

ACCEPTED ARTICLE

In Vitro Research on Antimicrobial Activity of Native Anatolian Honey Bee Products Against *Paenibacillus larvae* Strains

Emine Sonmez^a, Meral Kekecoglu^{a, b}, Arif Bozdeveci^c, and Sengul Alapy Karaoglu^c

^a Düzce University, Beekeeping Research Development and Application Centre, 81620 Düzce, Türkiye.

^b Düzce University, Faculty of Science, Department of Biology, 81620 Düzce, Türkiye.

^c Recep Tayyip Erdogan University, Faculty of Art and Science, Department of Biology, 53100 Rize, Türkiye.

***Corresponding author; e-mail:eminesonmez@duzce.edu.tr**

ABSTRACT

One of the most damaging diseases in beekeeping worldwide is American Foulbrood. The causative agent of the disease is *Paenibacillus larvae*, which can remain in spore form in the environment for decades and does not lose its virulence. In the management of this disease, it is inevitable to find an alternative method to the use of antibiotics and burning the hives. In this study, after determining the total phenolic (TPC) and total flavonoid content (TFC) of seven different Anatolian honey bee products (bee venom, bee bread, pollen, royal jelly, propolis, queen bee larvae, drone brood larvae), in vitro antimicrobial activities of these products against two different *P. larvae* strains were tested. As a result of Folin-Ciocalteu and AlCl₃ colorimetric methods, there were significant differences between the samples, and the highest content values were obtained from the propolis samples. The antimicrobial activity results showed that, *P. larvae* strains were susceptible to all bee products except queen bee larvae and drone brood larvae. The most significant inhibition was obtained from Anatolian bee venom with the lowest MIC dose 6.25 µg/mL. Bacterial strains showed susceptibility to Anatolian bee bread with an effective dose of 7.81 µg/mL following bee venom. This study is an important first step in identifying new active compounds for the use of in-hive natural products in the development of new preventive treatments against AFB disease, alternative to conventional antibiotic treatments.

Key words: Bee products, *Paenibacillus larvae*, American Foulbrood, Antimicrobial activity.

INTRODUCTION

One of the reasons why the expected yield from beekeeping is not always achieved at the desired level is the bacterial diseases that bee colonies are exposed to. These diseases affect honey bees in their larval and adult stages and cause significant economic losses. Among the bacterial diseases seen in honey bee larvae, American Foulbrood (AFB) and European

40 Foulbrood (EFB) are highly contagious and dangerous (Forsgren, 2010; Moharrami *et al.*,
41 2022). The World Organization for Animal Health (WOAH) has accepted these diseases in the
42 list of notifiable diseases and affect veterinary public health worldwide, posing a serious threat
43 to the safe international trade of honey bees and their products (Genersch, 2010). These diseases
44 are the most important causes of colony losses and low yields. The disease is highly virulent
45 and dangerous not only for individual larvae but also for the entire colony (Morse and Calderon,
46 2000). Disease agents can be encountered at any stage of the bee's life cycle, but are most
47 commonly encountered during the egg stage (Rauch *et al.*, 2009).

48 The AFB disease agent *P. larvae* is a Gram (+) and spore-forming bacterium. *P. larvae* spores
49 are highly resistant to heating, adverse conditions, and chemical agents. These spores
50 contaminate both honey and pollen, and are transmitted to larvae through contaminated food
51 (Genersch, 2010). Some *Paenibacillus* species have been reported to be opportunistic human
52 infections and can cause spoilage in pasteurized dairy products (Grady *et al.*, 2016). *P.*
53 *lentimorbus* and *P. popilliae* cause infection in scarab beetle grubs, while *P. larvae* can cause
54 infection in honey bee (*Apis mellifera*) larvae.

55 Although the use of antibiotics in beekeeping in European countries is prohibited, the
56 European Food Safety Authority (EFSA) reported that there were antibiotic residues in honey
57 samples (Chung *et al.*, 2017; Savarino *et al.* 2020). The use of Tylovet and Lincomix has been
58 approved in the USA to control this disease while Pennox 50 (oxytetracycline) and Terramycin
59 (oxytetracycline hydrochloride) are present for controlling either foulbrood diseases (Mosca *et*
60 *al.*, 2023). In order to control the bacteria that cause the disease, it has become necessary to
61 search for new drugs with different mechanisms of action against the development of resistance
62 resulting from the use of inappropriate chemicals (Alpay Karaoğlu, 2014). Antibiotics are only
63 effective on the vegetative form of the *P. larvae*. Antibiotic administration may temporarily
64 hide or suppress symptoms, but then the disease may reappear more severely (Borum, 2014).

65 Natural products such as plant extracts, plant essential oils, antimicrobial peptides, and
66 propolis are shown as alternative options (Raut and Karuppaiyil 2014; Alvarenga *et al.*, 2021;
67 Wang 2021). Cases in the advanced stages of the disease are difficult to treat. However, if the
68 disease has just started and is diagnosed early, there is a chance of prevention of transmission
69 and spread. The hive with suspected disease should be removed from the apiary urgently and
70 quickly (Borum, 2014).

71 Bee products such as propolis, bee venom, honey and royal jelly are used in "Apitherapy" in
72 many countries. Due to the role of bees in pollinating flowers, beekeeping is one of the
73 indispensable agricultural activities all over the world (Etxegarai-Legarreta and Sanchez-

74 Famoso, 2022). It is thought that apitherapy products will be useful against bee diseases for the
75 sustainability of beekeeping activities with a healthier and higher yield (Sevim *et al.*, 2021;
76 Šedivá *et al.*, 2018; Naglaa *et al.*, 2020). Propolis is known as a strong antimicrobial substance,
77 consisting of a mixture of different pollen, oils, special resins, and waxy collected by honey
78 bees from the buds and sprouts of plants. It is used to close holes and cracks in the hive, repair
79 honeycombs, glue honeycombs together, polish honeycomb eyes, narrow the hive entrance,
80 protect from bee diseases, and prevent their development by neutralizing disease agents (Wagh,
81 2013). The effect of propolis against microorganisms is its most important biological feature.
82 It ensures that fungi and bacteria remain at a lower level in the hive. Propolis is a natural bee
83 product that has been used by humans since ancient times due to its pharmacological properties
84 (Wagh, 2013; Bogdanov, 2012). Pollen is the male reproductive unit that forms on the antennae
85 of flowering plants and is involved in fertilization.

86 Honey bees collect pollen from flowers with their feet and deposit it on their hind legs. It
87 mixes the pollen with digestive enzymes and some nectar and stores it in the honeycomb cells
88 (Bogdanov, 2011a). Depending on the source, pollen has biological effects such as being
89 antimicrobial, antitumoral (prostate and breast cancers), antioxidant, antiaging, anti-
90 osteoporosis, anti-anemia, anti-diarrhea, memory enhancer, probiotic, regenerative,
91 performance-enhancing, and aphrodisiac (Bogdanov, 2011a).

92 Drone brood larvae are obtained by collecting between 3-7 days of age of larvae (Bărnuțiu *et*
93 *al.*, 2013). There are many androgenic hormones, sugars, amino acids, fatty acids, and a small
94 amount of minerals in its content (Altan *et al.*, 2013). Due to the androgenic hormones, it
95 contains, it is used to increase sperm count, as an aphrodisiac, and in bodybuilding (Mărgăoan
96 *et al.*, 2017). Bee venom is produced in the venom glands of worker bees and stored in the
97 venom bag (Bogdanov, 2011b). Newly emerged bees from the honeycomb cells have very little
98 ability to produce venom and reach their highest capacity when they are 12 days old. Melittin
99 is a peptide consisting of 26 amino acids that is the most abundant in bee venom (Rady *et al.*,
100 2017). Melittin is a cytolytic peptide that is nonspecific and can attack the lipid bilayer, thus
101 leading to toxicity. This peptide is a powerful agent that increases membrane permeability, and
102 with this feature, it causes antibacterial, antifungal, antiviral and anticancer activity (Kohno *et*
103 *al.*, 2014; Pandidan and Mechler, 2019).

104 Until now, there is limited information available regarding antimicrobial properties of
105 Anatolian bee products against *P. larvae*, even though it is well known for its strong inhibitory
106 effects against other Gram (+) bacteria (Sonmez *et al.*, 2023, 2022; Kekecoglu *et al.*, 2021;
107 2022; Popova *et al.*, 2005; Erkmen and Ozcan 2008). Owing to these reason the aim of the

108 present study was to test the antimicrobial activity of seven different bee products obtained
109 from Anatolian honey bees (*A. mellifera anatoliaca*, Yığılca ecotype) against the pathogen *P.*
110 *larvae*, which causes serious economic losses in the beekeeping industry.

111

112 MATERIALS AND METHOD

113 Sample Preparation

114 All bee products (royal jelly (RJ), drone brood larvae (DBL), queen bee larvae (QBL), bee
115 venom (BV), bee pollen (BP), bee bread (BB), and propolis samples) used in the study were
116 produced and analyzed at Düzce University Beekeeping Research Development and
117 Application Center (DAGEM), Düzce, Turkey. All samples obtained from three randomly
118 selected healthy, similar conditions and free of pesticides colony. The hive type is wooden
119 Langstroth, and the bee species forming the colony was Yığılca ecotype belonging to the *A.*
120 *mellifera anatoliaca*. Raw propolis samples were pulverized using a laboratory type blender
121 (Waring, commercial blender). These samples were weighted as 50 gr and 500 mL of 96%
122 ethanol (Sigma-Aldrich) was transferred into the samples. The resulting mixture was shaken at
123 150 rpm for 72 h and then filtered using filter paper. In order to remove the ethanol in the
124 filtrate, the samples were kept in the evaporator (IKA RV10) at 50-60 °C for 10 minutes. The
125 amount was determined by weighing the remaining resinous part, and stock solutions were
126 obtained using 70% ethyl alcohol with each sample containing 10% propolis content (0.1 g/mL)
127 (Kekecoglu *et al.*, 2021).

128 For collection of RJ sample 3-day-old larvae in the queen bee cells were pulled out of the cells
129 with the help of tweezers. Fresh royal jelly remaining in the cells was collected into opaque
130 bottles using a spatula and immediately stored at -18 °C. The obtained royal jelly samples were
131 diluted with distilled water in sterile Eppendorf tubes. DBL and QBL were obtained from
132 directly the opened or unsealed eyes of the honeycomb on the day of 4-9 and 5-7 after hatching,
133 respectively. Each sample was homogenized with a tissue homogenizer and then freeze-dried
134 at -70 °C. For dehydration, the samples were kept at 0.1 bar at -55 °C for 72 hours (Sonmez *et*
135 *al.*, 2023). The obtained lyophilized samples were stored at -20 °C until further experiments.
136 To dissolve the homogenates, 70% ethyl alcohol was transferred into 5 mg of sample and this
137 mixture was vortexed for 15 min and then shaken at room temperature for 8 hours. The BV
138 sample was obtained by the method previously mentioned by Sonmez *et al.* (2022). For BB and
139 BP samples 0.4 g of each bee product was weighted and dissolved in the same volume of 70%
140 ethanol, and methanol. Samples were shaken for 2 h at room temperature to obtain the

141 maximum amount of bioactive components. Finally, maximum dissolution and sterile
142 homogenates were obtained and used in further studies.

143

144 **Bacterial culture and Growth conditions**

145 The bacterial samples used in the study (*P. larvae* ATCC 9545 (ERIC I) and *P. larvae* DSM
146 25430 (ERIC II)) were commercially purchased. *P. larvae* strains were revived from the culture
147 collection in the microbiology research laboratory of Recep Tayyip Erdoğan University. The
148 chemicals and bacteria growth media used in the study were purchased commercially.

149 Bacterial strains were inoculated on MYPGP agar (Mueller-Hinton broth (10 g.L⁻¹), yeast
150 extract (15 g.L⁻¹), K₂HPO₄ (3 g.L⁻¹), sodium pyruvate (1 g.L⁻¹) (Fisher), glucose (2%) (Merck),
151 and agar 14 g.L⁻¹) and incubated at 37 °C for 3-4 days in a 5% CO₂ incubator. After the bacteria
152 were revived, single colonies were taken and pure cultures were cultured on MYPGP agar and
153 then overnight cultures were prepared from pure cultures (Sevim *et al.*, 2021).

154

155 **Determination of Antimicrobial Activity**

156 The antibacterial activities of the samples used in the study were tested against *P. larvae*
157 ATCC 9545 (ERIC I) and *P. larvae* DSM 25430 (ERIC II) strains using the agar-well diffusion
158 method (Fünfhaus *et al.*, 2018). Bacterial density was prepared as McFarland 0.5 (10⁸ CFU/mL)
159 and spread over the entire surface of the MYPGP agar medium with a sterile cotton swab. Five
160 millimetre wells were made/prepared at 2 cm intervals with the help of a sterile cork borer in
161 the agar plates. 50 microliters of the test samples were poured into the wells in the overlaid
162 plates and the plates incubated at 37 °C for 48 h in 5% CO₂. Antimicrobial activity was
163 evaluated by calculating the net inhibition zone, diameters in millimeters (Sevim *et al.*, 2021).

164 Minimal inhibition concentration values (MIC) were determined using the microdilution
165 technique (CLSI, 2015; Alpay Karaoğlu *et al.*, 2022). Test samples were serially diluted in
166 microplate wells containing MYPGP liquid medium. Turbidity suspensions of 0.5 McFarland
167 (10⁸ CFU/mL), were prepared from overnight cultures of *P. larvae* strains. After 10 µL of the
168 bacterial suspensions were poured into each well containing the test samples, microplates were
169 incubated in a 5% CO₂ incubator at 37 °C for 48 h. Ampicillin (10 µg/mL) was used as standard
170 control, ethanol (99%) and methanol as solvent control. The wells at the lowest concentration
171 without bacterial growth were determined as the MIC values (CLSI, 2015) and the antimicrobial
172 effect of each bee product was tested in triplicate.

173

174 **Determination of Total Phenolic Content**

175 The total phenolic content of honey bee products was determined by using the Folin Ciocalteu
176 method according to the published protocols with minor changes (Singleton *et al.*, 1999). After
177 20 mL of methanol extract from each sample was mixed with 680 mL of dH₂O, 0.5 mol/L
178 Folin-Ciocalteu reagent was added to this mixture. In the next step, the mixture was vortexed
179 for 2 min and after 400 mL of 10% Na₂CO₃ was added, it was kept at room temperature for 2
180 h. The absorbance of the samples was measured at 760 nm, and the results were given in mg
181 gallic acid equivalents (GAE) per gram of sample.

182
183

184 **Determination of Total Flavonoid Content**

185 Total flavonoid amounts of propolis, BP and BB were determined by making minor changes
186 in the AlCl₃ colorimetric method described in Fukumoto and Mazza, (2000). Each sample was
187 taken into volumetric bottles of 2 mL and 20 mL of methanol and 1 mL of 5% AlCl₃ were
188 added. After the mixture was incubated for 30 min at room temperature, the absorbance value
189 was measured at 420 nm. Each sample value was expressed as mg quercetin equivalent/g (mg
190 QE/g).

191
192

192 **Statistical analysis**

193 Each tested parameter for each sample were done in triplicate and as descriptive statistics,
194 mean, standard deviation, median, minimum and maximum values were obtained. Mann-
195 Whitney U test was performed to determine the variation of inhibition zone and MIC values
196 according to bacterial strains, and Kruskal-Wallis H test was performed to determine the
197 variation according to bee products. Spearman correlation coefficient was used for the
198 relationship between variables. The significance level was taken as .05. Data were analyzed
199 with SPSS 26.

200
201

201 **RESULTS**

202 Bee products obtained from DAGEM significantly inhibited the growth of *P. larvae* strains in
203 cultures with different MIC doses. The obtained results are summarized in Table 1. The zones
204 of inhibition varied between 0-28 mm demonstrating that many of the samples inhibited the
205 bacterial strains on the agar medium. In the agar well method, the largest inhibition zone was
206 obtained from BV and propolis A with a diameter of 28 and 26 mm respectively. DBL and QBL
207 did not create any inhibitory zones against the tested pathogens.

208 Among the honey bee products, the lowest MIC values of 3.125 µg/mL were recorded for BV
 209 while, DBL and QBL samples, that were not able to inhibit the growth of the pathogens showed
 210 no activity during the MIC test either.

211 In present study we detected an important antimicrobial effect from Anatolian BB samples
 212 and the MIC results of BB varied according to the solvent used. The obtained MIC values were
 213 7.81 µg/mL for ethanolic extract against both *P. larvae* strains. The effectiveness values
 214 obtained from the methanolic extract were 15.62 and 31.25 µg/mL for ATCC 9545 and DMG
 215 9820 strains, respectively.

216

217

218 **Table 1.** Agar well diffusion and MIC values of the Anatolian honey bee products
 219 against *P. larvae* strains.

Bee products	<i>Paenibacillus larvae</i> ATCC 9545		<i>Paenibacillus larvae</i> DMG 9820	
	Inhibition zone (mm)	MIC (µg/mL)	Inhibition Zone (mm)	MIC (µg/mL)
Bee venom	28	3.125	28	3.125
Royal jelly	8	250	8	250
Bee bread (Ethanol)	22	7.81	20	7.81
Bee bread (Methanol)	18	15.62	16	31.25
Pollen (Ethanol)	14	31.25	15	31.25
Pollen (Methanol)	15	31.25	15	31.25
Propolis A	26	7.81	26	7.81
Propolis B	24	15.62	24	15.62
Drone brood larvae	-	-	-	-
Queen bee larvae	-	-	-	-

220

221 The MIC values of RJ was highest (250 µg/mL) compared to other tested honeybee products.

222 The MIC values of samples A and B of the propolis were different and sample A (7.81 µg/mL)
 223 had lower MIC values than sample B (15.62 µg/mL). **Inhibition zone and MIC values were**

224 **not significantly different according to bacterial strains (U=.000; p=1.000). Inhibition zone**

225 **and MIC values differed significantly according to bee products (U=14.955; p<.037 and**

226 **U=15; p=.036, respectively). The inhibition zone obtained from BV was higher than RJ,**

227 **and the MIC value was lower and significant (Table 2).**

228

229

Table 2: Correlation Analysis of the variables.

	Inhibition Zones	MIC	Total Phenolic	Total Flavonoid
Inhibition Zones	-	.206	.562	.299
MIC	.445	-	-.410	.554
Total Phenolic	.023	.115	-	.262
Total Flavonoid	.261	.026	.327	-

230 * The above-diagonal Spearman correlation coefficient is the p value for the below-diagonal correlation
 231 coefficient.
 232

233 In Table 3, the total phenolic (TPC) and flavonoid content (TFC) of honey bee products are
 234 presented. According to the results of seven different samples analyzed with the Folin–
 235 Ciocalteu method, the sample with the highest total phenolic content was Propolis A with a
 236 value of 166.30 mg GAE/g. The lowest amount of phenolic substance was determined from the
 237 BV sample. Determination of total phenolic content was done for BB, pollen and propolis
 238 samples. The highest total phenolic substance content was detected in the propolis A sample,
 239 as was the total phenolic content. The honey bee product containing the lowest flavonoid
 240 component was determined as BV with a value of 0.03 mg QE/g.

241 **Moreover according to the statistical analysis results a significant positive correlation was**
 242 **obtained between inhibition zones and total phenolic (r⁵⁶²; p=.023) and between MIC**
 243 **and total flavonoids (r=.554; p=.026) (Table 2).**

244
 245 **Table 3.** Total phenolic and flavonoid content of Anatolian honey bee products.

	Total Phenolic content (mg GAE/g)	Total flavonoids (mg QE/g)
Bee venom	.82 ± .08 [.79 (.76-.91)]	.03 ± .01 [.03 (.02-.04)]
Royal jelly	3.87 ± 0.16 [3. 94 (3.69-3.98)]	0.89 ± 0.11 [.90 (.78-.99)]
Bee bread	9.06 ± 0.18 [9 (8.92-9.26)]	2.11 ± 0.21 [2.01 (1.97-2.35)]
Pollen	8.82 ± 0.89 [8.67 (8.01-9.78)]	3.90 ± 0.11 [3.90 (3.79-4.01)]
Drone brood larvae	10.86 ± .18 [10.84 (10.65-11.08)]	.08 ± .085 [.04 (.03-.21)]
Queen bee larvae	11.05 ± .06 [11.05 (11.01-11.09)]	.15 ± .06 [.15 (.11-.19)]
Propolis A	166.30 ± 1.50 [165.94 (165.01-167.95)]	83.01± 0.18 [82.92 (82.89-83.22)]
Propolis B	152.76 ± 0.59[152.68 (152.21-153.39)]	81.70 ± 0.55 [81.64 (81.18-82.28)]

246 * $\bar{x} \pm sd$ [Median (Min – Max)]

247

248 DISCUSSION

249 Honey bees are an important part of the food supply chain for both pollination and commercial
 250 beekeeping activities. Although honey bees are among the most important pollinators, their
 251 lives are under threat because they are infected with various pathogens. The most important of
 252 these pathogens is *P. larvae* that causes AFB (Dickel *et al.*, 2022). The management of this
 253 disease is the burning of the diseased hives today, or prophylactic feeding of antibiotics to the
 254 hives practiced in some countries (Genersch, 2010). However, the resistance developed by
 255 bacteria against the use of antibiotics and the residues in foods has become an increasing global
 256 problem worldwide. Also the use of antibiotics is not effective against these bacterial spores

257 and their use is related to the alteration of gut microbiota and the modification of the
258 development of bee behavior (Raymann and Moran, 2018; Ortiz-Alvarado *et al.*, 2020). In order
259 to prevent this disease in honey bees, it is necessary to develop sustainable and non-chemical
260 solutions, and alternatives to the use of antibiotics and burn the hives. Antimicrobial peptides
261 are thought to be one of the mechanisms that affect the resistance of honey bees to AFB
262 infection of colonies (Evans, 2004; Decanini *et al.*, 2007; Chan *et al.*, 2009). These natural
263 antimicrobial peptides found in snake, scorpion, and BV cause inhibit the pathogens by
264 breaking their membranes, moreover the bacteria do not develop resistance to these peptides
265 (Ventola, 2015). In addition to these natural peptides, many researchers reported that the
266 resistance of colonies to AFB was associated with larval feeding (Šedivá *et al.*, 2018). In line
267 with these data, this study, it was aimed to test the effectiveness of bee products, which are
268 known to be natural antimicrobial agents, against *P. larvae*. All tested bee products except DBL
269 and QBL significantly inhibited the growth of two different strains of *P. larvae* at rates ranging
270 from 6.25 to 62.5 µg/mL. Among these important bee products, BV was the most effective
271 against both bacterial strains at the lowest dose. Studies about the antimicrobial activity of BV
272 against bacterial strains that cause AFB are very limited. Lee *et al.* (2016) investigated the
273 antimicrobial effect of one of the BV peptides, secapin (AcSecapin-1) against *P. larvae* and
274 reported the MIC₅₀ value as 11.13 µM. Fernández *et al.* (2014) tested the efficacy of BV against
275 five different strains of *P. larvae* and they obtained MIC values between 3.12 to 8.33 µg/mL. It
276 was reported in a previous study that Anatolian BV is highly effective against yeast like fungi,
277 Gram (+) and Gram (-) bacteria (Sonmez *et al.*, 2022). The present study, Anatolian BV
278 significantly affected the growth and development of *P. larvae* strains and were effective
279 against the pathogen at very low MIC dose (for both strains 6.25 µg/mL).

280 In this study, another bee product that is significantly effective against *P. larvae* was BB. To
281 our knowledge no such study were present in the literature that tests the effectiveness of BB
282 against this honeybee pathogen. Hence, our studies could be of significant highlighting the
283 efficiency of BB against this pathogenic bacteria. However, Iorizzo *et al.* (2020) isolated
284 *Lactobacillus plantarum* strains from BB and investigated its antimicrobial effect against *P.*
285 *larvae*. They reported that isolated *Lactobacillus* strains were able to inhibit *P. larvae* growth.
286 Considering the compatibility with the previous study (Iorizzo *et al.*, 2020), the low MIC values
287 obtained from this study may be an indication that the probiotic bacteria in the content of BB
288 play an active role in the defense of the immune system of the honey bees against these bacteria.

289 Like all insects, honeybees produce antimicrobial peptides to defend themselves against
290 pathogens (Ilyasov *et al.*, 2013). The most important of these antimicrobial peptides are low

291 molecular weight proteins and peptides in RJ (Ramanathan *et al.*, 2018). Bíliková *et al.* (2001)
292 tested the efficacy of one of these peptides, royalicin, against *P. larvae* and other Gram (+)
293 bacteria using disk diffusion method and reported that this peptide inhibits the growth of this
294 pathogenic bacteria. In a similar study, Bachanová *et al.* (2002) suggested that royalicin and
295 other peptides are responsible for activity against *P. larvae* and other Gram (+) bacteria. In
296 another study, Hornitzky, (1998) reported that RJ had a bactericidal effect against the vegetative
297 form of *P. larvae* after application of 5 min. Šedivá *et al.* (2018) investigated the antibacterial
298 effects of trans-10-hydroxy-2-desenoic acid (10-HDA), an important fatty acid of RJ, against
299 *P. larvae* strains, including all Enterobacterial Repetitive Intergenic Consensus (ERIC)
300 genotypes and they reported that 10-HDA showed higher activity against these genotype with
301 decreasing pH. 10-HDA is an important component of RJ responsible for antimicrobial activity,
302 and it has been reported in previous studies that this fatty acid derivative was found at a high
303 level in Anatolian RJ (Sonmez *et al.*, 2023). Anatolian RJ, whose effectiveness was tested in
304 this study, was also found to be effective against two different *P. larvae* strains. This high
305 inhibition activity was thought to be due to its 10-HDA content, and it can be suggested that
306 this bee product may have a broad-spectrum protective effect in microbial infections occurring
307 in the hive.

308 Propolis has been used for many years due to its high biological activity. However, this high
309 efficiency could not be evaluated to form a useful model about honey bee diseases that damage
310 the beekeeping industry. Özkırım *et al.* (2014) investigated the antimicrobial activity of 18
311 ethanolic extracts of propolis samples against 10 different *P. larvae* isolates and they reported
312 that the bacterial strains were susceptible to all tested samples. Chen *et al.* (2018) tested the
313 efficacy of Taiwan green propolis on some Gram (+) bacteria and *P. larvae* using different
314 extraction methods and showed that the average MIC value was 20 µg/mL. Fangio *et al.* (2019)
315 and Antunez *et al.* (2008) reported that ethanolic extracts of propolis samples formed different
316 inhibition zones with values varying between 20-30 mm against *P. larvae* by disk diffusion
317 method. Sevim *et al.* (2021) tested the potential antimicrobial activity of Anatolian propolis
318 against *P. larvae* PB35 and SV35 strains and determined the MIC value as 74.87 µg/mL. It has
319 been reported in previous studies that Anatolian propolis is effective against both Gram (+) and
320 Gram (-) bacteria because of its high phenolic and flavonoid content (Kekecoglu *et al.*, 2021,
321 2022; Velikova *et al.*, 2000; Uzel *et al.*, 2005; Katircioglu and Mercan, 2006). In present study,
322 two different Anatolian propolis samples (A-B), which were tested for their effectiveness
323 against the pathogen that causes severe honey bee and crop loss in hives, also caused high
324 inhibition with low rates of MIC values (7.81 and 15.62 µg/mL respectively). Considering the

325 total phenolic and flavonoid content of Anatolian propolis examined in this study, it is not
326 surprising that a very low effective dose was obtained. For this reason Anatolian propolis
327 samples may have the potential to be used as an alternative disinfectant solution to the use of
328 antibiotics in hives.

329 For many years, besides its nutritional properties, the biological properties of BP and the
330 therapeutic effects resulting from this activity have been known worldwide (Soares de Arruda
331 *et al.*, 2021). However, no study that tested the effectiveness of this protein and lipid-rich
332 product against *P. larvae*. Grubbs *et al.* (2021) reported that the Actinobacteria strain of the
333 genus *Streptomyces* isolated from pollen stores exhibited significant inhibitory activity against
334 *P. larvae*. In this study, BP samples, whose antimicrobial effect was evaluated by using two
335 different solvents, were also effective against this pathogen with low MIC doses (31.25 µg/mL).
336 Hence, for the very first time we show that BP well known for its high nutritional value, acts as
337 a strong antimicrobial control agent against the *P.larvae* that causes bee larval disease.

338 The total phenolic and flavonoid content and amounts of honey bee products vary according
339 to the collected geographical region, collection time, vegetation cover, climate and bee race
340 (Campos *et al.*, 2015; Arruda *et al.*, 2013). It is known that these bioactive components, which
341 differ in each product, are also responsible for antimicrobial activity (Fatima *et al.*, 2014, Al-
342 Juhaimi *et al.*, 2022, Kekecoglu *et al.*, 2021). In previous studies, it was reported that there is a
343 positive correlation between total phenolic substance and antimicrobial activity (Pereira *et al.*,
344 2007; Estevinho *et al.*, 2008; Nazzaro *et al.*, 2013). Soares de Arruda *et al.* (2021) reported that
345 they observed moderate and weak correlations between total phenolics, total flavonols and
346 antibacterial activity parameters. However, Morais *et al.* (2011) in their study, showed that there
347 was no relationship between total phenolic substance and antimicrobial activity, and the extract
348 containing a lower percentage of phenolic substances was more effective against
349 microorganisms. In this study, although a positive correlation was obtained between total
350 phenolic substance and antimicrobial activity among propolis samples, no correlation was
351 found between antimicrobial activity with RJ, BB, pollen, QBL, and DBL.

352 AL-Ani *et al.* (2018) reported that the bioactivities obtained from propolis and other bee
353 products are not only due to the content of phenolic-flavonoid substances, but also due to the
354 synergistic effect between these biologically active substances. With these results, it can be
355 concluded that the antimicrobial activity is not only due to the total phenolic and flavonoid
356 substances, but also to the synergistic effect of the different components in these natural
357 products.

358

359 **CONCLUSION**

360 In conclusion, in this study, very effective antimicrobial activity results were obtained from
361 different bee products against pathogenic bacteria that cause serious damage to honey bee
362 colonies. In particular, bee venom has a good potential to inhibit AFB destruction in colonies.
363 The obtained MIC values were evaluated as an important result showing that these natural
364 products have the potential to be used in the control of AFB disease. It is recommended that
365 these products should be used as a preventative in larval feeding or hives before disease
366 transmission.

367

368

369

370 **REFERENCES**

371 Al-Ani, I., Zimmermann, S., Reichling, J. and Wink, M. 2018. Antimicrobial activities of
372 European propolis collected from various geographic origins alone and in combination with
373 antibiotics. *Medicines*, **5(1)**: 2.

374 Al-Juhaimi, F. Y., Özcan, M. M., Mohamed Ahmed, I. A., Alsawmahia, O. N., Özcan, M. M.,
375 Ghafoor, K. and Babiker, E. E. 2022. Bioactive compounds, antioxidant activity, fatty acid
376 composition and antimicrobial activity of propolis from different locations in Turkey. *Journal*
377 *of Apicultural Research.*, **61(2)**: 246-254.

378 Alpay Karaoğlu, Ş., Yaylı, N., Erik, İ., Korkmaz, B., Akpınar, R., Bozdeveci, A., Suyabatmaz,
379 Ş., Batan, N., Yeşilyurt, A., Kaya, S., Nisbet, C. and Güler, A. 2022. Biological Activity and
380 Phytochemical Analysis of *Dicranum scoparium* against the Bacterial Disease for Honey Bee.
381 *Chemistry & biodiversity*, **19(7)**: e202100887.

382 Alpay Karaoğlu, Ş., Tosun, G., Yılmaz, Y., Bozdeveci, A., Özdemir T., Terzioğlu, S.,
383 Akpınar, R., Nispet, C. and Yaylı, N. 2014. *Paenibacillus larvae* Isolation., Characterization.,
384 and Susceptibility to Antibiotics and Plant Extracts from American Foulbrood Diseased
385 Specimens. 4th International Muğla Beekeeping and Pine Honey Congress Muğla Turkey, PP.
386 153.

387 Altan, Ö., Yücel, B., Açıkgöz, Z., Şeremet, Ç., Kösoğlu, M., Turgan, N. and Özgönül, A. M.
388 2013. Apilarnil reduces fear and advances sexual development in male broilers but has no effect
389 on growth. *British poultry science*, **54(3)**: 355-361.

390 Alvarenga, L., Cardozo, L. F. M. F., Borges, N. A., Chermut, T. R., Ribeiro, M., Leite Junior,
391 M., Shiels, P. G., Stenvinkel, P. and Mafra, D. 2021. To bee or not to bee? The bee extract
392 propolis as a bioactive compound in the burden of lifestyle diseases. *Nutrition*, **83**: 111094.

- 393 American Veterinary Medical Association. The Veterinarian's Role in Honey Bee Health.
394 Available online: <https://www.avma.org> (accessed on 5 October 2022).
- 395 Antunez, K., Harriet, J., Gende, L., Eguaras, M. and Zunino, P. 2008. Efficacy of natural
396 propolis extract in the control of American Foulbrood. *Veterinary Microbiology*, **131**: 324–331.
- 397 Arruda, V. A. S. D., Freitas, A. D. S. D., Barth, O. M., Estevinho, L. M. and Almeida-
398 Muradian, L. B. 2013. Propriedades biologicas do polen apícola de coqueiro., coletado na
399 Regi~ao Nordeste do Brasil. *Magistra*, **25**: 27–36.
- 400 Bankova, V. S., de Castro, S. L. and Marcucci, M. C. 2000. Propolis: recent advances in
401 chemistry and plant origin. *Apidologie*, **31(1)**: 3-15.
- 402 Bărnuțiu, L. I., Mărghitaș, L., Dezmirean, D., Bobiș, O., Mihai, C. and Pavel, C. 2013.
403 Physicochemical composition of Apilarnil (bee drone larvae). *Lucrări Științifice-Seria*
404 *Zootehnie*, **59**: 199-202.
- 405 Bíliková, K., Wu, G. and Šimúth, J. 2001. Isolation of a peptide fraction from honeybee royal
406 jelly as a potential antifoulbrood factor. *Apidologie*, **32(3)**: 275-283.
- 407 Bogdanov, S. 2011a. Pollen: nutrition., functional properties., health: a review. *Bee Product*
408 *Science*, **1**: 34.
- 409 Bogdanov, S. 2011b. Bee Venom: Composition., health. Medicine., a review. *Bee Prod Sci.*,
410 **1**: 1-20.
- 411 Bogdanov, S. 2012. Propolis: biological properties and medical applications. *The propolis*
412 *book*, **2**: 1.
- 413 Borum, E. 2014. Diagnosis of Infection., Fighting and Protection Methods in Foulbrood
414 Infection of Honeybees. *Uludag Bee Journal*, **14(1)**: 44-55.
- 415 Campos, M. G., Almaraz-Abarca, N., Matos, M. P., Gomes, N. M., Arruda, V. A. S., Barth,
416 O. M., Freitas, A. S. and Almeida-Muradian, L. B. 2015. Zea mays L. Pollen: An approach to
417 its quality control. *Journal of Agricultural Science and Technology B*, **5(8)**: 513–522.
- 418 Chan, Q. W. T., Melathopoulos, A. P., Pernal, S. F. and Foster, L. J. 2009. The innate immune
419 and systemic response in honey bees to a bacterial pathogen., *Paenibacillus larvae*. *BMC*
420 *Genomics*, **10**: 387.
- 421 Chen, Y. W., Ye, S. R., Ting, C. and Yu, Y. H. 2018. Antibacterial activity of propolins from
422 Taiwanese green propolis. *Journal of food and drug analysis*, **26(2)**: 761-768.
- 423 Chung, H. S., Lee, Y. J., Rahman, M. M., Abd El-Aty, A. M., Lee, H. S., Kabir, M. H., ... and
424 Shim, J. H. 2017. Uptake of the veterinary antibiotics chlortetracycline, enrofloxacin, and
425 sulphathiazole from soil by radish. *Science of the total Environment*, **605**: 322-331.

426 CLSI (Clinical and Laboratory Standards Institute) 2015. Methods for Dilution Antimicrobial
427 Susceptibility Tests for Bacteria That Grow Aerobically., Approved Standard—Tenth Edition.
428 CLSI document M07-A10 (ISBN 1-56238-987-4 [Print]., ISBN 1-56238-988-2 (Accessed
429 date: 18.06. 2021.).

430 Decanini, L. I., Collins, A. M. and Evans, J. D. 2007. Variation and heritability in immune
431 gene expression by diseased honeybees. *J Hered.*, **98**: 195–201.

432 Dickel, F., Bos, N. M. P., Hughes, H., Martín-Hernández, R., Higes, M., Kleiser, A. and
433 Freitak, D. 2022. The oral vaccination with *Paenibacillus larvae* bacterin can decrease
434 susceptibility to American Foulbrood infection in honey bees—A safety and efficacy
435 study. *Frontiers in Veterinary Science*, **9**: 1494.

436 Erkmen, O. and Özcan, M. M. 2008. Antimicrobial effects of Turkish propolis, pollen, and
437 laurel on spoilage and pathogenic food-related microorganisms. *Journal of medicinal
438 food*, **11(3)**: 587-592.

439 Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G. and Pereira, E. 2008. Antioxidant and
440 antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and
441 Chemical Toxicology*, **46**: 3774–3779.

442 Etxegarai-Legarreta, O. and Sanchez-Famoso, V. 2022. The role of beekeeping in the
443 generation of goods and services: The interrelation between environmental, socioeconomic, and
444 sociocultural utilities. *Agriculture*, **12(4)**: 551.

445 Evans, J. D. 2004. Transcriptional immune responses by honey bee larvae during invasion by
446 the bacterial pathogen., *Paenibacillus larvae*. *J Invertebr Pathol.*, **85**: 105–111.

447 Fangio, M. F., Orallo, D. E., Gende, L. B. and Churio, M. S. 2019. Chemical characterization
448 and antimicrobial activity against *Paenibacillus larvae* of propolis from Buenos Aires province
449 Argentina. *Journal of Apicultural Research*, **58(4)**: 626-638.

450 Fatima, J., Baserisalehi, M. and Nima, B. 2014. Antimicrobial activity and chemical screening
451 of propolis extracts. *American Journal of Life Sciences*, **2(2)**: 72–75.

452 Fernández, N. J., Porrini, M. P., Podaza, E. A., Damiani, N., Gende, L. B. and Eguaras, M. J.
453 2014. A scientific note on the first report of honeybee venom inhibiting *Paenibacillus larvae*
454 growth. *Apidologie*, **45**: 719-721.

455 Forsgren, E. 2010. European foulbrood in honey bees. *Journal of invertebrate pathology*, **103**:
456 5-9.

457 Fukumoto, L. R. and Mazza, G. 2000. Assessing antioxidant and prooxidant activities of
458 phenolic compounds. *Journal of Agricultural and Food Chemistry*, **48(8)**: 3597–3604.

459 Fünfhaus, A., Göbel, J., Ebeling, J., Knispel, H., Garcia-Gonzalez, E. and Genersch, E. 2018.
460 Swarming motility and biofilm formation of *Paenibacillus larvae*, the etiological agent of
461 American Foulbrood of honey bees (*Apis mellifera*). *Scientific reports*, **8(1)**: 8840.

462 Genersch, E. 2010. American Foulbrood in honeybees and its causative agent., *Paenibacillus*
463 *larvae*. *Journal of invertebrate pathology*, **103**: 10-19.

464 Grady, E. N., MacDonald, J., Liu, L., Richman, A. and Yuan, Z. C. 2016. Current knowledge
465 and perspectives of *Paenibacillus*: a review. *Microbial cell factories*, **15**: 1-18.

466 Grubbs, K. J., May, D. S., Sardina, J. A., Dermenjian, R. K., Wyche, T. P., Pinto-Tomás, A.
467 A., Clardy, J. and Currie, C. R. 2021. Pollen *Streptomyces* produce antibiotic that inhibits the
468 honey bee pathogen *Paenibacillus larvae*. *Frontiers in Microbiology*, **12**: 632637.

469 Ilyasov, R. A., Gaifullina, L. R., Saltykova, E. S., Poskryakov, A. V. and Nikolaenko, A. G.
470 2013. Defensins in the honeybee antiinfectious protection. *Journal of evolutionary*
471 *biochemistry and physiology*, **49**: 1-9.

472 Iorizzo, M., Testa, B., Lombardi, S. J., Ganassi, S., Ianiro, M., Letizia, F. *et al.* 2020.
473 Antimicrobial activity against *Paenibacillus larvae* and functional properties of
474 *Lactiplantibacillus plantarum* strains: Potential benefits for honeybee health. *Antibiotics*, **9(8)**:
475 442.

476 Katircioglu, H. and Mercan, N. 2006. Antimicrobial activity and chemical compositions of
477 Turkish propolis from different regions. *African journal of biotechnology*, **5(11)**.

478 Kekecoglu, M., Sonmez, E., Acar, M. K. and Karaoglu, S. A. 2021. Pollen Analysis.,
479 Chemical Composition and Antibacterial Activity of Anatolian Chestnut Propolis Collected
480 From Yığılca Region. *Biology Bulletin*, **48**: 721-728.

481 Kekecoglu, M., Sönmez, E., Dorkaç, P. and Eroglu, N. 2022. Determination of In Vitro
482 Antimicrobial Activities of Different Propolis Samples from Düzce-Yığılca Region against
483 Oral Microorganisms. *KSU J. Agric Nat.*, **25(2)**: 234-242.

484 Kohno, M., Horibe, T., Ohara, K., Ito, S. and Kawakami, K. 2014. The membrane-lytic
485 peptides K8L9 and melittin enter cancer cells via receptor endocytosis following subcytotoxic
486 exposure. *Chem Biol.*, **21**: 1522-32.

487 Lee, K. S., Kim, B. Y., Yoon, H. J., Choi, Y. S. and Jin, B. R. 2016. Secapin., a bee venom
488 peptide., exhibits anti-fibrinolytic., anti-elastolytic., and anti-microbial
489 activities. *Developmental & Comparative Immunology*, **63**: 27-35.

490 Moharrami, M., Mojgani, N., Bagheri, M. and Toutiaee, S. 2022. Role of Honey Bee Gut
491 Microbiota in the Control of American Foulbrood and European Foulbrood Diseases. *Archives*
492 *of Razi Institute*, **77(4)**: 1331.

- 493 Morais, M., Moreira, L., Feás, X. and Estevinho, L. M. 2011. Honeybee-collected pollen from
494 five Portuguese Natural Parks: Palynological origin., phenolic content., antioxidant properties
495 and antimicrobial activity. *Food and Chemical Toxicology*, **49(5)**: 1096-1101.
- 496 Mărgăoan, R., Mărghitaş, L. A., Dezmarean, D. S., Bobiş, O., Bonta, V., Cătană, C., et al.
497 2017. Comparative Study on Quality Parameters of Royal Jelly., Apilarnil and Queen Bee
498 Larvae Triturate. Bulletin of the University of Agricultural Sciences & Veterinary Medicine
499 Cluj-Napoca. *Animal Science & Biotechnologies*, **74(1)**: 51-58.
- 500 Morse, R. A. and Calderon, N. W. 2000. The value of honey bee pollination in the United
501 States. *Bee Cult.*, **128**: 1–15.
- 502 Mosca, M., Bubnic, J., Giannetti, L., Fortugno, L., Pietropaoli, M., Manara, V., ... and
503 Formato, G. 2023. Adoption of Partial Shook Swarm in the Integrated Control of American and
504 European Foulbrood of Honey Bee (*Apis mellifera* L.). *Agriculture*, **13(2)**: 363.
- 505 Naglaa, F. B., Wael, M. M. and Mohamed, E. H. 2020. Controlling of some honeybee *Apis*
506 *mellifera* (Hymenoptera: Apidae) colonies diseases by bee venom. *Egypt. J. Plant Protec. Res.*
507 *Inst*, **3(4)**: 1139-1150.
- 508 Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R. and De Feo, V. 2013. Effect of
509 Essential Oils on Pathogenic Bacteria. *Pharmaceuticals*, **6**: 1451–1474.
- 510 Ortiz-Alvarado, Y., Clark, D. R., Vega-Melendez, C. J., Flores-Cruz, Z., Domingez-Bello, M.
511 G. and Giray, T. 2020. Antibiotics in hives and their effects on honey bee physiology and
512 behavioral development. *Biology open*, **9(11)**: bio053884.
- 513 Özkırım, A., Çelemlı, Ö. G., Schiesser, A., Charistos, L. and Hatjina, F. 2014. A comparison
514 of the activities of Greek and Turkish propolis against *Paenibacillus larvae*. Journal of
515 *Apicultural Research*, **53(5)**: 528-536.
- 516 Pandidan, S. and Mechler, A. 2019. Nano-viscosimetry analysis of the membrane disrupting
517 action of the bee venom peptide melittin. *Sci Rep.*, **9**: 10841.
- 518 Pereira, J. A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P., Ferreira, I., Ferreres, F., Bento,
519 A., Seabra, R. and Estevinho, L. 2007. Walnut (*Juglans regia* L.) leaves: phenolic compounds.,
520 antibacterial activity and antioxidant potential of different cultivars. *Food and Chemical*
521 *Toxicology*, **45**: 2287–2295.
- 522 Popova, M., Silici, S., Kaftanoglu, O. and Bankova, V. 2005. Antibacterial activity of Turkish
523 propolis and its qualitative and quantitative chemical composition. *Phytomedicine*, **12(3)**: 221-
524 228.
- 525 Rady, I., Siddiqui, I. A., Rady, M. and Mukhtar, H. 2017. Melittin., a major peptide component
526 of bee venom., and its conjugates in cancer therapy. *Cancer letters*, **402**: 16–31.

527 Rauch, S., Ashiralieva, A., Hedtke, K. and Genersc, E. 2009. Negative correlation between
528 individual-insect-level virulence and colony-level virulence of *Paenibacillus larvae*, the
529 etiological agent of American foulbrood of honeybees. *Appl Environ Microbiol.*, **75**: 3344–
530 3347.

531 Ramanathan, A. N. K. G., Nair, A. J. and Sugunan, V. S. 2018. A review on Royal Jelly
532 proteins and peptides. *Journal of Functional Foods*, **44**: 255-264.

533 Raut, J. S. and Karuppayil, S. M. 2014. A status review on the medicinal properties of essential
534 oils. *Ind Crops Prod.*, **62**: 250-64.

535 Raymann, K. and Moran, N. A. 2018. The role of the gut microbiome in health and disease of
536 adult honey bee workers. *Current opinion in insect science*, **26**: 97-104.

537 Savarino, A. E., Terio, V., Barrasso, R., Ceci, E., Panseri, S., Chiesa, L. M. and Bonerba, E.
538 2020. Occurrence of antibiotic residues in Apulian honey: Potential risk of environmental
539 pollution by antibiotics. *Italian journal of food safety*, **9(1)**: 14-19.

540 Šedivá, M., Laho, M., Kohútová, L., Mojžišová, A., Majtán, J. and Klaudivy, J. 2018. 10-
541 HDA., a major fatty acid of royal jelly., exhibits pH dependent growth-inhibitory activity
542 against different strains of *Paenibacillus larvae*. *Molecules*, **23(12)**: 3236.

543 Sevim, E., Bozdeveci, A., Pinarbaş, M., Kekeçoğlu, M., Akpınar, R., Keskin, M., Kolaylı, S.
544 and Karaoğlu, S. A. 2021. Antibacterial Effects of Anatolian Propolis on *Paenibacillus larvae*.
545 *U Bee J.*, **21(2)**: 177-186.

546 Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. 1999. Analysis of total phenols
547 and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods*
548 *in enzymology.*, Lester., P., Ed. Academic Press. PP. 152-178.

549 Soares de Arruda, V. A., Viera dos Santos, A., Figueiredo Sampaio, D., da Silva Araujo, E.,
550 de Castro Peixoto, AL., Estevinho, L. M. and de Almeida-Muradian, L. B. 2021. Brazilian bee
551 pollen: phenolic content., antioxidant properties and antimicrobial activity. *Journal of*
552 *Apicultural Research*, **60(5)**: 775-783.

553 Sonmez, E., Kekecoglu, M., Bozdeveci, A. and Alpay Karaoglu, S. 2022. Chemical profiling
554 and antimicrobial effect of Anatolian honey bee venom. *Toxicon*, **213**: 1-6.

555 Sonmez, E., Kekecoglu, M., Sahin, H., Bozdeveci, A. and Alpay Karaoglu, S. 2023. An
556 evaluation of the chemical composition and biological properties of Anatolian Royal Jelly.,
557 drone brood and queen bee larvae. *European Food Research and Technology*, **249(5)**: 1391-
558 1401.

559 Sonmez, E., Kekecoglu, M., Sahin, H., Bozdeveci, A. and Alpay Karaoglu, S. 2023.
560 Comparing the biological properties and chemical profiling of chestnut bee pollen and bee
561 bread collected from Anatolia. *Brazilian Journal of Microbiology*, 1-11.

562 Uzel, A., Önçağ, Ö., Çoğulu, D. and Gençay, Ö. 2005. Chemical compositions and
563 antimicrobial activities of four different Anatolian propolis samples. *Microbiological research*,
564 **160(2)**: 189-195.

565 Velikova, M., Bankova, V., Marcucci, M. C., Tsvetkova, I. and Kujumgiev, A. 2000.
566 Chemical composition and biological activity of propolis from Brazilian meliponinae.
567 *Zeitschrift für Naturforschung C*, **55(9-10)**: 785-789.

568 Ventola, C. L. 2015. The antibiotic resistance crisis: Part 1: causes and threats. *Pharmacol*
569 *Ther.*, **40**: 277–283.

570 Wagh, V. D. 2013. Propolis: a wonder bees product and its pharmacological
571 potentials. *Advances in Pharmacological and Pharmaceutical Sciences*, **2013**: 1-11.

572 Wang, C., Hong, T., Cui, P., Wang, J. and Xia, J. 2021. Antimicrobial peptides towards
573 clinical application: Delivery and formulation. *Adv Drug Deliv Rev.*, **175**: 113818.

574

575

576

577

578