginosa	7.92±0.66	7.23±0.81	7.48±0.88	8.76±0.54	8.62±0.43	30.67±0.74	25.33±0.83	N.T.
E.coli	7.22±0.82	7.00±0.21	7.74±0.33	8.62±0.52	8.25±0.66	22.00±0.23	17.00±0.00	N.T.
S.cerevisiae	7.62±0.23	7.32±0.43	7.09±0.43	7.52±0.32	12.30±0.43	N.T.	N.T.	17.00±0.32
C.albicans	6.00±0.00	7.12±0.00	7.56±0.71	7.00±0.61	13.00±0.12	N.T.	N.T.	17.89±0.54

-: noinhibition, NT: Not tested , *Listeriamono cyto*genes ATCC®7677, *Bacillus subtilis* B209, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC®10876) *Pseudomonas aeruginosa* ATCC®27853,*Citrobacter freundii* ATCC® 43864 (-), *Escherichia coli,* ATCC®25922, *Klebsiella pneumoniae* ATCC®13883, yeast *Saccharomyces cerevisiae* ATCC 976 , and fungi, *Candida albicans* ATCC®10231

The largest inhibitory zones were observed with the extracts of 3 against, for bacteria and fungi (Table 4).

DISCUSSION

The use of bee products as food supplements and in supportive treatment is increasing day by day. It is stated in studies that the products obtained from beehives have many effects on health (Münstedt and Bogdanov, 2009; Hegazi, 2012; Habrykaet al., 2016; Alvarez-Suarez, 2017, Kocot et al., 2018). However, the biological active properties of these products should be determined before they are used. Although brood cover wax material is a valuable product obtained from bublumbees beehives, biochemical characterization of it has not performed before. Thus, results of our study need to be compared with liter-

ature data obtained for honey beehive products. In a study conducted by Keskin and Ozkok (2020), it was stated that the amount of total phenolic substance of honey bee pollen and bee bread obtained from the same hive were 5.57-6.93 mg GAE/g, respectively. According to Kolaylı et al. (2020), the total phenolic content of different honey and propolis samples was determined. According to that study, it was stated that the total phenolic content of different honey samples varied between 2.42 and 10.51 mg GAE/g, while the propolis samples ranged between 10.33 and 23.21 mg GAE/g (Kolaylı et al., 2020). When the total phenolic content of bee nests is compared with other products in the honey bee beehive product, it is seen that honey, bee bread and pollen have a similar amount of total phenolic content, while propolis contains relatively higher total phenolic substance. Abdallah et al. (2020) reported that the total flavonoid content in two honey samples harvested from Algeria varied between 0.68 and 0.93 mg QE/g. Keskin and Ozkok (2020) stated that the total flavonoid content of pollen and bee bread samples was 2.11 and 2.27 mg QE/g, respectively. In the study on propolis standardization conducted by Keskin and Kolaylı (2018), it is stated that the average total amount of flavonoid content in propolis samples collected from different regions of Turkey is 2%. Wang et al. (2016) determined that the total phenolic and flavonoids content of Korean propolis ranged from 49 to 239 mg gallic acid equivalent (GAE)/g EEP Brazilian, Chinese, and Australian samples, 127–142 mg GAE/g EEP) and from 21 to 50 mg quercetin equivalent (QE)/g EEP Brazilian, Chinese, and Australian samples, 33–53 mg QE/g EEP), respectively.

In the light of these data, bee nest extracts can be an alternative to honey, pollen and bee bread in terms of total flavonoid content. Aliyazicioglu et al. (2013) stated in their study that the FRAP values of different propolis samples varied between 182.1 and 325.4 µM Trolox/g propolis. Keskin and Ozkok (2020) stated in their study that pollen and bee bread FRAP values were 64.56 and 83.62 µmol $FeSO_4.7H_2O$/g sample, respectively. Can et al. (2015) stated in their study that the FRAP values of propolis samples harvested from Azerbaijan varied between 170.27 and 437.90 µM Trolox/g, and the DPPH• SC_{50} values between 15 and 198 mg/ml. Saral (2018) stated in a study that the FRAP values of honey samples collected from the Eastern Black Sea region ranged from 1.25 to 49.92 µmol $FeSO_4$/g sample. Gul and Pehlivan (2018) collected honey samples from different regions of Turkey in their study. Accordingly, they found that FRAP values ranged between 0.0022- 0.0091 mg/100 g honey and DPPH• values varied between 12.01 mg/ml and 65.52 mg/ml. Ozkok et al. (2021) stated in their study that half of 23 different propolis samples were rich in quercetin. Malkoç et al. (2019) stated that Anzer honey is rich in p-coumaric acid (63-1405 µg/100g), t-cinnamic acid (4-65 µg/100g) and pinocembrin (3000-6420 µg/100g). When honey and propolis are compared with the amounts of p-coumaric, quercetin, pinocembrin and t-cinnamic acid, it is clear that bee nests contain more of these components. In a study, the bioactivity of different pollen samples were compared (Margaoan et al., 2021). Accordingly, they determined that the total amount of phenolic substance varied between 16.40 and 41.17 mg GAE/g and the flavonoids content varied between 2.39 and 7.17 mg QE/g. They determined that the highest value of DPPH• was 2.93 mmolTrolox/g and 9.64 mmol Trolox/g for the TEAC. Akbulut and Akkemik (2018) compared the bioactive properties of honey, pollen and propolis samples in their study. They stated that the total phenolic substance amount of honey samples extracted with ethanol varied between 28-32 mg GAE/g. On the other hand, Nakajima et al. (2009) compared the antioxidant properties of different bee products in their study. Accordingly, it was stated that the antioxidant activity of propolis was higher than the antioxidant activity of pollen. It was emphasized that this difference was due to the amounts of phenolic components such as coumaric acid and Artepillin C. Karadal et al. (2018) compared the antioxidant and antibacterial activities of different hive products in their study. Accordingly, it was emphasized that propolis samples had the highest total phenolic content. It was stated that honey, pollen and propolis samples all showed antibacterial activity, but propolis had the strongest antibacterial activity. Adaškevi˘ciute et al. (2019) compared the total phenolic, flavonoid content and antioxidant activities of bee pollen with other hive products. Accordingly, it was stated that pollen samples had higher total flavonoid content than other products.

CONCLUSION

Bumblebee (*Bombus terrestris L.*) is a bee specie that has an important place in pollination. In this study, the biochemical characterization of bumblebee nest wax covers, which are produced in a controlled manner, was studied for the first time and their antimicrobial activities were determined. It is clear that the nest wax cover produced by Bumblebee, which is

thought to be involved only in pollination, has almost equivalent activity to *Apis mellifera* L. bee race hive products such as honey and bee pollen. This shows that bumblebee nest wax cover extract can be an alternative product for apitherapy applications.

CONFLICT OF INTEREST

None declared by the authors.

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