Araştırma Makalesi https://doi.org/10.46810/tdfd.776424 Research Article

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Determination of Biological Activity, Lipophilic and Volatile Organic Compounds of Bingöl Propolis Isolates

Dilhun Keriman ARSERİM-UCAR[1*], İbrahim Halil GECİBESLER[2], Mert SUDAGIDAN[3], İnan DURSUN[4,5], Veysel SÜZERER[6], Ramazan SOLMAZ[2]

[1] Bingöl University, Faculty of Health Sciences, Nutrition and Dietetics Department, Bingöl, Turkey
[2] Bingöl University, Faculty of Health Sciences, Occupational Health and Safety Department, Bingöl, Turkey
[3] Konya Food and Agriculture University, Kit-Argem R&D Center, Konya, Turkey
[4] Bingöl University, Unit of Pilot University Coordination Center, Bingöl, Turkey
[5] Bingöl University, Center of Research and Application Center, Bingöl, Turkey
[6] Bingöl University, Department of Pharmacy Sciences, Vocational School of Health Services, Bingöl, Turkey
Dilhun Keriman ARSERİM-UCAR ORCID No: 0000-0001-8011-5059
İbrahim Halil GECİBESLER ORCID No: 0000-0002-4473-2671
Mert SUDAGIDAN ORCID No: 0000-0002-3980-8344
İnan DURSUN ORCID No: 0000-0003-1717-8166
Veysel SÜZERER ORCID No: 0000-0001-5383-4613
Ramazan SOLMAZ ORCID No: 0000-0002-9295-1203

**Sorumlu yazar: kerimanarserim@gmail.com*

(Alınış: 02.08.2020, Kabul: 21.10.2020, Online Yayınlanma: 23.10.2020)

Keywords
Bingöl Propolis, Propolis Isolates, Propolis Essential Oil, Biological Activity, Volatile Organic Compounds

Abstract: In this study, the biological activity and chemical composition of Bingöl propolis were investigated. The isolates, including ethanol isolate (EI), hexane isolate (HI), and essential oil isolate (EOI), were prepared from raw propolis. The lipophilic and volatile organic compounds in isolates were analyzed by Gas Chromatography-Mass Spectrometry (GS-MS) measurements. Bingöl propolis isolates showed antimicrobial activity against reference pathogen bacterial strains, including *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (NRRL-B-3711) *Escherichia coli* (ATCC 25922), *Salmonella enterica* subsp. *enterica serovar* Typhimurium (ATCC 14028), while *B. cereus* was observed to be the most susceptible bacteria to all tested propolis isolates. It was observed that EI showed the highest antimicrobial and antioxidant activity compared to other isolates. The presence of bioactive components in the lipophilic and essential oil isolates of Bingöl propolis may have contributed to the biological activity of propolis. Bingöl propolis isolates (EI and EOI) obtained in this study have the potential to be used as natural preservatives in food systems.

Bingöl Propolis İzolatlarının Biyolojik Aktivitesinin, Lipofilik ve Uçucu Organik Bileşenlerinin Belierlenmesi

Anahtar Kelimeler
Bingöl Propolisi, Propolis İzolatları, Propolis Uçucu Yağı, Biyolojik Aktivite, Uçucu Organik Bileşenler

Öz: Çalışmada Bingöl Propolis izolatlarını biyolojik aktivitesi ve kimyasal bileşimi araştırılmıştır. Propolisin etanol izolatı (Eİ), hekzan izolatı (Hİ) ve uçucu yağ izolatı (UYİ) ham propolisten hazırlanmıştır. İzolatların lipofilik ve uçucu organik bileşenleri Gaz Kromatografisi- Kütle Spektrometresi (GC-MS) ile analiz edilmiştir. Bingöl Propolis izolatları *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (NRRL-B-3711) *Escherichia coli* (ATCC 25922), *Salmonella enterica* subsp. *enterica serovar* Typhimurium (ATCC 14028) referans patojen bakteri suşlarına karşı antimikrobiyal aktivite gösterirken, test edilen tüm propolis izolatlarına en duyarlı bakteri *B. Cereus* olduğu gözlenmiştir. Eİ izolatının diğer izolatlara kıyasla en yüksek antimikrobiyal ve antioksidan aktivite gösterdiği belirlenmiştir. Bingöl propolisinin lipofilik ve uçucu yağ izolatlarında biyoaktif bileşen varlığının, propolisin biyolojik aktivitesine katkıda bulunduğu değerlendirilmiştir. Çalışma kapsamında elde edilen Bingöl propolisi (Eİ ve UYİ) izolatlarının gıda sistemlerinde doğal koruyucular olarak kullanılabilme potansiyeline sahip olduğu belirlenmiştir.

1. INTRODUCTION

Bee products had attracted considerable attention due to their health benefits as a traditional medicine from ancient times. Propolis is one of the most investigated bee products because of its broad array of biological activities, including antimicrobial, antioxidant, anticancer, anti-inflammatory, antiviral, antitumoral activity, and wound healing [1]. Bee propolis which is called ‘’Bee-glue’’ is a natural resinous substance collected by honey bees from various plant sources for protecting their hives [2]. Propolis composed of 50% resins and balsams, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances [3-4]. Geography and botanical origins, honey bee species, and harvesting seasons have a bearing on the chemical composition, constitution, color, smell of propolis, as well as biological activity [4-6]. Propolis, considered to be a potential source of naturally occurring compound, contains a wide variety of chemical compounds such as sugars, alcohols, aldehydes, aliphatic acids/esters, amino acids, steroids, aromatic esters/acids, volatile organic compounds, ketones, chalcones and dihydrochalcones, flavanones, flavones and flavonols, hydrocarbon esters, ethers, terpenoids, hydroxy and keto waxes and waxy acids [2, 7-8]. The major extracted bioactive compound is varied in amount and type depends on the used extraction process associated with used solvents type and polarity. Biologic activity of propolis extracts from different solvents, including water, ethanol, and other solvent fractions and constituents have been already reported in several studies [6, 9-12]. However, information regarding the propolis oils constituents [13-15] and biological activity such as antifungal [16] and antimicrobial activity [17] were scarce. In addition, propolis extracts antibacterial activity has been documented in several studies, such as against food-borne pathogens [5], plant bacterial pathogens [18], honeybee pathogen *Paenibacillus larvae* [19], specific oral pathogens [20] and also *varroa mite* [21]. Not only the extract but also propolis oil and the volatile constituents exhibited high antimicrobial activity. The study of Melliou et al. [22] reported the antibacterial activity of volatile components of propolis against six bacteria and three fungi. The research performed by Sinott et al.[23] demonstrated the effect in vitro anthelmintic activity of propolis essential oil.

Propolis contains natural antioxidant compounds that capable of reducing activity, chelating properties, and hydrogen atom transfer act as a defensive mechanism against free radicals [7]. The antioxidant activity of propolis extracts from different solvents, including ethanol extracts [24-25] and water extracts [7, 11], possessed potent antioxidant activity. Antioxidant capacity of propolis associated with its high contents of phenolic and flavonoids (bioactive) compounds, which has great potential as natural and safe antioxidants for food applications.

To the best of our knowledge, the biological activity and volatile constituents of propolis oil from Bingöl province have not been reported. This work is aimed to investigate the biological activity of Bingöl propolis isolates. Moreover, propolis isolates of Bingöl province were tested for antioxidant and antibacterial activity against reference pathogen bacteria, including *S.aureus*, *B. cereus*, *E.coli*, and *S.thyphimurium*. Furthermore, volatile organic compounds and lipophilic components of Bingöl propolis isolates were investigated.

2. MATERIAL AND METHODS

2.1. Sampling

The propolis in the beehives were harvested by beekeepers from the central locations (39º 00′ 739″ N and 40º 15′ 190″ E) of Bingöl province at an altitude of 1810 m during June-July 2019. The harvested propolis samples were taken into amber glass flasks and stored at -20 °C. Lamiaceae, Fabaceae, Asteraceae, Apiaceae, Boraginaceae, and Brassicaceae were the dominant plant families in the localities where propolis samples were collected [26-27].

2.2. Isolate Preparation Processes

2.2.1. Essential oil isolate

To obtain the volatile organic compounds of Bingöl propolis, 2 kg of powdered raw propolis was dissolved in 2.2 L of distilled water in a 5 L glass flask, and was added on it. Clevenger apparatus was mounted on the glass flask, and a jacket heater was used for heat treatment [28]. Extraction was continued for 4 hours to obtain volatile propolis components. Cooled circulator (-25°C) used for the condensation process. The essential oil obtained at the end of extraction was taken to the organic n-hexane phase and dried with anhydrous sodium sulfate. The amount of propolis essential oil (EOI) obtained was recorded as 187 mg. Propolis essential oil was taken in a brown colored vial covered with aluminum foil on the outside and stored at + 4°C for biological activity and chemical analysis.

2.2.2. Organic isolates

To obtain the *n*-hexane isolate, 100 g of powdered propolis was added to the glass flask, and 500 mL of *n*-hexane was added. Extraction was carried out at 22 °C using a magnetic stirrer. The same processes were repeated three more times, and the extraction process was terminated after repetitions [29] . At the end of the extraction process, *n*-hexane extract and propolis pulp were separated using filter paper. The extraction process was continued by adding 500 mL of ethanol on the remaining propolis pulp. The extraction with ethanol procedure was the same as the physical conditions in the *n*-hexane extraction procedure. At the end of extraction, propolis pulp and ethanol extract were separated by filtration. The solvents in the n-hexane and ethanol extracts were removed under low pressure so that the heat treatment temperature did not exceed 35°C with the aid of the rotary evaporator. The amounts of dry isolates obtained as a result of solvent removal from n-hexane and ethanol extracts were recorded as 3.48 g

and 7.85 g, respectively. Dry isolates were taken in amber vials covered with aluminum foil and stored for bioactivity and chemical analysis at + 4°C.

2.3. Esterification Process

The esterification process of the *n*-hexane and ethanol isolates was carried out according to the appropriate literature [30]. 0.5 g of each of *n*-hexane and ethanol isolates were taken into separate glass flasks. 5 mL of methanol: sulphuric acid: toluene (2:1:1;v:v:v) was added and treated for 12 hours at 160 rpm in a 50°C water bath. At the end of the period, 3 ml of distilled water was added to the reaction medium and mixed. It was shaken vigorously using 3 mL of diethyl ether to remove the esterification fraction in the reaction mixture. After phase separation, anhydrous sodium sulfate was added to the organic phase to remove moisture from the organic phase. After the organic phase was filtered through 0.22 µm PTFE filter, it was taken into amber vials and ready for chemical analysis.

2.4. Lipophilic and Volatile Component Analysis

Agilent brand (7890A GC and 5975C MS) gas chromatography-mass spectrometer GC-MS was used to analyze the lipophilic and volatile components contained in Bingöl propolis. In the mass analysis, the FID detector was used simultaneously with an MS detector. SGE GC capillary column (100m x 250 µm x 0.25 µm) was used as the column. The lipophilic component analysis conditions were as follows: sample injection volume was performed as 1 µL in non-split mode. The flow rate of the carrier gas from the column was set to 1 mL/min. The column oven temperature is kept constant at 120°C for 8 minutes, then it starts from this temperature and reaches 252 °C in 3°C/min increments and is kept there for 10 min. The total analysis time was 62 minutes, for volatile organic components was performed by GC-MS spectrometer, which is used in the analysis of lipophilic components. The volatile organic component analysis conditions were as follows: the injection volume of the sample was 1 µL by selecting 1/10 split mode. The column furnace temperature was initially kept constant at 40 °C for 1 min, then reaches 150°C in an increment of 4°C/min and was held there for 5 min. It then reaches 255°C in increments of 4°C/min, where 10.5 min was expected. The total analysis time was 79 min. MS results were determined by comparing with Wiley and NIST libraries in the memory of the device [31].

2.5. Antimicrobial Activity Test

The antibacterial activity test of the propolis ethanol isolate (EI), propolis essential oil isolate (EOI) and hexane isolate of propolis (HI) against Gram (+) bacteria; *S.aureus* (ATCC 25923), *B. cereus* (NRRL-B-3711) and Gram (-) bacteria; *E.coli* (ATCC 25922), *Salmonella enterica* subsp. *enterica serovar* Typhimurium (ATCC 14028) was performed as described by the CLSI (The Clinical and Laboratory Standards Institute) method [32]. Bacterial cultures were grown on Tryptic Soya Agar (TSA, Oxoid) at 37 ºC for 18 h. Bacterial suspensions were adjusted to McFarland 0.5 turbidity in 10 mL sterile 0.9% (w/v) NaCl. The screening of antibacterial activity of propolis isolates (EI, EOI, and HI) was performed by the microdilution method to determine the minimum inhibitory concentrations. A solution of 1 mg/mL propolis isolates samples were prepared with DMSO (Dimethyl sulfoxide, Sigma) as 10% (w/w of propolis). Müller-Hilton broth containing DMSO with bacteria without sample was used as a positive control, and samples without bacteria were used as negative controls. The serial dilutions were prepared with MHB (Müller-Hilton broth, Oxoid). All dilutions were done duplicate. The microwell plates were incubated at 37 ºC for 24 h; at the end of incubation, the absorbance (OD) of each well was measured using a microplate reader (SpectraMax ®, Plus 384, USA) at 600 nm.

2.6. Antioxidant Activity Test

The antioxidant activity of propolis isolates, including EI, EOI, and HI, was determined using $ABTS^{\bullet+}$ (2,2'-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay described by Re et al. [33]. The ABTS reagent at 7 mM concentration was prepared in deionized water. $ABTS^{\bullet+}$ stock solution reacted with 2.45 mM potassium persulfate and standing in the dark at room temperature for 12-16 h. $ABTS^{\bullet+}$ radical solution was diluted with ethanol: water (1:1,v/v) to obtain an absorbance of 0.75-0.80 at 734 nm. A solution of 2 mg/mL isolate sample were prepared in dimethyl sulfoxide: ethanol (2:1, v/v). All determinations for samples and standards were performed in triplicate. The reaction between $ABTS^{\bullet+}$ and isolate samples was conducted in 96 well microplates. The reaction mixture contained 275 µL of diluted $ABTS^{\bullet+}$ and 5 µL sample or Trolox standard. All samples were incubated at 30°C for 6 min before reading. The absorbance was obtained after incubation using a microplate reader (SpectraMax®, Plus 384, USA) at 734 nm. The antioxidant capability to scavenge the $ABTS^{\bullet+}$ represented as percentage ABTS scavenging activity that was calculated by the following equation:

$$\text{ABTS scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100 \quad (1)$$

The sample concentrations providing % inhibition was obtained by calibration curve. The direrent ABTS concentrations (0.003125; 0.00625; 0.0125; 0.025; 0.05; 0.1 mM) was used to obtain the calibration curve, determined by linear regression (r^2:0.9985).

2.7. Statistical Analyses

Statistical analysis was performed with Minitab 17 software version, and data analyses were conducted by one-way analysis of variance (ANOVA) by Tukey's

comparison test, $p<0.05$ was considered as significant. The data were recorded as mean ± standard deviation.

3. RESULT AND DISCUSSION

3.1. Volatile Organic Compounds

Gas chromatography-mass spectrometry analysis results showed that Bingöl propolis contains 37 components in terms of volatile organic components (Table1). Bingöl propolis was found to be rich especially in compounds such as simple terpenoids (linalool (1.82%) and prenol (1.20%)), monoterpenes (*α*-pinene (2.92%), *γ*-terpinene (2.61%) and (+)-2-carene (1.37%)), sesquiterpenes (*α*-copaene (4.47%), (+)-*δ*-cadinene (6.69%), *γ*-muurolene (2.62%) ve zonarene (3.10%)) and sesquiterpenoids (epi-*α*-cadinol (2,35%), *γ*-eudesmol (3.37%), *α*-eudesmol (5.14%) ve *β*-eudesmol (5.62%). In addition, other organic components such as octanal (3.76%), nonanal (8.99%), decanal (12.58%), and prenyl acetate (2.13%), which are found in fragrance components in natural foods such as fruits, vegetables, and plants, were also predominantly analyzed [34]. Whereas, differently, the *p*-vinylanisole (6.64%), which has an aromatic compound, was detected as a dominant component. Moreover, the dimethylvinylcarbinol, which is famous as a pheromone component in bee and other insect species, was also analyzed at a rate of 2.08% (Figure 1).

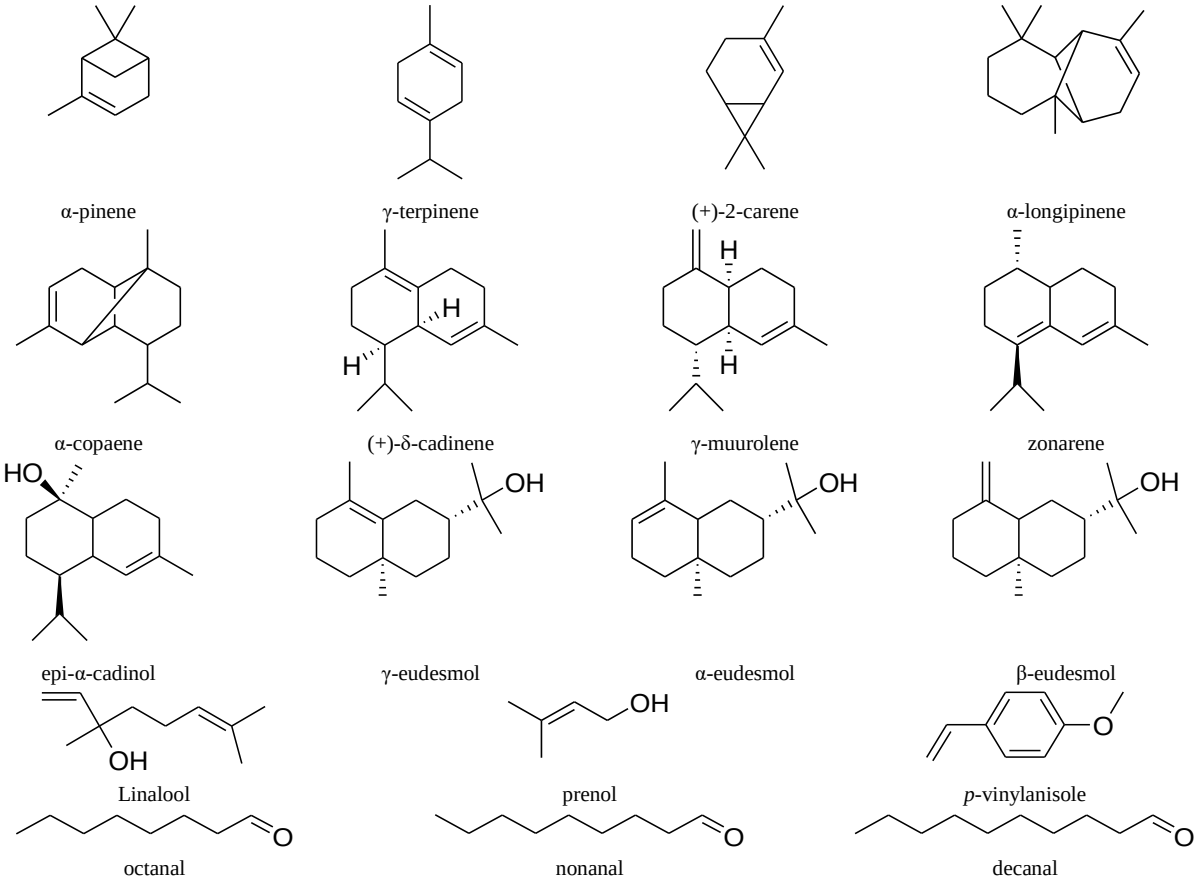

Figure 1. Chemical structures of volatile organic compounds predominantly in Bingol propolis

Table 1. Volatile organic compounds in the essential oil of Bingol propolis

RT	Compound name	RA (%)
13.732	Dimethylvinylcarbinol	2.08
14.757	α-Pinene	2.92
15.197	γ-Terpinene	2.61
16.496	(+)-2-Carene	1.37
19.781	Prenyl acetate	2.13
21.514	Prenol	1.20
22.092	Octanal	3.76
23,608	Pentylbenzene	0.97
24,730	Nonanal	8.99
26.098	(Z,Z)-α-Farnesene	0.13
26.532	Linalool	1.82
27.282	Decanal	12.58
28.489	Zonarene	3.10
28.953	α-Longipinene	1.11
29,153	α-Cubebene	0.04
29.857	α-Ylangene	0.09
29.926	α-Muurolene	0.75
30.475	α-Copaene	4.47
30.858	(+)-δ-Cadinene	6.69
32.071	α-Terpineol	0.66

33.279	p-Vinylanisole	6.64
34.200	cis-Calamenene	0.97
36.019	α-Acetoxytoluene	0.93
36,443	10,11-Epoxy-Calamenene	0.71
36.506	1-(N-Methyl-2-Amino-Phenyl)-3-Methyl-1,3-Butadiene	0.18
36.609	α-Calacorene	0.84
42,468	Isoledene	0.75
43,710	1,6-Dimethylnaphthalene	0.04
44,705	Epi-α-Cadinol	2.35
45.077	γ-Eudesmol	3.37
46.136	γ-Muurolene	2.92
46.571	3,4-Dimethoxystyrene	2.13
46.903	α-Cadinol	2.21
47.927	α-Eudesmol	5.14
48.654	β-Eudesmol	5.62
55.079	Ethyl (2E)-3-[2-(Diethoxyphosphoryl)-4 (Dimethylamino)Phenyl]-2-Propenoate	0.22
58.198	1,13-Tetradecadiene	0.31

*RT: retention time; RA: relative abundant

3.2. Lipophilic Compounds

The lipophilic components of *n*-hexane and ethanol isolates, which are non-esterified forms (HI and EI isolate, respectively) and esterified forms (EHI and EEI isolates respectively) analyzed by GC-MS spectrometer. According to the results of the analysis, Bingol propolis had a total of 66 lipophilic components (Table 2). GC-MS analysis of both esterified forms and non-esterified forms of the Bingöl propolis isolates showed that they had a very broad lipophilic component content (figure 2). EHI and EIE isolates with esterified forms were obtained to determine the fatty acids and other lipophilic components by volatilizing the lipids contained in Bingöl propolis. HI and EI isolates with non-esterified forms were provide to analyze other organic compounds such as steroid and saponin that Bingöl propolis contains. Bingöl propolis has been found to contain lauric acid (0.24-0.48%), myristic acid (0.51-0.78%), stearic acid (1.75-1.86%) and palmitic acid (8.86-9.34%) components from the saturated fatty acids in EHI and EIE isolates, while it contains oleic acid (10.87-13.19%), linoleic acid (0.85-0.92%) and linolenic acid (0.36-0.43%). The eicosane (27.32%) and docosane (24.33%) components of the HIE isolate, the heneicosane (38.33%), pentacosane (9.12%) and 17-pentatriacontene (10.33%) components in the EIE isolate were the major components, while the tetracosane (13.18-13.7%) component was represented as the major component in both isolates. Similarly, in the HI isolate, the eicosane (30.41%), docosane (15.5%) and tetracosane (18.0%) components were dominant components, while the EI isolate was predominantly containing acyclic hydrocarbons such as pentacosane (9.63%), tetracosane, nonadecane (13.92%), 17-pentatriacontane (22.84%) and 1-nonadecene (8.19%). Unlike these components, , α-amyrone (9.16%) and β-amyrin (24.28%) components were also found to be the dominant components in the EI isolate. Considering the lipophilic component variety of Bingöl propolis, it was quite remarkable that it was rich in unsaturated fatty acids such as oleic acid (omega 9), which provides significant benefits to human health in the daily diet, especially in EHI and EEI isolates. These unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid have been reported to show broad-spectrum biological activity [35-37] . In addition, the many beneficial effects of α-amyrone and β-amyrin and sesquiterpenoids, which are dominant in EI isolate, have also been reported [38-40].

Table 2. Lipophilic components contained in Bingol propolis

RT	Compound Name	EHI	EEI	HI	EI
12.560	2-Nitrocinnamyl alcohol				1.05
15.709	2-Methyl 1-dodecanol	0.02			
16.121	Octadecene	0.05			
16.212	1-Tetradecene	0.13	0.03		
16.178	Pentafluoropropionic acid, hexadecyl ester			0.1	
16.335	Cyclotetradecane		0.05		
18.433	δ-Cadinene	0.21	0.2	0.1	
18.770	γ-Muurolene			0.13	
21.116	Lauric acid (C 12:0)	0.48	0.24		
22.078	(E)-9-Octadecene	0.3	0.12		
26.512	Myristic acid (C 14:0)	0.78	0.51		
27.742	Ethyl myristate	0.19			
28.732	Carbonic acid, dodecyl isobutyl ester		0.08		
30.409	2,5-Dimethyl-4-methoxyphenol			0.03	
30.521	(-)-α.-Amorphene				0.25

30.670	β-Patchoulene				0.14
31.925	Palmitic acid (C 16:0)	9.34	8.86		
32.440	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene			0.24	
33.012	Ethyl palmitate	1.7			
33.075	γ-Curcumene			0.28	
33.737	6-α-cadina-4,9-diene			0.49	
33.779	α-Selinene		0.11		
34.080	γ-Eudesmol				0.43
34.437	α-Cadinol		0.24	0.63	
34.923	(E)-Methyl cinnamate	0.17	0.11		
35.473	α-Eudesmol	0.24	0.15	0.95	0.97
36.165	β-Eudesmol			0.81	1.05
36.583	Pentadecane			2.97	
36.834	Stearic acid (C 18:0)	1.75	1.86		
37.430	Oleic acid (C 18:1; cis-9; omega 9)	10.87	13.19		
38.276	Ethyl elaidate	1.77			
38.311	(Z)-13-Octadecenal			0.25	
38.791	Linoleic acid (C 18:2; cis, cis-9,12; Omega 6)	0.85	0.92		
38.854	2-Nonadecanone			0.89	
39.478	(Z,Z) 2-Methyl-3,13-octadecadienol	0.09			
39.621	Tetracosane	0.16			
39.713	N-[4-Bromo-N-Butyl] 2-piperidinone			0.27	
39.781	Cyclohexadecane		0.22		
40.559	α-Linolenic acid (C 18:3; all Cis - 9,12,15; Omega 3)	0.36	0.43		
41.761	Eicosane	27.32	1.20	30.41	
43.426	Tricosane	0.14			
44.027	Heneicosane	0.43	38.33	0.3	
44.717	α-Copaene				0.40
45.200	2-[(E)-9-Octadecenyloxy]ethanol	0.15			
45.177	1,13-Tetradecadiene			0.56	
45.206	1,9-Tetradecadiene		0.13		
45.461	Sesquisabinene hydrate				0.29
46.182	Valencene				0.69
49.011	Octadecane		0.27	11.24	
49.314	Methyl 12-oxo-9-dodecenoate	0.41			
50.836	Docosane	24.33		15.5	
52.301	Nonacosane	1.41			
53.365	Pentacosane		9.12	0.76	9.63
53.411	2-Heptadecenal	0.9			
54.830	Tetracosane	13.7	13.18	18.0	5.70
58.164	1,12-Tridecadiene				1.01
58.572	9-Nonadecene			13.54	
59.013	17-Pentatriacontene		10.33		
59.983	Nonadecane				13.92
60.586	Cis-1-chloro-9-octadecene			0.29	
61.565	2,5-Dımethyl-4-Methoxyphenol	0.01			

61.410	Benzyl cinnamate			0.33	
68.252	1-Nonadecene				8.19
69.430	α- Amyrone				9.16
71.679	17-Pentatriacontene				22.84
75.936	β-Amyrin				24.28

*RT: Retention time; EHI: estrificated *n*-hexane isolate; EEI: estrificated ethanol isolate; HI: *n*-hexane isolate; I: ethanol isolate.

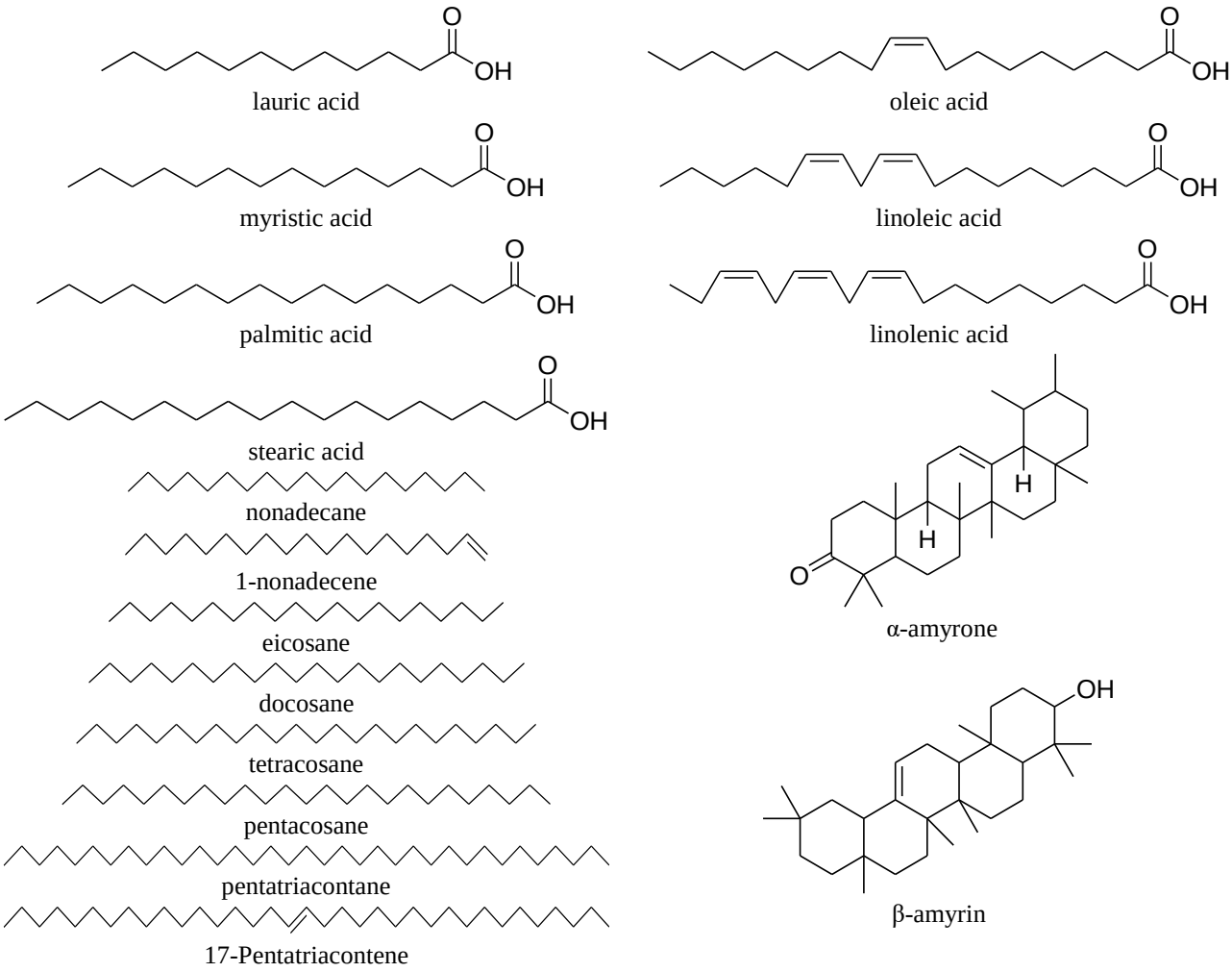

Figure 2. Chemical structures of lipophilic components in Bingol propolis

The compounds determined in the Bingöl propolis include 17-pentatriacontene, 1-nonadecene, nonadecane, octadecane, 2-nonadecanone were also reported in propolis samples collected from 21 diferent parts of Turkey [41], benzyl cinnamate, methyl cinnamate, *α*-pinene, *α*-copaene, *β*-eudesmol, *α*-eudesmol, nonacosane, heneicosane, 2-nonadecanone was found in the Hatay propolis samples [42]. In addition, Bingöl propolis contains the fatty acids of Anatolian propolis, including; lauric acid, myristic acid, palmitic acid, oleic acid, stearic acid, and linoleic acid [43].

3.3. Antimicrobial Activity Test

The antimicrobial activity of propolis isolates were determined using the microdilution method to determine the minimum inhibitory concentrations. The results obtained on the susceptibility of *S.aureus*, *B. cereus*, *E.coli*, and *S.thyphimurium* to the isolates, including HI, EI, and EOI, are illustrated in Figure 3. Also, the MICs of each sample can be seen in Table 3.

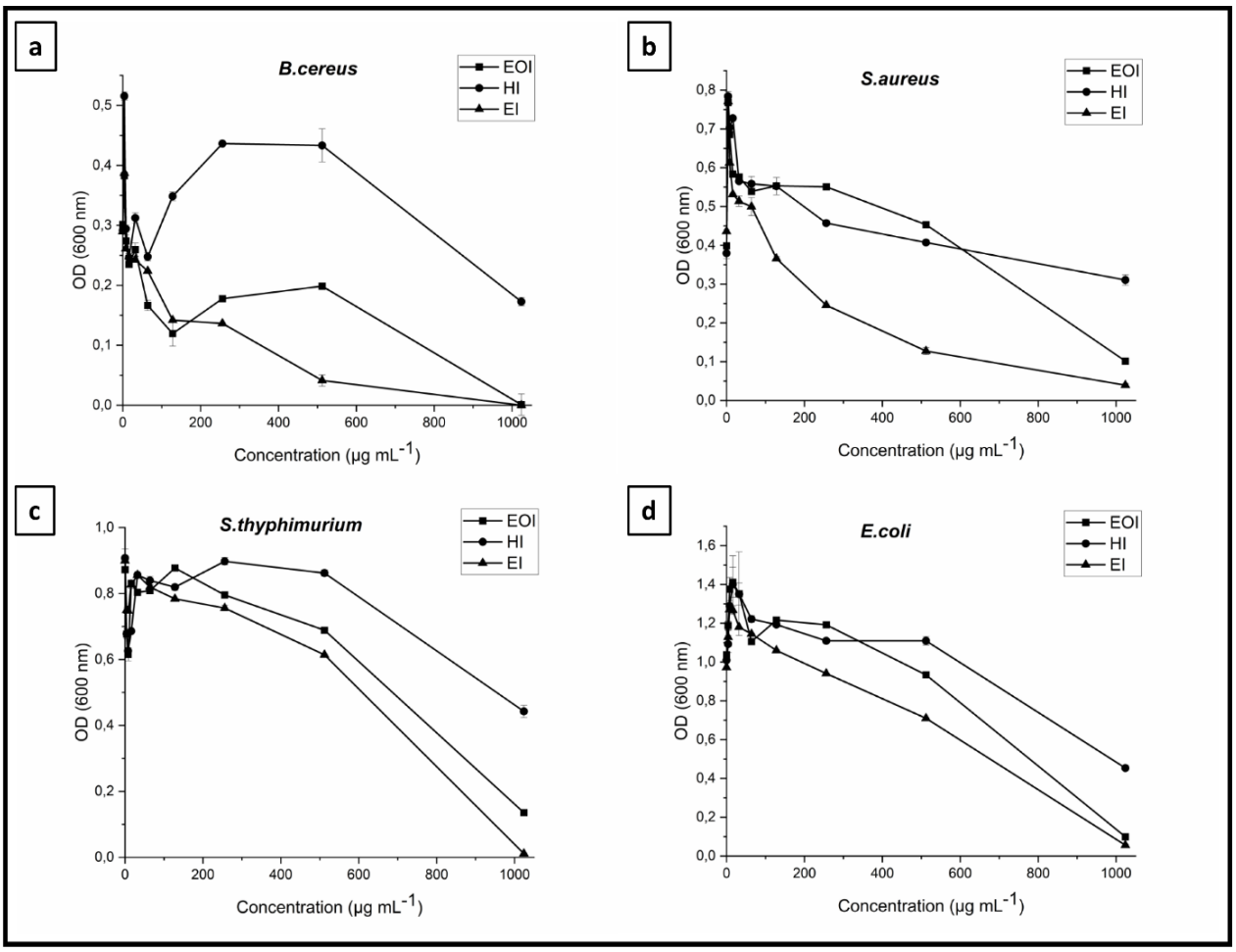

Figure 3. The antibacterial activity of the different concentrations of propolis essential oil isolate (EOI), hexane isolate of propolis (HI), and ethanol isolate of propolis (EI) against (a) *B.cereus*, (b) *S.aureus*, (c) *E.coli*, (d) *S.thyphimurium.* OD: optical density

Table 3. Antibacterial effects of propolis samples on various reference bacterial pathogens

	Propolis concentration		
Microorganism	**El (µg mL^{-1}) MIC**	**EOI (µg mL^{-1}) MIC**	**HI (µg mL^{-1}) MIC**
B. Cereus NRRL-B-3711	<1024	1024	>1024
S.aureus ATCC 25923	>1024	>1024	>1024
E.coli ATCC 25922	>1024	>1024	>1024
S.thyphimurium ATCC 14028	>1024	>1024	>1024

EI: Propolis Ethanol Isolate, EOI: Essential Oil Isolate, HI: Hexane Isolate

All tested propolis samples showed microbial inhibition against all assessed pathogens. *In vitro*, the antibacterial activity of propolis isolates against Gram (+) and Gram (-) bacteria showed variation toward the tested propolis isolates. The chemical composition, volatile and nonvolatile components, and the applied concentration affect the antimicrobial efficacy [22,43-45]. From the results of our study, the higher susceptibility of Gram-(+) bacteria than Gram-(-) bacteria was observed for EI, and EOI, the most sensitive bacteria was *B. cereus* for all tested propolis samples. The EI sample was the most effective one on tested bacteria among the samples. The EOI showed significant inhibition than the HI. The Propolis extraction method is one of the significant factors in propolis biological activity [6, 46-47]. The antimicrobial activity was concentration-dependent, and these results were in agreement with documented results in the literature [5-6, 46]. Previous studies have reported that propolis contains a high amount of phenolics and flavanoids compounds these compounds are associated with antimicrobial activity [44-45, 47]. The *in vitro* antibacterial efficacy (bactericidal or bacteriostatic) of propolis depended on concentration, treatment time, and mode of bacterial action; however, the exact action mechanism is not yet known [5,17,48-49]. Microbial inoculum levels such as low, intermediate, or high also had a significant effect on the propolis antibacterial activity [50]. The antimicrobial activity of the propolis from different parts of the world had been reported. It is able to inhibit and retard the growth of the wide range of microorganisms, including bacteria, yeast, and moulds [5,46,51-52].

3.4. Antioxidant Activity Test

$ABTS^{\bullet+}$ (2,2'-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging method was used to determine the antioxidant capacity of propolis isolates

(EI, EOI and HI). $ABTS^{\bullet+}$ scavenging capability of propolis isolates are shown in Table 4.

Table 4. Antioxidant activity of propolis isolates (EI, EOI, and HI)

Propolis isolates	**$ABTS^{\bullet+}$ scavenging (mmol TE (g dry Isolate) $^{-1}$)**
Ethanol Isolate (EI)	6.05±0.23 [a]
Essential Oil Isolate (EOI)	0.46±0.04 [b]
Hexane Isolate (HI)	0.15±0.002 [b]

a-b: Different letters between rows indicate different values ($p < 0.05$) according to the Tukey test.

The EI had higher cation radical scavenging activity compared with the EOI and HI samples. However, the EOI and HI samples antioxidant activity was not statistically ($p < 0.05$) significant. Propolis contains a wide variety of chemical compounds, including phenolic compounds, flavonoids, aromatic esters, diterpenic acids, which are the responsible for the biological activity of propolis [9,50]. Antioxidant activity assays have different mechanisms, principles including electron-donating and hydrogen atom transfer with using different reference compounds [53]. Propolis contains phenolic compounds, flavones, and other compounds capable of reducing activity, chelating properties, and hydrogen atom transfer [7]. Previous studies have shown that Turkish propolis antioxidant activity was associated with its high amount of phenolic profile [54]. Propolis extracts have multifunctional properties that can prevent food spoilage from microbial and chemical reactions and improve the quality and safety of food production. Food applications of propolis had been documented that propolis extracts could be a promising candidate as a natural preservative in food applications [55], as well as the volatiles from the propolis fractions had significant potential for preserving the food quality [56].

4. CONCLUSION

This research is a primary screening step for Bingöl propolis. According to our results, the EI of Bingöl propolis exhibited high antioxidant and antimicrobial activity among tested samples. The biological activity of the EOI was better than the HI. Several factors affect the propolis biologic activity; the extraction method is one of the significant factors on propolis biological activity. However, further investigations are required to point out with dose-dependent studies and different kinds of bacteria strains for antimicrobial activity. Also, the other antioxidant activity assays could be used for propolis isolates. Further experiments are needed to elucidate the composition of Bingöl Propolis isolates. Researches are underway the Mission Differentiation and Specialization Aimed at Regional Development Program ongoing projects at Bingöl University.

Acknowledgment

The authors gratefully acknowledge the financial support from the Presidency of the Council of Higher Education, Presidency of Turkey Strategy and Budget, coordinated by The Scientific Research Projects Coordination Unit of Bingöl University (Project Number: 2017K124000-BÜBAP and PİKOM-Arı.2019.012/ BÜ-THBKP-ARI). The authors also thank Bingöl University, the Center of Research and Application Center for their contributions.

REFERENCES

[1] Pasupuleti VR, Sammugam L, Ramesh N, Gan SH. Honey, Propolis, and Royal Jelly: A Comprehensive Review of Their Biological Actions and Health Benefits. Oxidative Medicine And Cellular Longevity. 2017;2017.

[2] Anjum IS, Ullah A, Khan AK, Attaullah M, Khan H, Ali H, et al. Saudi Journal of Biological Sciences Composition and functional properties of propolis (bee glue): A review. Saudi Journal of Biological Sciences. 2019; 26(7):1695-1703.

[3] Isla MI, Paredes-Guzman JF, Nieva-Moreno MI, Koo H, Park YK. Some chemical composition and biological activity of northern Argentine propolis. Journal of Agricultural Food Chemistry. 2005;53(4):1166–72.

[4] Sobočanec S, Šverko V, Balog T, Šarić A, Rusak G, Likić S, et al. Oxidant/antioxidant properties of Croatian native propolis. Journal of Agricultural Food Chemistry. 2006;54(21):8018–26.

[5] Kim YH, Chung HJ. The effects of Korean propolis against food-borne pathogens and transmission electron microscopic examination. New Biotechnology. 2011;28(6):713–8.

[6] Seibert JB, Bautista-Silva JP, Amparo TR, Petit A, Pervier P, Dos Santos Almeida JC, et al. Development of propolis nanoemulsion with antioxidant and antimicrobial activity for use as a potential natural preservative. Food Chemistry. 2019;287:61–7.

[7] Gülçin I, Bursal E, Şehitoğlu MH, Bilsel M, Gören AC. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food and Chemical Toxicology. 2010;48(8–9):2227–38.

[8] Marcucci MC. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie. 1995;26(2):83–99.

[9] Ristivojević P, Dimkić I, Guzelmeric E, Trifković J, Knežević M, Berić T, Yesilada E, Milojković-Opsenica D, Stanković, S. (2018). Profiling of Turkish propolis subtypes: Comparative evaluation of their phytochemical compositions, antioxidant and antimicrobial activities. LWT-Food Science and Technology, 2018;95, 367-379.

[10] Kumazawa S, Hamasaka T, Nakayama T. Antioxidant activity of propolis of various geographic origins. Food Chemistry. 2004;84(3):329–39.

[11] Nagai T, Inoue R, Inoue H, Suzuki N. Preparation and antioxidant properties of water extract of propolis. Food Chemistry. 2003;80(1):29–33.

[12] Ramón-Sierra J, Peraza-López E, Rodríguez-Borges R, Yam-Puc A, Madera-Santana T, Ortiz-

Vázquez E. Partial characterization of ethanolic extract of Melipona beecheii propolis and in vitro evaluation of its antifungal activity. Brazilian Journal of Pharmacognosy. 2019;29(3):319–24.
[13] de Albuquerque IL, Alves LA, Lemos TLG, Dorneles CA, de Morais MO. Constituents of the essential oil of brazilian green propolis from Brazil. J Essent Oil Res. 2008;20(5):414–5.
[14] Bankova V, Boudourova-Krasteva G, Popov S, Sforcin JM, Funari SRC. Seasonal variations in essential oil from brazilian propolis. Journal of Essential Oil Research. 1998;10(6):693–6.
[15] Kusumoto T, Miyamoto T, Higuchi R, Doi S, Sugimoto H, Yamada H. Isolation and structures of two new compounds from the essential oil of Brazilian propolis. Chemical Pharmaceutical Bulletin. 2001;49(9):1207–9.
[16] Ioshida MDM, Young MCM, Lago JHG. Chemical composition and antifungal activity of essential oil from brazilian propolis. Journal of Essent Oil Bearing Plants. 2010;13(5):633–7.
[17] Oliveira AP, França HS, Kuster RM, Teixeira LA, Rocha LM. Chemical composition and antibacterial activity of Brazilian propolis essential oil. Journal of Venomous Animals and Toxins including Tropical Diseases. 2010;16(1):121-30.
[18] Basim E, Basim H, Özcan M. Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. Journal of Food Engineering. 2006;77(4):992–6.
[19] Mihai CM, Mârghitaş LA, Dezmirean DS, Chirilâ F, Moritz RFA, Schlüns H. Interactions among flavonoids of propolis affect antibacterial activity against the honeybee pathogen Paenibacillus larvae. Journal of Invertebrate Pathology. 2012;110(1):68–72.
[20] Koru O, Toksoy F, Acikel CH, Tunca YM, Baysallara M, Guclu AU, et al. In vitro antimicrobial activity of propolis samples from different geographical origins against certain oral pathogens. Anaerobe. 2007;13:140–5.
[21] Garedew A, Schmolz E, Lamprecht I. Microcalorimetric and respirometric investigation of the effect of temperature on the antivarroa action of the natural bee product-propolis. Thermochim Acta. 2003;399(1–2):171–80.
[22] Melliou E, Stratis E, Chinou I. Volatile constituents of propolis from various regions of Greece - Antimicrobial activity. Food Chemistry. 2007;103(2):375–80.
[23] Sinott FA, Sena-Lopes Â, Leal KS, Thais de Oliveira Silva M, Cardoso de Freitas M, Quintana de Moura M, et al. Essential oil from Brazilian Red Propolis exhibits anthelmintic activity against larvae of Toxocara cati. Expperimental Parasitology. 2019;200:37–41.
[24] Ahn M, Kumazawa S, Usui Y, Nakamura J. Food Chemistry Antioxidant activity and constituents of propolis collected in various areas of China. 2007;101:1383–92.
[25] Hochheim S, Guedes A, Faccin-Galhardi L, Rechenchoski DZ, Nozawa C, Linhares RE, et al. Determination of phenolic profile by HPLC–ESI-MS/MS, antioxidant activity, in vitro cytotoxicity and anti-herpetic activity of propolis from the Brazilian native bee Melipona quadrifasciata. Brazilian Journal of Pharmacognosy. 2019;29(3):339–50.
[26] Yapar, Y., & Behçet, L.. The Flora Of Hiro Plateau (Adaklı-Bingöl/Turkey) And Its Surroundings. Biol Divers Conserv. 2018;3:126–40.
[27] Behçet L, Yapar Y. Important plants at the Matan Mountain (Bingöl) flora with regard to beekeeping. Biol Divers Conserv. 2019;12:149–59.
[28] Erdoğan MK, Geçibesler İH, Behçet L. Chemical constituents, antioxidant, antiproliferative and apoptotic effects of a new endemic Boraginaceae species: Paracaryum bingoelianum. Results in Chemistry. 2020;2:100032.
[29] Gecibesler IH. Antioxidant Activity and Phenolic Profile of Turkish Celtis tournefortii. Chemistry of Natural Compounds. 2019;55(4):738–42.
[30] Gecibesler IH, Yaglıoglu AS, Gul F, Temirturk M, Demirtas I. Phytochemicals of Chrysophthalmum montanum (DC.) Boiss. Roots and Their Antiproliferative Activities Against HeLa and C6 Cell Lines. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2019;89(1):145–54.
[31] Geçibesler IH, Kiliç Ö, Demirtas I. Appraisal of biological activities of crude extracts with subfractions and phytochemical content of endemic Nepeta nuda L. subsp. lydiae from Turkey. Progress in Nutrition. 2017;19(1):121–9.
[32] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; (CLSI). 2018. M100–S28.
[33] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999;26(9-10):1231-7.
[34] Gecibesler IH, Kocak A, Demirtas I. Biological activities, phenolic profiles and essential oil components of Tanacetum cilicicum (BOISS) Grierson. Nat Prod Res .2016;30:2850–2855.
[35] Bhattacharjee B, Pal PK, Chattopadhyay A, Bandyopadhyay D. Oleic acid protects against cadmium induced cardiac and hepatic tissue injury in male Wistar rats: A mechanistic study. Life Sci.2020; 244:117324.
[36] Kusumah D, Wakui M, Murakami M, Xie X, Yukihito K, Maeda I. Linoleic acid, α-linolenic acid, and monolinolenins as antibacterial substances in the heat-processed soybean fermented with Rhizopus oligosporus. Biosci Biotechnol Biochem.2020; 84:1285–1290.
[37] Gecibesler IH, Erdogan M. A new nutraceutical resource from a rare native plant growing in Turkey and for its spectro-chemical and biological insights: Endemic Diplotaenia bingolensis (Apiaceae). Spectrochim Acta - Part A Mol Biomol Spectrosc .2019.223:117358.
[38] Abdel-Raouf N, Al-Enazi NM, Al-Homaidan AA,

Ibraheem IBM, Al-Othman MR, Hatamleh AA. Antibacterial β-amyrin isolated from Laurencia microcladia. Arabian Journal Chemistry. 2015;8(1):32–7.

[39] Monzote L, Cuesta-Rubio O, Fernandez MC, Hernandez IM, Fraga J, Pérez K, et al. In vitro antimicrobial assessment of Cuban propolis extracts. Memorios do Instituto Oswaldo Cruz. 2012;107(8):978–84.

[40] Papachroni D, Graikou K, Kosalec I, Damianakos H, Ingram V, Chinou I. Phytochemical analysis and biological evaluation of selected african propolis samples from Cameroon and Congo. Natural Products Communications. 2015;10(1):67–70.

[41] Celemli OG. Chemical Classification of Propolis Samples Collected from Different Regions of Turkey in Geographical Region Base. Hacettepe Journal Biology Chemistry. 2015;43(1):49–57.

[42] Sahinler N, Gul A,Copur G. Chemical Composition and preservative effcet of Turkish Propolis on egg quality during storage. Asian Journal of Chemistry. 2009;21(3):1877-1886

[43] Uzel A, Sorkun K, Önçağ Ö, Çoğulu D, Gençay Ö, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Research. 2005;160(2):189–95.

[44] Gargouri W, Osés SM, Fernández-Muiño MA, Sancho MT, Kechaou N. Evaluation of bioactive compounds and biological activities of Tunisian propolis. Food Science and Technology. LWT. 2019;111:328–36.

[45 Regueira Neto MS, Tintino SR, da Silva ARP, Costa M do S, Boligon AA, Matias EFF, et al. Seasonal variation of Brazilian red propolis: Antibacterial activity, synergistic effect and phytochemical screening. Food and Chemical Toxicology. 2017;107:572–80.

[46] Kalogeropoulos N, Konteles SJ, Troullidou E, Mourtzinos I, Karathanos VT. Chemical composition, antioxidant activity and antimicrobial properties of propolis extracts from Greece and Cyprus. Food Chemistry. 2009;116(2):452–61.

[47] Kubiliene L, Laugaliene V, Pavilonis A, Maruska A, Majiene D, Barcauskaite K, et al. Alternative preparation of propolis extracts: Comparison of their composition and biological activities. BMC Complementary and Alternative Medicine. 2015;15(1):1–7.

[48] Agüero MB, Svetaz L, Sánchez M, Luna L, Lima B, López ML, et al. Argentinean Andean propolis associated with the medicinal plant Larrea nitida Cav. (Zygophyllaceae). HPLC-MS and GC-MS characterization and antifungal activity. Food Chemical and Toxicology. 2011;49(9):1970–8.

[49] Mirzoeva OK, Grishanin RN, Calder PC. Antimicrobial action of propolis and some of its components: The effects on growth, membrane potential and motility of bacteria. Microbiological Research. 1997;152(3):239–46.

[50] Petruzzi L, Corbo MR, Campaniello D, Speranza B, Sinigaglia M, Bevilacqua A. Antifungal and antibacterial effect of propolis: A comparative hit for food-borne pseudomonas, enterobacteriaceae and fungi. Foods. 2020;9(5):559.

[51] Freires IA, Queiroz VCPP, Furletti VF, Ikegaki M, de Alencar SM, Duarte MCT, et al. Chemical composition and antifungal potential of Brazilian propolis against Candida spp. Journal de mycologie medicale. 2016;26(2):122–32.

[52] Yang S, Peng L, Cheng Y, Chen F, Pan S. Control of citrus green and blue molds by Chinese propolis. Food Science and Biotechnology. 2010;19(5):1303–8.

[53] Gulcin İ. Antioxidants and antioxidant methods: an updated overview. Archives of Toxicology. 2020;94:651–715.

[54] Ozdal T, Ceylan FD, Eroglu N, Kaplan M, Olgun EO, Capanoglu E. Investigation of antioxidant capacity, bioaccessibility and LC-MS/MS phenolic profile of Turkish propolis. Food Research International. 2019;122:528–36.

[55] Pobiega K, Kraśniewska K, Gniewosz M. Application of propolis in antimicrobial and antioxidative protection of food quality – A review. Trends in Food Science& Technology. 2019;83:53–62.

[56] Atungulu Griffiths G, Toshitaka U, Fumihiko T, Daisuke H. Effect of vapors from fractionated samples of propolis on microbial and oxidation damage of rice during storage. Journal of Food Engineering. 2008;88(3):341–52.