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AYHAN TEMİZ

AYLA ŞENER

ASLI ÖZKÖK TÜYLÜ

KADRİYE SORKUN

BEKİR SALİH

TÜBİTAK

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Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two foodborne pathogens, *Salmonella* Enteritidis and *Listeria monocytogenes*

Ayhan TEMİZ[1], Ayla ŞENER[1], Aslı ÖZKÖK TÜYLÜ[2], Kadriye SORKUN[2], Bekir SALİH[3]

[1]Department of Food Engineering, Faculty of Engineering, Hacettepe University, 06800 Beytepe, Ankara - TURKEY

[2]Department of Biology, Faculty of Science, Hacettepe University, 06800 Beytepe, Ankara - TURKEY

[3]Department of Chemistry, Faculty of Science, Hacettepe University, 06800 Beytepe, Ankara - TURKEY

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Abstract: The aim of the present study was to investigate the antibacterial activities of 25 propolis samples collected from various geographical regions of Turkey against 2 food-borne pathogens, *Salmonella* Enteritidis ATCC 13076 and *Listeria monocytogenes* ATCC 1462. The chemical compositions of ethyl alcohol extracts of the propolis (EEP) samples were determined by gas chromatography coupled to mass spectrometry. The main components of EEP samples were flavonoids, aromatic acid esters, aromatic alcohols, aromatic acids, aliphatic carboxylic acids, terpens, and aliphatic carboxylic acid esters. Antibacterial activities of the EEP samples were tested at 2 different dilutions of 1:10 and 1:100 (v/v). All EEP samples at 1:10 dilution showed high antibacterial activity against the test bacterial strains and no viable bacteria were determined after incubation. The *S.* Enteritidis strain was found to be resistant toward EEP samples at 1:100 dilution ratios. However, most of the EEP at 1:100 dilution ratios had high antibacterial activity against the *L. monocytogenes* strain. While some of EEP at 1:100 dilution ratios killed all viable cells of *L. monocytogenes*, some EEP carried out 1-6 log reductions in the viable cells of this strain. Antibacterial activity of propolis depends on the chemical composition and types of bacteria. The propolis samples had a marked antibacterial action against the gram-positive strain and limited activity against gram-negative one depending on EEP concentration.

Key words: Propolis, antibacterial activity, food pathogens, *Salmonella* Enteritidis, *Listeria monocytogenes*

Türkiye'nin farklı coğrafik bölgelerinden toplanan propolis örneklerinin *Salmonella* Enteritidis ve *Listeria monocytogenes*'e karşı antibakteriyal aktiviteleri

Özet: Bu çalışmanın amacı, Türkiye'nin çeşitli coğrafik bölgelerinden toplanan 25 propolis örneğinin, iki gıda patojenine (*Salmonella* Enteritidis ATCC 13076 ve *Listeria monocytogenes* ATCC 1462) karşı antibakteriyal aktivitelerini araştırmaktır. Propolis örneklerinin etil alkol ekstraktlarının (EEP) kimyasal kompozisyonları, kütle spektorometresine birleştirilmiş gaz kromatografisi ile belirlenmiştir. EEP örneklerinin ana bileşimleri flavonoidler, aromatik asit esterleri, aromatik alkoller, aromatik asitler, alifatik karboksilik asitler, terpenler ve alifatik karboksilik asit esterleri olarak tespit edilmiştir. EEP örneklerinin antibakteriyal aktiviteleri, 1:10 ve 1:100 (v/v) şeklinde iki farklı seyreltmesi denenmiştir. EEP örneklerinin hepsi 1:10 seyreltmede denenen bakteri suşlarına karşı yüksek antibakteriyal aktivite göstermiş ve inkübasyon sonrasında canlı bakteri varlığı belirlenmemiştir. *S.* Enteritidis suşunun, 1:100 seyreltme oranında EEP örneklerine karşı dirençli olduğu gözlenmiştir. Ancak, 1:100 seyreltme oranında EEP örneklerinin çoğunun *L.*

monocytogenes suşuna karşı yüksek antibakteriyal aktiviteye sahip olduğu görülmüştür. Bazı EEP örnekleri 1:100 seyreltme oranında ortamdaki canlı *L. monocytogenes* hücrelerinin tümünü öldürürken, bazı EEP örnekleri bu suş üzerinde 1-6 log düzeyinde azalmalar gerçekleştirmiştir. Propolisin antibakteriyal aktivitesi, kimyasal kompozisyonuna ve bakteri türüne bağlıdır. Propolis örneklerinin EEP konsantrasyonuna bağlı olarak, gram-pozitif suşa karşı önemli düzeyde antibakteriyal aktiviteye sahip olduğu tespit edilirken, gram-negatif suşa karşı sınırlı düzeyde aktivite gösterdiği belirlenmiştir.

Anahtar sözcükler: Propolis, antibakteriyal aktivite, gıda patojenleri, *Salmonella* Enteritidis, *Listeria monocytogenes*

Introduction

Bee glue called propolis is a dark sticky resinous substance collected by bees from leaf buds, twigs, trunk wounds, and trees such as *Castanea sativa*, *Populus* spp., and *Aesculus hippocastanum*. Bees attach the propolis to their hind legs, and carry it back to their colony, where it is combined with bees wax and used by worker "hive" bees to seal and sterilize the colony nest (1). Propolis contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids, and their esters, phenolic aldehydes and alcohols), terpenoids, steroids, amino acids, and inorganic compounds (2).

Propolis has been used in folk medicine to maintain health since ancient times (3). Many biological properties including antibacterial, antifungal, antiviral, local anesthetic, anti-inflammatory, antioxidant, hepatoprotective, immunostimulating, and cytostatic activities have been ascribed to propolis. Therefore, propolis is available commercially in different formulated forms such as tablets, capsules, toothpaste, mouthwash preparations, face creams, ointments, lotions, and solutions (2,4). The medical applications of propolis have led to increased interest in its chemical composition as well as its origin (5,6).

In fact, it is known that plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, which are found in vitro to have antimicrobial properties (7). The antimicrobial activity of propolis against a wide range of bacteria, fungi, and viruses has been investigated since the late 1940s and it showed variable activity against different microorganisms (8-20). Many researchers have studied the antibacterial activity of propolis and its extracts against gram-positive and gram-negative bacteria. They found that propolis had antibacterial activity against a wide range of gram-positive strains but had limited or no activity against gram-negative strains (2,9,10,12-15,19,20).

Salmonella spp. and *Listeria monocytogenes* are 2 important food-borne pathogen bacteria. Salmonellae are gram-negative, facultative anaerobic, non-spore forming bacteria belonging to the family Enterobacteriaceae. Salmonellosis is the most prevalent food-borne disease caused by *Salmonella* spp. and serotypes in many countries. In general, foods of animal origin such as beef, chicken, turkey, pork, eggs, milk, and their products have been implicated in the outbreaks of human salmonellosis (21,22). *S.* Enteritidis (*Salmonella enterica* subsp. *enterica* serovar Enteritidis) is reported to be one of the most frequently isolated serotypes in foods and it is closely associated with raw, undercooked, or contaminated eggs and egg products. There has been an increased incidence of gastrointestinal infections caused by *S.* Enteritidis (23,24). *L. monocytogenes*, the causative agent of listeriosis, is a gram-positive, facultative anaerobic, psychrotrophic, non-spore forming bacterium. It is widely distributed in the natural environment and consequently present in various animal products and in vegetables. Many types of heat-processed or ready-to-eat foods, such as raw and pasteurized milk and dairy products (particularly cheeses), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish are common sources for this bacterium (22,25).

The aim of this study was to determine the antibacterial activities of 25 propolis samples collected from various geographical regions of Turkey against 2 important food-borne pathogens, *S.* Enteritidis and *L. monocytogenes*.

Materials and methods

Propolis samples

Twenty-five propolis samples belonged to *Apis mellifera* colonies were collected from different regions of Turkey. The samples were collected using propolis traps and stored in the freezer until further processing. Geographical regions and some other properties of the propolis samples are listed in Table 1.

Preparation of ethanol extracts of propolis (EEP)

Propolis sample was hardened in a freezer and ground in a handy grinder. Then 100 g of the sample was dissolved in 300 mL of 96% ethanol. This mixture was incubated for 4 weeks at 30 °C in a tightly closed bottle with periodically stirring. After incubation, supernatant was filtered twice with Whatman No. 4 then with No. 1 filter papers. The final filtered solution (concentrated EEP, Table 1) was diluted in 1:10 ratio (w/v) with 96% ethanol and this solution was called EEP. For GC-MS analysis, a portion of the EEP was evaporated to dryness. Then about 5 mg of residue was mixed with 75 µL of dry pyridine and 50 µL bis(trimethylsilyl) trifluoroacetamide, heated at 80 °C for 20 min and then the final supernatant was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) (19,20).

GC-MS analysis

A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with a mass detector (MS5973, Hewlett-Packard) was used for the analysis of the diluted EEP samples. Experimental conditions of the GC-MS system were as follows: a DB 5MS column

Table 1. Propolis samples tested in the study.

Propolis sample location	Symbol	Concentrated EEP solution (0.01 g/mL)
Kazan-Ankara	AKA	9.45
Mamak-Ankara	AMA	0.75
Bartın	B	3.56
Osmangazi-Bursa	BOS	4.25
Tahtaköprü 1-Bursa	BTK 1	2.9
Tahtaköprü 2-Bursa	BTK 2	0.5
Tahtaköprü 3-Bursa	BTK 3	0.046
Tahtaköprü 4-Bursa	BTK 4	4.25
Kemaliye-Erzincan	EKE	7.8
Gümüşhane	G	11.8
Akşehir-Konya	KAK	4.2
Mersin	M	3.1
Muğla Merkez	MUM	5
Muğla 1	MU 1	5
Muğla 2	MU 2	2
Muğla 3	MU 3	3.4
Dalaman-Muğla	MUDA	9.1
Fethiye 1-Muğla	MFE 1	7
Fethiye 2-Muğla	MFE 2	5.7
Fethiye 3-Muğla	MFE 3	7
Fethiye 4-Muğla	MFE 4	5.7
Ordu	O	0.074
Rize Anzer 1	RAN 1	3.5
Rize Anzer 2	RAN 2	4.3
Yalova	Y	1.6

(30 m × 0.25 mm and 0.25 μm of film thickness) was used and flow rate of mobile phase (He) was set at 0.7 mL/min. In the gas chromatography part, temperature was kept at 50 °C for 1 min. After this period, the temperature was increased to 150 °C with a 10 °C/min heating ramp and then kept at 150 °C for 2 min. Finally, temperature was increased to 280 °C with a 20 °C/min heating ramp and then kept at 280 °C for 30 min (19,20).

Bacterial strains

S. Enteritidis ATCC 13076 and *L. monocytogenes* ATCC 1462 were used to investigate antibacterial activity of EEP samples. Bacterial cultures were sub-cultured in Brain Heart Infusion (BHI) broth at 37 °C for 24 h.

Antimicrobial activity test

Determination of antimicrobial activity was performed on the basis of macrodilution method of National Committee for Clinical Laboratory Standards (NCCLS) with our little modifications (26). Each of the EEP samples was diluted in the ratio of 1:10 and 1:100 (v/v) by using sterile distilled water. One milliliter of the diluted EEP solution was added to 9 mL of BHI broth medium and then 0.1 mL of the overnight test bacterium culture was inoculated into this medium. The initial number of test bacterium in the inoculated medium was determined by pour plate method using tryptic soy agar (TSA). Then the inoculated BHI broth medium tubes were incubated at 37 °C for 24 h and viable cell number was determined by pour plate method using TSA. Antibacterial activity of the test EEP was evaluated as the reduction in the number of viable bacteria after the incubation.

Results and discussion

In this study, the antibacterial activities of 25 different EEP samples were tested at 2 different dilutions (1:10 and 1:100) against *S.* Enteritidis ATCC 13076 and *L. monocytogenes* ATCC 1462.

The viable cell numbers of the bacteria at initials and after 24 h incubation are given in Table 2.

The results showed that all EEP samples at higher concentration (1:10 dilution) showed a strong bactericidal effect on both bacterial strains, i.e. no viable bacteria were determined after 24 h of incubation of the inoculated medium. However, EEP samples at lower concentrations (1:100 dilutions) had variable inhibitory action on the growth of the bacterial strains. Eight EEP samples (B, BTK 1, BTK 2, BTK 3, BTK 4, G, M, and MFE 1) at this dilution ratio had a bactericidal effect on *L. monocytogenes*. On the other hand, 6 EEPs (AKA, BOS, EKE, MU 2, MFE 2 and MFE 3) caused 4-5 log reductions, 3 EEP samples (AMA, MFE 4 and RAN 2) caused 2-3 log reductions, and 2 EEPs (O and RAN 1) caused < 2 log reductions in the viable cell numbers of *L. monocytogenes*. The remaining 6 EEP samples (KAK, MUM, MU 1, MU 3, MUDA, and Y) had no inhibitory effect on the growth of this bacterium (Table 2).

Compared with *L. monocytogenes*, the growth of *S.* Enteritidis was weakly inhibited by only a small number of EEP samples at lower concentrations. Only 5 EEP samples (B, BTK 2, BTK 3, MFE 1, and MFE 3) showed a very weak antibacterial activity against this bacterium within the range of 0.19-0.69 log reductions in the viable cell number. The other 20 EEP samples had no inhibitory effect on the growth of *S.* Enteritidis (Table 2).

Seven organic components were dominantly detected in the EEP samples (Table 3). Flavonoids were the only shared component found in all EEP samples with different levels. However, there was no clear correlation between the amount of flavonoids and the antibacterial effect of EEP samples. Five out of 6 EEP samples that were inactive on *L. monocytogenes* at 1:100 dilution ratio contained relatively low levels of flavonoids. However, sample Y, which was also inactive on *L. monocytogenes* at 1:100 dilution ratio, had high amounts of flavonoids.

Quercetin, chrysin, 4',5-dihydroxy-7-methoxyflavanone, and 3,4',7-tri methoxyflavanone were the flavonoids detected in EEP samples (Table 4). Quercetin was detected in all EEP samples at different levels, except for sample MU 1, which was inactive on *L. monocytogenes* at 1:100 dilution. Chrysin was found in some of the EEP samples, which were active on *L. monocytogenes* at 1:100 dilution (12 out of 19 EEP samples). However, it was detected in the EEP samples (6 samples), which were inactive on *L. monocytogenes* at 1:100 dilution. 4',5-Dihydroxy-7-

Table 2. Antibacterial activity of EEP samples against *S.* Enteritidis and *L. monocytogenes*.

Symbol of EEP	Dilution Ratio	*S.* Enteritidis ATCC 13076		*L. monocytogenes* ATCC 1462	
		Initial cell number (log cfu/mL)	Cell number after 24 h incubation log cfu/mL)	Initial cell number (log cfu/mL)	Cell number after 24 h incubation (log cfu/mL)
AKA	1:10	7.19	< 1.00*	6.67	< 1.00
	1:100	7.02	9.19	6.76	1.30
AMA	1:10	6.00	< 1.00	6.65	< 1.00
	1:100	7.25	7.68	6.34	3.14
B	1:10	7.08	< 1.00	6.39	< 1.00
	1:100	7.92	7.23	6.44	< 1.00
BOS	1:10	6.79	< 1.00	5.49	< 1.00
	1:100	7.49	7.70	6.38	1.48
BTK 1	1:10	7.05	< 1.00	6.56	< 1.00
	1:100	7.19	7.97	6.22	< 1.00
BTK 2	1:10	6.95	< 1.00	5.77	< 1.00
	1:100	7.22	6.59	5.15	< 1.00
BTK 3	1:10	7,20	< 1.00	6.60	< 1.00
	1:100	7,13	6,91	6.72	< 1.00
BTK 4	1:10	6.42	< 1.00	6.28	< 1.00
	1:100	7.22	8,09	6.52	< 1.00
EKE	1:10	7.08	< 1.00	6.81	< 1.00
	1:100	6.60	6.97	6.93	1.00
G	1:10	6.97	< 1.00	6.16	< 1.00
	1:100	6.87	7.05	7.29	< 1.00
KAK	1:10	6.46	< 1.00	6.60	< 1.00
	1:100	7.17	7.62	6.77	7.29
M	1:10	7.16	< 1.00	5.60	< 1.00
	1:100	7.29	7.81	6.64	< 1.00
MUM	1:10	7.09	< 1.00	6.40	< 1.00
	1:100	7.39	8.37	6.88	8.21
MU 1	1:10	7.16	< 1.00	6.59	< 1.00
	1:100	7.29	8.52	6.58	7.89
MU 2	1:10	7.04	< 1.00	6.05	< 1.00
	1:100	7.27	7.97	6.89	2.11
MU 3	1:10	7.12	< 1.00	6.56	< 1.00
	1:100	7.31	8.36	6.53	8.94
MUDA	1:10	7.42	< 1.00	6.77	< 1.00
	1:100	7.29	7.95	6.80	8.36
MFE 1	1:10	6.60	< 1.00	5.00	< 1.00
	1:100	7.59	7.40	6.75	<1.00
MFE 2	1:10	6.64	< 1.00	6.11	<1.00
	1:100	7.34	8.32	6.74	1.78
MFE 3	1:10	7.20	< 1.00	6.06	< 1.00
	1:100	7.13	6.91	6.66	1.85
MFE 4	1:10	6.19	< 1.00	6.75	< 1.00
	1:100	7.24	7.76	6.95	4.13
O	1:10	7.93	< 1.00	6.66	< 1.00
	1:100	7.12	7.90	6.85	5.38
RAN 1	1:10	7.10	< 1.00	5.90	< 1.00
	1:100	7.16	8.16	5.75	5.15
RAN 2	1:10	7.23	< 1.00	5.04	< 1.00
	1:100	7.24	7.46	6.91	3.55
Y	1:10	8.99	< 1.00	6.44	< 1.00
	1:100	7.10	7.56	7.02	9.40

< 1.00*: No colony formation determined in 10^{-1} dilution of the sample

methoxyflavanone was detected in some of the EEP samples, which were active on *L. monocytogenes* at 1:100 dilution (7 out of 19 EEP samples). However, it was not found in the 6 EEP samples, which had no antibacterial activity on *L. monocytogenes* at 1:100 dilution. 3,4',7-Tri methoxyflavanone was detected in only sample B, which was bactericidal for *L. monocytogenes* at 1:100 dilution.

The results of the study showed that EEP samples had strong antibacterial effect on both food-borne pathogens, *S.* Enteritidis (a gram-negative bacterium) and *L. monocytogenes* (a gram-positive bacterium), depending on EEP concentration. A higher EEP concentration was necessary for the inhibitory effect on *S.* Enteritidis (Table 2). These results are in good agreement with the results of several previous studies in which a marked inhibitory effect of propolis on gram-negative bacteria at its higher concentrations was observed (8,19,20).

In a study by Abdulsalam et al. in 1989, antibacterial activity of an ethanol extract of a propolis sample was tested against some gram-positive and gram-negative bacterial species (8). All gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *S. epidermis*, and *Streptococcus pyogenes*) were inhibited by 100 ppm EEP in the growth medium whereas 8

Table 3. Chemical components of the EEP samples (percent out of detected total organic content in our experimental conditions).

EEP	Aromatic Alcohols	Aromatic Acid Esters	Aromatic Acids	Flavonoids	Aliphatic Carboxylic Acids	Terpenes	Aliphatic Carboxylic Acid Esters
AKA	1.47	0.46	2.87	12.79	1.55	-*	-
AMA	1.14	0.37	0.2	8.01	2	0.21	-
B	2.9	2.71	1.2	16.44	2.17	-	-
BOS	2.32	5.83	-	6	0.41	-	-
BTK 1	-	1.24	-	35.37	-	-	0.74
BTK 2	1.26	1.64	1.84	28.39	3.96	-	2.17
BTK 3	-	-	-	28.53	-	-	-
BTK 4	1.09	2.4	2.61	33.36	1	-	0.50
EKE	-	-	0.31	11.84	0.8	-	-
G	0.71	0.54	0.88	11.7	0.26	-	-
KAK	0.46	0.31	1.04	5.12	-	0.24	-
M	1.79	2.76	0.19	7.86	0.69	0.22	-
MUM	-	0.10	-	4.38	2.78	0.13	1.03
MU 1	-	88.36	-	0.16	2.37	-	-
MU 2	0.21	2.95	2.61	10.9	1.77	0.15	0.45
MU 3	-	60.03	0.53	7.18	1.42	-	0.22
MUDA	-	61.05	0.15	1.73	0.66	0.3	0.17
MFE 1	-	32.8	-	12.96	1.26	-	0.27
MFE 2	-	-	-	13.2	-	-	-
MFE 3	-	36.67	0.47	14.16	0.81	0.41	-
MFE 4	0.47	5.56	0.63	23.47	3.04	-	1.56
O	0.13	-	-	5.84	0.98	-	1.60
RAN 1	0.42	0.35	1.39	0.40	1.44	-	-
RAN 2	1.22	1.23	3.48	15.22	0.54	-	-
Y	1.31	0.58	3.71	26.11	0.79	-	0.27

-*: Not determined

Table 4. Flavonoid composition of EEP samples (percent out of detected total organic content in our experimental conditions) and antibacterial effect of EEP samples against *L. monocytogenes* at 1:100 dilution.

Antibacterial effect	Flavonoids			
	3,4',7-Tri methoxyflavanone	Chrysin	4',5-Dihydroxy-7 methoxyflavanone	Quercetin
Bactericidal: Complete reduction in the number of viable bacteria				
B	0.31	-*	1.13	15
BTK 1	-	12.50	-	22.87
BTK 2	-	8.85	0.75	18.79
BTK 3	-	9.51	-	19.02
BTK 4	-	11.24	0.74	21.38
G	-	-	-	11.7
M	-	-	-	7.86
MFE 1	-	4.93	-	8.03
5-6 log reductions in the number of viable bacteria				
AKA	-	5.98	0.26	6.55
BOS	-	-	1.78	4.22
EKE	-	0.14	0.63	11.07
MU 2	-	2.85	-	8.05
MFE 2	-	-	-	13.20
MFE 3	-	5.80	-	8.36
2-3 log reductions in the number of viable bacteria				
AMA	-	0.56	0.84	6.61
MFE 4	-	8.70	-	14.77
RAN 2	-	-	-	15.22
1.5- < 2 log reductions in the number of viable bacteria				
O	-	2.22	-	3.62
RAN 1	-	-	-	0.40
No antibacterial effect				
KAK	-	3.27	-	1.85
MUM	-	1.82	-	2.56
MU 1	-	0.16	-	-
MU 3	-	2.42	-	4.76
MUDA	-	0.70	-	1.03
Y	-	8.61	-	17.5

-*: Not determined

gram-negative bacterial species were inhibited at higher concentrations (*Enterobacter cloacae* and *Proteus vulgaris* at 400 ppm; *Pseudomonas aeruginosa*, *Serratia marcescens* and a *Serratia* spp. at 800 ppm; and *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* at 1200 ppm).

Cihangir et al. determined the minimal inhibitory concentration (MIC) of ethanol extract of the propolis samples collected from different regions of Turkey against 3 gram-positive (*S. aureus*, *B. subtilis*, and a β-hemolytic *Streptococcus* sp.) and 3 gram-negative (*E. coli, Salmonella typhi*, and a *Proteus* sp.) bacterial strains. The MIC values of EEP samples for gram-negative bacteria were higher than those of gram-positive bacteria 10 to 100 times depending on the regions of EEP samples (19).

Uzel et al. studied 4 Anatolian propolis samples and they observed that gram-positive bacteria were susceptible to low propolis concentration and gram-negative bacterial growth was only inhibited at higher propolis concentrations (20).

The results of the present study clearly indicated that antibacterial activity of the propolis samples was dependent on the chemical composition and especially the concentration of the active components and compounds of the samples (Tables 3 and 4).

It was revealed that the chemical composition of propolis was very complex and dependent on the flora in the area where it was collected (27-30). Therefore, the variations in the antibacterial activity of the EEP samples used in this study, which are from different origins, are not surprising.

Aga et al. determined the MIC values of 3 antimicrobial compounds isolated from Brazilian propolis. The MIC values of 2 compounds (3,5 diprenyl-4-hydroxycinnamic acid and 3-prenyl-4-dihydrocinnamoloxycinnamic acid) for *Enterobacter aerogenes* were higher than those of *B. cereus* by about 2-fold, but the values of one compound (2,2-dimethyl-6-carboxyethenyl-2H-1-benzopyran) were identical for both strains. It was indicated that 3,5 diprenyl-4-hydroxycinnamic acid was likely to be one of the major antimicrobial compounds in Brazilian propolis (31).

French researchers found that propolis showed bacteriostatic activity towards *B. subtilis, P. vulgaris*, and *B. alvei*. The effect was less marked towards *Salmonella gallinarum*, *S. pullorum*, and *S. dublin*, and negligible towards different strains of *E. coli*. Galangin and pinocembrin were isolated from the propolis and found to be partly responsible for the activity. Galangin at a concentration of 0.065 mg/mL inhibited *B. subtilis*. A higher level (0.080 mg/mL) of it inhibited the growth of *B. alvei* and *P. vulgaris* but twice this concentration was needed to inhibit *S. gallinarum* growth (30).

In conclusion, EEP samples have strong antibacterial effect on both food-borne pathogens, *S.* Enteritidis and *L. monocytogenes*, if an appropriate EEP concentration is used. Antibacterial activity of propolis depends on the chemical composition and especially the concentration of the active components and compounds of the samples. These findings confirm that the antibacterial activity of propolis may be attributed to the synergism between flavonoids and other components and compounds of EEP samples. This study offers useful information for the usage of propolis as a natural antimicrobial agent to control microbial growth in food products and might provide an alternative to chemical preservatives.

Corresponding author:
Ayhan TEMİZ
Department of Food Engineering,
Faculty of Engineering,
Hacettepe University,
06800 Beytepe, Ankara - TURKEY
E-mail: temiz@hacettepe.edu.tr.

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