MELLIFERA RESEARCH ARTICLE

An *In vitro* Study on Antimicrobial and Antioxidant Activity of Propolis from Rize Province of Turkey

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ABSTRACT

In this study antibacterial, antifungal and antioxidant activities of propolis samples from the Rize province of Turkey in different solvents were investigated. A total of fifteen microorganisms belonging to Gram-positive *(Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus salivarius)*, Gram-negative bacteria *(Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enteritidis)* and a fungi *(Candida albicans)* were studied using a disc-diffusion and minimal inhibition concentration (MIC) methods. Ethanol extracts of propolis (EEP), acetone extracts of propolis (AEP), ethyl acetate extracts of propolis (EAEP) and methanol extracts of propolis (MEP) showed the highest antimicrobial activity against *S. mutans, L. monocytogenes, M. luteus, B. licheniformis and C. albicans.* While dimethyl sulfoxide extracts of propolis (DMSOEP) has the weakly activity against some test organism. The most sensitive microorganisms to propolis were *E. coli, B. licheniformis, S. mutans, L. monocytogenes and B. cereus* in the test microorganisms.
Finally, according to the results shown by GC-MS, at least one substance was dissolved in EAEP and also by us, was found to have the highest antioxidant effect in the EAEP and AEP and the highest antimicrobial effect in the AEP.

Key words: Antimicrobial activity, antioxidant activity, propolis, GC-MS

Introduction

Propolis, a resinous substance collected by *Apis mellifera* bees from various plant sources and mixed with secreted beeswax, is a multifunctional material used by bees in the construction, maintenance, and protection of their hives [1-3]. Propolis is a complex resinous bee product with a physical appearance that varies widely, depending on many factors. The color may be cream, yellow, green, light or dark brown. Some samples have a friable, hard texture, while other samples may be elastic and gummy. Bees use propolis for diverse purposes, one of them is to seal the openings in the hive. A medicine containing vaseline and propolis (propolisin vasogen) was used for wound treatment during the Boer war [4,5]. Propolis composition is extremely complex. The main constituents are beeswax, resin and volatiles. The insects secrete beeswax,

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while the latter two constituents are obtained from plants. Distinction of flora from one origin to another provide variable source of propolis for bees and also cause color changes. The main visited plant species are poplar (*Populus* spp.), beech (*Fagus sylvatica*), horse chestnut (*Aesculus hippocastanum*), birch (*Betula alba*), alder (*Alnus glutinosa*) and various conifer trees [6]. The most favorable poplar species are *Populus alba*, *Populus nigra* and *Populus tremula* [7,8]. Similarly other plants used for the production of propolis are *Eucalyptus* species and *Baccharis dracunculifolia* [9,10].

In the present study, we investigated the antibacterial, antifungal and antioxidant activities of acetone, ethyl acetate, ethanol, methanol, dimethyl sulfoxide and water extracts of propolis samples.

Materials and methods

Propolis samples and preparation of extracts

Crude propolis samples were collected from Rize province of Turkey during October and November 2006. The samples were stored in air-tight glass containers in dark at -20°C until they were used. Propolis extracts were prepared by stirring 30 g samples in 150 mL of 95 % ethanol, acetone, ethyl acetate, methanol, water and dimethyl sulfoxide for a week at 4°C respectively. The extracts were filtered through 45 µm membrane filter, and then the solution was dried with an evaporator. The crude extracts were stored at -20°C until used.

Test strains and culture media

Strains of bacteria and fungi were obtained from ATCC (American Type Culture Collection, Rockville, Maryland), NCTC (National Collection of Type Culture, England), NRRL (Agricultural Research Service, United States of America), RSHE (Refik Saydam Hıfzısıhha Institute, Turkey). Antimicrobial activities of propolis extracts in different solvents were assayed against *Bacillus cereus* ATCC 11778, *Bacillus licheniformis* NRRL B-1001, *Bacillus subtilis* NRRL B-209, *Candida albicans* ATCC 25922, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 5041, *Listeria monocytogenes* NCTC 5348, *Micrococcus luteus* NRRL B-1018, *Proteus vulgaris* NRRL B-123, *Pseudomonas aeruginosa* NRRL B-2679, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 6538, *Streptococcus mutans* RSHE 676, *Streptococcus pneumoniae* ATCC 10015 and *Streptococcus salivarius* RSHE 606. The species of bacteria were grown in Mueller Hinton Agar (Merck) and Mueller Hinton Broth (Merck). *C. albicans* was grown in Sabouraud Dextrose Broth (Difco) and Sabouraud Dextrose Agar (Oxoid). The concentrations of bacterial suspensions were adjusted to 10^8 cells/ml, while those of fungal suspensions to 10^7 cells/ml.

Antifungal and antibacterial assay

Antibacterial and antifungal activities were measured using the method of disc diffusion on agar plates [11]. In order to test antibacterial and antifungal activity, the fractions of propolis samples were dissolved in six different solvents. For bacterial Mueller Hinton Agar medium (Merck 20 ml) and Sabouraud Dextrose Agar (Oxoid 20 ml) for fungus were poured into each 150 mm petri dishes. All bacterial strains were grown in Mueller Hinton Broth medium (Merck) for 24 h, at 37°C and *C. albicans,* was grown in Sabouraud Dextrose Broth (Difco) at 27°C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with Mueller Hinton Broth medium (Merck) for bacteria and for

fungi Sabouraud Dextrose Broth (Difco). Suspension (100 µL) with approximately 10^8 bacteria and fungi per millilitre was placed in petri dishes, over agar and dispersed. Then, 6 mm diameter sterile blank discs (Oxoid) were placed on agar to load 15 µL of each propolis samples (20 mg/mL). One hundred units of nystatin was used as a positive control for fungus, ampicillin and cephazolin obtained from a local pharmacy and alcohol as a negative control for bacteria. Inhibition zones were determined after incubation at 27°C for 48 h. The study was conducted in three replicates. All measurements were done in triplicate.

Minimum inhibition concentration (MIC)

The agar dilution method, described by Vanden Berghe and Vlietinck was used for antibacterial screening with slight modifications. Instead of 96 well plates, 24 well tissue culture plates (Corning) were used [12]. The crude extracts were dissolved in 95% ethanol and physiological Tris buffer (Amresco) 1:4 and mixed with an equal amount of 3% agar solution at 45°C to a final concentration of 4, 2, 1 and 0,5 mg of extract/ml. An amount of 400 µl from the solutions was transferred into each well of the tissue culture plate (Corning). After solidification each well was inoculated with 10µl of freshly prepared bacterial suspension of 10^8 bacterial/ml and incubated at 37°C for 24 h. For bacteria ampicillin and cephazolin obtained from a local pharmacy, were used at 4, 2, 1 and 0,5 mg/ml (1 g/mL stock) as positive control, for fungus nystatin and 95% alcohol was used as negative control. The bacterial and fungal growth were assessed by a stereo microscope after the incubation period. All the assays were performed in triplicate.

GC-MS analysis

Propolis, grated after cooling, was extracted for 24 h with 95% ethanol (1:10, w/v) at room temperature. The extract was evaporated to dryness. About 5mg of the residue was mixed with 50 µl of dry pyridine and 75 µl bis (trimethylsilyl) trifluoracetamide, heated at 80 °C for 20 min and analyzed by GC-MS. The GC-MS analysis was performed with a Shimadzu Gas Chromatograph 2010 Plus linked to Shimadzu 2010 mass spectrometer system equipped with a 23m long, 0.25mm id, 0.5 mm film thickness HP5-MS capillary column. The temperature was programmed from 100 to 310 °C at a rate of 5 °C/min. Helium was used as a carrier gas, flow rate 0.7 ml/min. Split ratio 1:80, injector temperature 280 °C, ionization voltage 70 eV. The identification was accomplished using computer searches on a NIST98 MS data library. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass spectral fragmentation. If available, reference compounds were co-chromatographed to confirm GC retention times. The components of propolis extracts in different solvents were determined by considering their areas as percentage of the total ion current. Some components remained unidentified because of the lack of authentic samples and library spectra of the corresponding compounds [13].

Ferric Reducing/Antioxidant Power Assay

For ferric reducing/antioxidant power (FRAP) used in the determination of total antioxidant activities, the improved TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) is preferred [14, 15]. The activities of the samples were determined as micromolar FRAP value, which was obtained by using ascorbic acid in the 62.5–1000 µM range to prepare the calibration plot (Figure 1). Briefly,

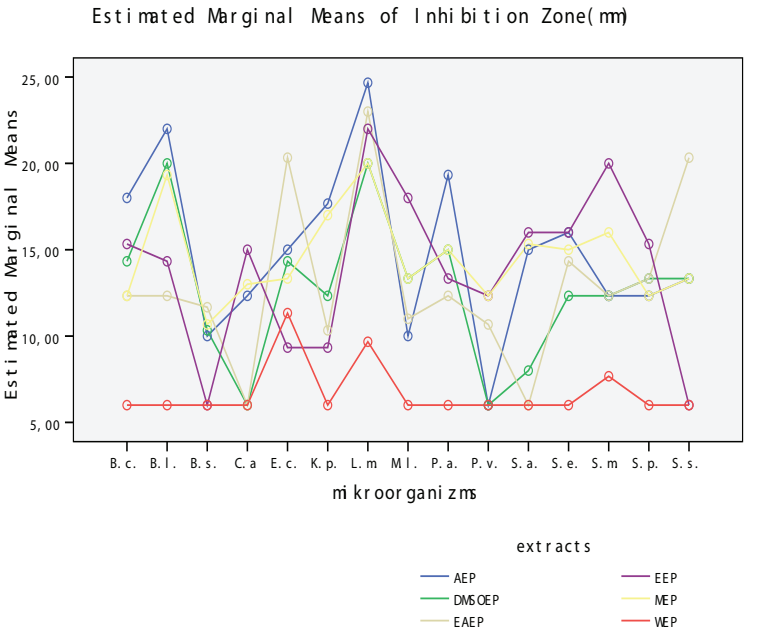

Figure 1. Profile Plot (Results of antimicrobial screening different solvent of Rize province propolis extracts determined by the agar diffusion method)

100 µL sample of same concentration for all the samples was mixed with 3.0 mL FRAP reagent (prepared by mixing acetate buffer, TPTZ, and FeCl3.6H2O solutions), and the absorbance was read at 595 nm against water blank at the end of 20 min incubation period. FRAP values were obtained by multiplying the µM concentration of ascorbic acid corresponding to the absorbance of the sample from calibration graph by two, the stoichiometric factor.

Statistical analysis

The data were analyzed by using SPSS for Windows (v.15.0). The differences between the means of the inhibition zones were tested with one-way variance analysis followed by Tukey's HSD test. The results were evaluated in the confidence limit of 0.05.

Results and Discussion

In the present study, the antimicrobial activity of acetone, ethyl acetate, ethanol, methanol, dimethyl sulfoxide and water propolis extracts from the Rize province of Turkey were investigated. The antibacterial and antifungal activity of propolis extracts in different solvents were initially evaluated by the disc diffusion method against nine Gram positive, five Gram negative bacteria and one fungus strain. The six tested compounds exhibited relatively strong antibacterial and antifungal activity. The results obtained in the disk diffusion assay regarding the growth inhibition zones of the tested microorganisms are shown in Table 1. In generally, methanol extract of propolis (MEP) samples more or less exhibit inhibitory action on the test organisms, but the samples showed a strong inhibitory effect on the growth of *L. monocytogenes* and *B. licheniformis* (20-19 mm/15 µl inhibition zone), among bacteria.

Table 1. Zones of inhibition (mm) showing the antimicrobial activity of propolis extracts

	Tested microorganisms mean zone of inhibition in mm ± standart derivation														
Extracts type	E.c	S.a.	S.s.	K.p.	S.e.	S.p.	B.c.	L.m.	S.m.	B.l.	M.l.	B.s.	P.a.	P.v.	C.a.
EAEP	20.33±1.57	6.00±0.00	20.00±1.00	10.33±0.57	14.33±0.57	13.00±1.00	12.00±0.00	22.66±0.57	12.33±0.57	11.66±0.57	11.33±0.57	11.00±0.00	12.33±0.57	10.33±0.57	6.00±0.00
EEP	9.00±1.00	16.00±0.00	6.00±0.00	8.66 ±0.57	16.00±0.00	15.33±1.52	15.00±0.00	22,33±0.57	20.00±1.00	14.33±0.57	18.00±1.00	6.00±0.00	13.00±0.00	12.00±0.00	15.33±0.57
AEP	15±0.00	14.66 ±0.57	13.00±2.00	17.00±1.00	16.00±0.00	12.00±1.00	17.33±1.15	24.33±0.57	11.67±0.57	21.66±0.57	10.00±1.00	10.00±0.00	19.33±0.57	6.00±0.00	12.00±1.00
MEP	13±0.00	14.67 ±0.57	13.00±0.00	17.00±0.00	15.00±3.00	11.67 ±0.57	12.00±0.00	20.33±0.57	16.33±0.57	19.33±0.57	13.00±1.00	9.67±0.57	15.00±0.00	12.00±01.00	13.00±0.00
DMSO	13.66±0.57	8.33±0.57	6.00±0.00	11.66±0.57	12.00±0.00	13.00±1.00	13.66±0.57	20.33±0.57	12.00±0.00	20.00±0.00	13.00±1.00	10.00±0.00	15.33±1.52	6.00±0.00	6.00±0.00
WEP	10.66±0.57	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	9.33±0.57	7.33±0.57	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
Means of column	13.67g	10.94c	11.83d	11.77d	13.22fg	11.83d	12.66ef	19.88ı	13.27fg	15.50h	11.88de	8.77a	13.50g	8.72a	9.72b
Ampicillin	15	10	10	13	35	28	27	25	25	30	35	36	28	28	NT
Cephazolin	15	10	10	10	36	22	23	32	30	25	35	38	24	28	NT
Nystatin	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	15
70% ethanol	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00

SD: standard deviation, 6.00 : no inhibition, NT: Not tested, Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumonia* ATCC 5041, *E. coli* ATCC 25922, *S. enteridis* ATCC 13076, *S. pneumonia* ATCC 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *B. licheniformis* NRRL-B1001, *M. luteus* NRRL- B1018, *B. subtillis* NRRL-B209, *P. aeruginosa* NRRL-B2679, *P. vulgaris* NRRL-B123 and *C. albicans*ATCC25922

On the other hand, antifungal activity was shown against *C. albicans* (13 mm/15 µl inhibition zone). However, ethanol extract of propolis (EEP) samples exhibited inhibitory action on the *C. albicans*. Moroever, the samples showed a strong inhibitory effect on the growth of *S. mutans, L. monocytogenes* and *M. luteus* among bacteria. EEP did not show activity only against *S. salivarius* and *B. subtilis.* The crude propolis sample obtained from (AEP) samples showed antibacterial and antifungal activity (24-18 mm/15 µl inhibition zone) against the *L. monocytogenes, B. cereus, P. aeruginosa* and *B. licheniformis*. whereas, no activity was observed against *P. vulgaris.* However, this extract showed weak antifungal activity against *C. albicans.*

Water of propolis (WEP) samples did not form an inhibitory zone against any of the microorganisms tested except for *S. mutans, L. monocytogenes* and *E. coli.* It showed weak antibacterial activity (7-11 mm/15 µl inhibition zone) against these bacteria. Dimethyl sulfoxide of propolis (DMSOEP) samples did not exhibit inhibitory action on the *S. salivarius and P. vulgaris.* However, DMSOEP samples weakly exhibited inhibitory action on *S. aureus,* and *B. subtilis*. At the same time, the samples highly showed inhibitory effect on the growth of *M. luteus,* and *L. monocytogenes* among bacteria but did not show antifungal effect on *C. albicans*. On the other hand, ethyl acetate of propolis (EAEP) samples weakly exhibited inhibitory action on *P. aeruginosa, P. vulgaris* and *B. subtilis* but the samples showed a strong inhibitory effect on the growth of*, L. monocytogenes, E. coli* and *S. salivarius* but did not show antifungal effect on *C. albicans* and *S. aurues.* Evaluation of MIC s of different solvent propolis extracts from the Rize province of Turkey by means of agar dilution experiment method is reported in Table 2.

Table 2. Antimicrobial activity of propolis extracts

Extracts Type	Tested Microorganisms Minimal Inhibitory Concentration (MIC) in mg/ml												
	E.c.	*S.a.*	*S.s.*	*K.p.*	*S.e.*	*S.p.*	*B.c.*	*L.m.*	*S.m.*	*B.l.*	*M.l.*	*B.s.*	*P.a.*	*P.v.*	*C.a.*
EAEP	1≤	2≤	1≤	4≤	2≤	2≤	2≤	1≤	4≤	4≤	4≤	4≤	2≤	4≤	-
EEP	4≤	2≤	-	4≤	1≤	1≤	1≤	1≤	1≤	2≤	1≤	-	2≤	4≤	1≤
AEP	2≤	2≤	2≤	1≤	1≤	1≤	1≤	1≤	4≤	1≤	4≤	4≤	1≤	-	4≤
MEP	2≤	2≤	2≤	1≤	2≤	2≤	2≤	1≤	2≤	1≤	4≤	4≤	1≤	4≤	4≤
DMSOEP	2≤	4≤	-	-	4≤	2≤	1≤	1≤	4≤	1≤	4≤	4≤	1≤	-	-
WEP	-	-	-	-	-	-	-	4≤	-	-	-	-	-	-	-

- : no inhibition, Microorganisms: *S. salivarius* RSHE 606, *S. aureus* ATCC 6538, *K. pneumoniae* ATCC 5041, *E. coli* ATCC 25922, *S. enteritidis* ATCC 13076, *S. pneumoniae* ATCC 10015, *B. cereus* ATCC 11778, *L. monocytogenes* NCTC 5348, *S. mutans* RSHE 676, *B. licheniformis* NRRL-B1001, *M. luteus* NRRL-B1018, *B. subtillis* NRRL-B209, *P. aeruginosa* NRRL-B2679, *P. vulgaris*NRRL- B123 and *C. albicans* ATCC 25922

Table 3. Chemical composition of propolis extracts (% Total ion current, GC-MS)

Compounds	WEP	EPE	EAEP	APE	MEP	DMSOEP	
	%TIC						Total line
Aliphatic Acids							
Oleic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl (E)-octadec-9-enoate		2.41	2.89	2.86			8.16
9-Octadecenoic acid (Z)-, ethyl ester **IUPAC name** ethyl (E)-octadec-9-enoate		0.99					0.99
9,12-Octadecadienoic acid (Z,Z)-, trimethylsilyl ester **IUPAC name** trimethylsilyl (9Z,12Z)-octadeca-9,12-dienoate		0.54					0.54
Octadecanoic acid **IUPAC name** Octadecanoic acid			0.11				0.11
Octadecanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl octadecanoate			1.33				1.33
2-Monostearin trimethylsilyl ether **IUPAC name** 1,3-bis(trimethylsilyloxy)propan-2-yl octadecanoate		0.50	0.18	1.78			2.46
Octadecanoic acid, 9,10,18-tris[(trimethylsilyl)oxy]-, methyl ester **IUPAC name** methyl 9,10,18-tris(trimethylsilyloxy)octadecanoate			0.31				0.31
Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl 2-trimethylsilyloxypropanoate					0.65		0.65
Propanoic acid, 2-(aminooxy) **IUPAC name** 2-(aminooxy) propanoate						0.39	**0.39**

Propanoic acid, 2-methyl-2-[(trimethylsilyl)oxy]-,trimethylsilyl ester **IUPAC name** trimethylsilyl 2-methyl-2-trimethylsilyloxypropanoate			0.17		1.70		1.87
1H-Indole-3-propanoic acid, 1-(trimethylsilyl)-, methyl ester **IUPAC name** methyl 3-(1-trimethylsilylindol-3-yl)propanoate			0.25				0.25
alpha.-(2,4,5-trichlorophenoxy) propionic acid, n- butyl ester **IUPAC name** butyl 2-(2,4,5-trichlorophenoxy)propanoate		0.51	0.12				0.63
4-hydroxyphenylpropionic acid-ditms **IUPAC name** trimethylsilyl3-(4-trimethylsilyloxyphenyl) propanoate			0.84				0.84
ethyl ester of 3-trimethylsilyl-propionic acid **IUPAC name** Ethyl3-(trimethylsilyl)propanoate					0.37		0.37
Hexadecanoic acid, ethyl ester **IUPAC name** ethyl hexadecanoate			0.20				0.20
Hexadecanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl hexadecanoate		1.77	2.93	0.70			5.40
Butanoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl 4-trimethylsilyloxybutanoate					1.09		1.09
Butanoic acid, 3-[(trimethylsilyl)oxy]-, methyl ester **IUPAC name** methyl 3-trimethylsilyloxybutanoate			0.24				0.24
Dodecanoic acid **IUPAC name** Dodecanoic acid			0.15				0.15
Dodecanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl dodecanoate			0.17				0.17

Aromatic Acids							
Benzenepropanoic acid, trimethylsilyl ester **IUPAC name** trimethylsilyl 3-hphenylpropanoatepenylpropanoic acid			0.15				0.15
Benzeneacetic acid, 2,4,5-tris[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** 2,4,5-Tris[(trimethylsilyl)oxy]benzeneacetic acid trimethylsilyl ester				4.93			4.93
Benzeneacetic acid, 3-methoxy-.alpha.,4-bis[(trimethylsilyl)oxy]-, ethyl ester **IUPAC name** ethyl 2-(3-methoxy-4-trimethylsilyloxyphenyl)-2-trimethylsilyloxyacetate			0.20				0.20
Cinnamic acid, p-(trimethylsiloxy)-, trimethylsilyl ester **IUPAC name** trimethylsilyl (E)-3-(4-trimethylsilyloxyphenyl)prop-2-enoate		1.25	0.58				1.83
Cinnamic acid, 3,4-dimethoxy-, trimethylsilyl ester **IUPAC name** trimethylsilyl (E)-3-(3,4-dimethoxyphenyl)prop-2-enoate		0.69	0.52				1.21
Cinnamic acid, 3,4-bis(trimethylsiloxy)-, methyl ester **IUPAC name** methyl (E)-3-[3,4-bis(trimethylsilyloxy)phenyl]prop-2-enoate			0.41				0.41
Cinnamic acid, p-methoxy-, trimethylsilyl ester_ **IUPAC name** trimethylsilyl (E)-3-(4-methoxyphenyl)prop-2-enoate			0.35				0.35
caffeic acid-tms-ether **IUPAC name** trimethylsilyl (E)-3-[3,4-bis(trimethylsilyloxy)phenyl]prop-2-enoate		1.97	2.64				4.61

trans-Caffeic acid, triTMS **IUPAC name** trimethylsilyl (E)-3-[3,4-bis(trimethylsilyloxy)phenyl]prop-2-enoate	7.15		0.37	23.56			31.08
ferulic acid-tms ether **IUPAC name** trimethylsilyl (E)-3-(3-methoxy-4-trimethylsilyloxyphenyl) prop-2-enoate		2.32	1.03	2.01			5.36
isoferulic acid-tms ether **IUPAC name** trimethylsilyl (E)-3-(4-methoxy-3-trimethylsilyloxyphenyl) prop-2-enoate			0.40				0.40
Benzoic acid **IUPAC name** Benzoic acid			0.33				0.33
Benzoic acid, 4-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl 4-trimethylsilyloxybenzoate			0.21				0.21
Benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester **IUPAC name** trimethylsilyl3-methoxy-4-trimethylsilyloxy benzoate			0.15				0.15
Benzoic acid trimethylsilyl ester **IUPAC name** Trimethylsilyl benzoate		0.95	0.48		1.94		3.37
ethanol, 1-(methylencyclopropyl)- **IUPAC name** 1-(2-methylidenecyclopropyl)ethanol	1.12						1.12
Ethanol, 2-(9-octadecenyloxy)-, (Z)- **IUPAC name** 2-[(Z)-octadec-9-enoxy]ethanol			0.10				0.10
3,7-Dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl- **IUPAC name** 5-[(trimethylsilyl)oxy]- tms-glycerol	36.89	2.98	0.30	3.66			43.83
Benzeneethanol **IUPAC name** 2-phenylethanol			0.12				0.12
Total	45.15	18.50	18.89	39.50	6.03	1.25	129.32

The ethanol of propolis (EEP) samples required an MIC of 1 mg/ml for *S. salivarius, E.* coli and *L. monocytogenes* while all propolis samples required an MIC of 4 mg/ml for *B. subtilis*. Only the methanol of propolis (MEP) samples required an MIC of 1mg/ml for *E. coli*. Lower MIC values (1.4 mg/ml) were required against *E. coli*, however none of WEP samples were required against all pathogens. WEP, the other extracts of all propolis samples were active on microorganisms. The most sensitive microorganism to propolis was *E. coli* in the gram-negative group and *Streptococcus mutans* in the gram-positive group. The least sensitive microorganism was *Streptococcus salivarius.*

A control test ran with standard antibiotics revealed that propolis samples from the Rize province of Turkey had a similar or greater inhibitory effect on *S. mutans*, *B. licheniformis L. monocytogenes, M. luteus*, and *C. albicans* growth. According to the results, it may be concluded that, in general, Gram-positive bacteria and fungus were more susceptible to all of propolis samples antibacterial action than Gram-negative bacteria. De novo synthesis of water-insoluble glucan is essential for the adherence of *Streptococcus mutans* and other oral microorganisms to the tooth surface, forming a barrier that prevents the diffusion of acids produced by the bacteria [16]. Extensive screening for biologically active compounds from natural sources with these effects has been performed. For example, except for the water extract of propolis (WEP), the other propolis inhibited the growth of *S. mutans*. Similar results have been reported in other studies, which support our findings that propolis is mainly active against Gram-positives [17,18]. The antimicrobial activity against all pathogens was evaluated. EEP, AEP, EAEP and MEP showed the highest antimicrobial activity against *S. mutans, L. monocytogenes, M. luteus, B. licheniformis* and *C. albicans* While DMSOEP had the weak activity against some test organisms. Except for *S. mutans*, WEP was not effective against all pathogens. However it has been reported that EEP is effective on Gram-negative bacteria at higher concentrations [19]. These results indicated that acetone extracts of all propolis samples were more active than the DMSO extracts of the same samples. However, our findings are not similar to those of other researchers, who found differences [20]. Our results are similar to Ugur and Arslan's results. According to Hegazi et al.[21]; The propolis samples show different antimicrobial activity due to it is complex resinous bee product with a physical appearance that varies widely, depending on many factors. This propolis is known as a healer and used for the treatment of various diseases in humans. Several compounds have been identified in propolis, and three distinct chemical groups have been reported to be present: (i) flavonoid aglycones, (ii) cinnamic acid derivatives, and (iii) terpenoids [22-24]. Flavonoids have been considered as the main biologically active compounds in propolis [1, 25, 26]. In our opinion, the qualitative and quantitative composition of propolis plays an important role in their biological activity.

Several studies regarding antimicrobial activity of propolis ethanolic extracts showed a positive correlation between flavonoid content and antibacterial properties of propolis [27]. The composition of raw propolis depends upon the plant source, bud exudates of different trees, generally *Populus* in the temperate zone [28]. Propolis contains wide variability of active compounds (flavonoids and phenolic acids). Variations in the flavonoid content of propolis are mainly attributable to the

difference in the preferred regional plants visited by honeybees [29]. Numerous researchers have reported that caffeic acids, flavonoids and phenolic esters are the main biologically active compounds in propolis [30-32]. However, our samples were found to be active against the gram negative bacteria. This activity can be a synergism between flavonoids, apigenin, chrysin, and/or other components in raw propolis samples. Besides, 5-[(trimethylsilyl)oxy]-tms-glycerol=36.89 value is ignored because it is bacteriostatic glycerol, and total column of acetone=39.50 are considered as the greatest value. Consequently, acetone of propolis extract showed the highest antibacterial effect.

The extracts (100 μL) were treated with FRAP reagent (3.0 mL), and the absorbance values measured at 595 nm after a 20 min incubation period were used to calculate the FRAP values from a calibration graph prepared with ascorbic acid (Figure 2). The corresponding ascorbic acid concentration value was multiplied by two to express the antioxidant capacities as μM FRAP (Figure 3). A higher FRAP value reflects higher antioxidant capacity. Thus, all the propolis extracts showed much higher antioxidant power in comparison to the standard antioxidant Trolox (500 μM). While the aqueous extract had the lowest activity, acetone extract showed the highest, an approximately 55 fold activity range. In order to show the relationship between the total extract table antioxidant content with the solvent polarity, the dielectric constant of the extraction solvents were plotted against the FRAP values obtained (Figure 4). Dielectric constant is an indicator of solvent polarity. As the solvent polarity increased, the total extracted antioxidants decreased as evident from lower FRAP values. Care must be taken that no solvent with practically nonpolar nature, such as hexane, was used in the tests; the lowest polarity solvent extracts may also be expected to show lower FRAP values. Finally, in our test, we found the highest antioxidant effect in the ethyl acetate extract of propolis and acetone extract of propolis.

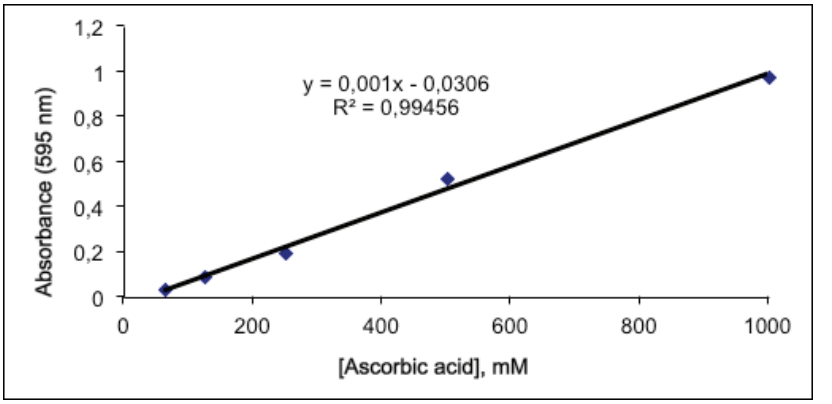

Figure 2. Calibration curve for FRAP test, prepared by ascorbic acid concentration plotted against the absorbance value measured at 595 nm in FRAP assay.

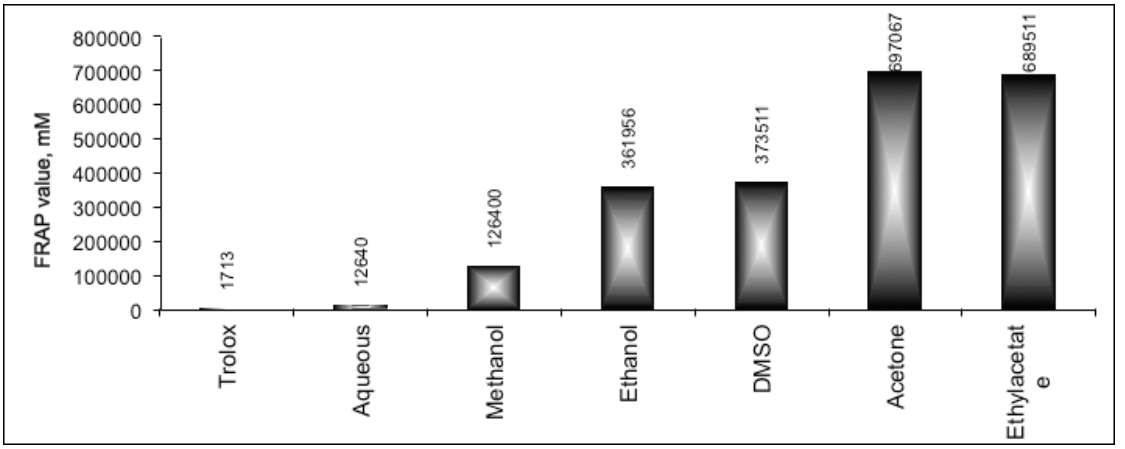

Figure 3. FRAP values of the extracts and the standard antioxidant Trolox (500 μM). Aqueous extracts were diluted 20 fold, and the others 400 fold in the assay, and the FRAP values were calculated by multiplying with the dilution factor.

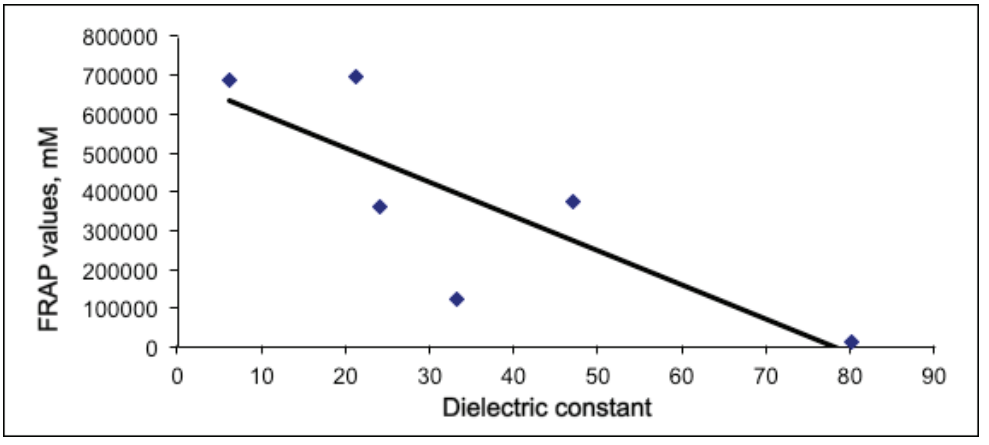

Figure 4. FRAP values of the propolis extracts plotted against the dielectric constantas of the extraction solvent

Türkiye'de Rize İlinden Elde Edilen Propolisin Antimikrobiyal ve Antioksidan Aktivitesi Üzerine İn Vitro Çalışma

ÖZ

Bu çalışmada Türkiye'nin Rize ilinden toplanan propolis örneklerinin farklı solventlerde antibakteriyal antifungal ve antioksidan aktiviteleri araştırılmıştır.Gram pozitif bakteriler *(Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus salivarius)*, Gram negatif bakteriler *(Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella enteritidis)* ve bir maya *(Candida albicans)* olmak üzere toplamda 15 mikroorganizma disk difüzyon ve minimum inhibisyon konsantrasyonu (MİK) yöntemi kullanilarak incelenmiştir. Propolisin etanol ekstraktı (EPE), propolisin aseton ekstraktı (APE), propolisin etil asetat ekstraktı (EAPE) ve propolisin metanol ekstraktı (MPE) *S. mutans, L. monocytogenesis, M. luteus, B. licheniformis ve C. albicans*'a karşı en yüksek antimikrobiyal aktivite göstermiştir. Dimetil sülfoksit Propolis Ekstraktı (DMSOPE) bazı test mikroorganizmalarına karşı zayıf aktivite göstermiştir. Propolise en duyarlı mikroorganizmalar *E. coli B. licheniformis, S. mutans, L. monocytogenes ve B. cereus* olmuştur.

Anahtar Kelimeler: Antimikrobiyal aktivite, antioksidan aktivite, propolis, GC-MS

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