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RESEARCH ARTICLE

In Vitro Activity of Turkish Propolis Samples Against Anaerobic Bacteria Causing Oral Cavity Infections

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Summary

The aim of this study was to evaluate the antimicrobial activity of propolis samples collected from different regions of Turkey against anaerobic bacteria causing especially oral cavity infections. A total of eleven anaerobic bacterial strains have been tested in this study. The strains were tested by agar dilution method for detecting minimum inhibitory concentration (MIC) and by macro dilution broth method for detecting minimum bactericidal concentration (MBC). Turkish propolis samples were found highly effective against all tested anaerobic bacteria compared with ethanol control, without statistical differences. The MIC and MBC of propolis samples ranged from 0.4-0.6 mg/ml to 108.1-186.2 mg/ml, respectively. *Actinomyces odontolyticus* was the most susceptible strains; whereas *Prevotella intermedia* was was the least susceptible strain to all tested propolis samples. Ilic/Erzincan (ER-I) propolis sample was the more effective against all tested anaerobic bacteria; whereas Bartin (BA) propolis sample was the less effective. Gram-positive anaerobic bacteria were detected to be the most sensitive to propolis samples; with the MIC values ranging from 0.4 to 6.1 mg/ml compared with Gram-negative anaerobic bacteria with MIC ranging from 5.8 to 108.1 mg/ml ($P<0.05$). As a result of, Turkish propolis samples had antibacterial activity against anaerobic bacteria especially causing oral cavity infections. Because of the high rate of resistance of the anaerobic bacteria isolated from oral cavity infections, standardized preparations of propolis are suggested to use in treatment of this kind of infections. However, further studies are needed to be performed on the clinical applications of propolis in oral cavity infections.

Keywords: *Turkish propolis, Anaerobic bacteria, Antimicrobial activity, MIC, MBC*

Oral Kavite Enfeksiyonlarına Neden Olan Anaerobik Bakterilere Karşı Türk Propolis Örneklerinin *In vitro* Antimikrobiyal Aktivitesi

Özet

Bu çalışmanın amacı, özellikle oral kavite enfeksiyonlarına neden olan anaerobik mikroorganizmalara karşı Türkiye'nin çeşitli bölgelerinden toplanan propolis örneklerinin antimikrobiyal aktivitesini değerlendirmektir. Bu çalışmada toplam onbir anaerobik mikroorganizma test edildi. İzolatların minimal inhibitör konsantrasyonlarını (MİK) tespit etmek için agar dilüsyon yöntemi, minimal bakterisidal konsantrasyonlarını (MBK) tespit etmek için makro tüp dilüsyon yöntemi kullanıldı. Türk propolis örnekleri istatistiksel fark olmaksızın etanol kontrolü ile karşılaştırıldığında tüm test edilen anaerobik mikroorganizmalara karşı etkili bulundu. Propolis örneklerinin MİK ve MBK'ları sırasıyla 0.4-0.6 mg/ml ile 108.1-186.2 mg/ml değerleri arasındaydı. Tüm test edilen propolis örneklerine karşı *Prevotella intermedia* en az duyarlı izolat iken iken, *Actinomyces odontolyticus* en fazla duyarlı izolat idi. Bartin (BA) propolis örneği tüm test edilen anaerobik mikroorganizmalara karşı en az etkili iken, İlic/Erzincan (ER-I) propolis örneği en fazla etkili idi. Propolis örneklerine MİK oranları 0.4-6.1 mg/ml ile Gram-pozitif anaerobik bakteriler, MİK oranları 5.8-108.1 mg/ml ile Gram-negatif bakteriler ile karşılaştırıldığında daha duyarlı oldukları tespit edildi ($P<0.05$). Sonuç olarak, Türk propolis örnekleri özellikle oral kavite enfeksiyonlarına neden olan anaerobik bakterilere karşı iyi derecede antimikrobiyal etkinliğe sahipti. Oral kavite enfeksiyonlarından izole edilen anaerobik bakterilerdeki yüksek direnç oranlarından dolayı, standardize edilerek hazırlanmış propolis bu tür enfeksiyonların tedavisinde kullanılmak için tavsiye edilmektedir. Ancak propolisin oral kavite enfeksiyonlarında klinik uygulamalarına yönelik ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar sözcükler: *Türk propolis örnekleri, Anaerobik bakteri, Antimikrobiyal aktivite, MİK, MBK*

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INTRODUCTION

Propolis is a natural resinous product collected by honeybees from plant exudates, beeswax and bee secretions [1]. Propolis has been used in folk medicine since it has many biological properties such as antimicrobial, anti-inflammatory, antioxidant, immunomodulatory activities, among others [2-4]. Biological activities of propolis are directly related to its chemical components and as well as its origin [5]. Propolis samples contain a variety of chemical components such as flavonoids, aromatic acids, diterpenic acids, and phenolic compounds [6]. The antimicrobial activity of propolis samples against a wide rage the bacteria, fungi, virus and invading larva has been reported from previous studies [7,8]. Since multi-drug-resistant Gram-positive and Gram-negative bacteria are important cause of community and hospital acquired infections, new and accurate antimicrobial agents are needed against these resistant bacteria [9].

The anaerobic microorganisms are closely involved in the pathogenesis of oral cavity infections [10]. More than 500 taxa of microorganisms have been isolated from the human oral flora. Anaerobic bacteria are predominant of the bacterial community in this flora [11]. Although mechanical debridement is important factor in the treatment of the periodontal and oral cavity infections, systemic antibiotics may be prescribed to prevent the spread of infection and the onset of serious complications [12]. However, bacterial species are getting resistant to antibiotics used in oral cavity infections [13]. Therefore, the development of new therapies for the treatment of infection caused by resistant microorganisms is necessary [14].

The aim of this study was to evaluate the activity of ethanol extract propolis (EEP) samples collected from different regions of Turkey against eleven anaerobic bacteria causing mainly periodontal diseases.

MATERIAL and METHODS

Propolis Samples

Propolis samples were collected from apiaries that belong to two phytogeographical regions of Turkey (Euro-Siberia, Irano-Turanian). The apiaries were selected according to criteria such as far from pollutants, fabricas. The samples were collected from Artvin, Bartın, Bursa, Erzincan, Tekirdağ, Yalova, Zonguldak regions. The symbols and the collecting areas of the samples are given in the *Table 1*. Phytogeoraphical region means classifying of the plants according to their spreading area. In the world there are 37 phytogeographical regions. In Turkey three of this regions are existing; European-Siberian phytogeographical region (include Black Sea region), Irano-Turanian phytogeographicak region (include Central Anatolia and East Anatolia Region), Mediterranean phytogeographical rehion (include Mediterranean region).

Preparation of Ethanol Extracts of Propolis

Ethanol extracts of propolis (EEP) was prepared as Kilic et al.[15]. Concentrated solution called EEP [obtained diluting the original EEP solution in 1:10, weight/volume (w/v)] was evaporated to dryness. About 5 mg of residue was mixed with 75 µl of dry pyridine and 50 ml bis (trimethylsilyl) trifuoroacetamide (BSTFA), heated at 80°C for 20 min, and then the final supernatant was analyzed by gas chromatography coupled to mass spectrometry (GC-MS) [16].

GC-MS Analysis

A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS5973, Hewlett-Packard) was used for the analysis of EEP samples. An experimental condition of GC-MS system was as follows: DB 5MS column (30 m x 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 for 1 min at 50°C then increased to 150°C with 10°C/min heating ramp. After this period, temperature was kept at 150°C for 2 min. Finally; temperature was increased to 280°C with 20°C/min heating ramp and then kept at 280°C for 30 min [15].

Bacterial Strains

A total of eleven anaerobic bacterial strains have been tested in this study: *Peptostreptococcus magnus* ATCC 29328, *Eubacterium lentum* ATCC 43055, *Lactobacillus acidophilus* ATCC 4356, *Actinomyces odontolyticus* ATCC 17929, *Prevotella intermedia* ATCC 25611, *Prevotella oralis* ATCC 33269, *Prevotella melaninogenica* ATCC 25845, *Porphyromonas gingivalis* ATCC 33277, *Fusobacterium nucleatum* ATCC 10953, *Bilophila wardsworthia* ATCC 51581, *Veillonella parvula* ATCC 10790. Before the study, all strains were kept in our laboratory and cryopreserved at -86°C. For each experiment, the bacteria were inoculated into Brucella agar plates supplemented with 0.5% yeast extract, hemine (5 µg/ml), menadione (1 µg/ml) and 5% horse blood. Incubation was performed at 37°C under anaerobic conditions for 5 days in anaerobic jar with gas generating kit (Mitsubishi Gas Chemical Company, Anaerogen, Oxoid, England).

Determination of the Minimum Inhibitory Concentration (MIC) of EEP Samples

The agar dilution method was performed 3 times for each strain as described by the National Committee for

Clinical Laboratory Standards (NCCLS) [17]. Serial two-fold dilutions of EEP were prepared in Brucella agar, which was supplemented with 5% sheep blood, menadione (1 µg/ml) and hemin (5 µg/ml) by the manufacturer. Agar dilutions ranged from 1/2 to 1/512 µg/ml. Two controls were used: (a) agar plates containing no EEP (b) agar plates containing ethanol at 1% final concentration. Each antimicrobial test was also re-performed with plates containing the culture medium plus ethanol as solvent control. The inoculums were prepared by picking three to five colonies of the test organism and inoculating them into 5 ml of enriched thioglycolate broth supplemented with vitamin K (1 µg/ml), hemin (5 µg/ml) and $NaHCO_3$ (1 mg/ml). The broth cultures were incubated over-night at 37°C and used to prepare an organism suspension in prereduced Brucella broth (Difco Laboratories, USA) equivalent in density to a 0.5 McFarland standard. Each plate was then inoculated with a multipoint inoculating device (Steers replicator), which delivered a final inoculum of approximately 105 CFU per spot. The inoculum size was verified by plating serial dilutions of the inoculum and performing colony counts. The plates were incubated at 37°C in an anaerobic jar with gas generating kit (90% N_2, 5% CO_2 and 5% H_2) for 48 h. Bacteroides fragilis ATCC 25285 was used as quality-control organism recommended by NCCLS [17].

Determination of Minimum Bactericidal Concentration (MBC) of EEP Samples

Determination of minimum bactericidal concentration (MBC) of EEP for the eleven reference strains of anaerobic bacteria was performed by macro dilution broth method as described by the NCCLS [17]. Serial two-fold dilutions of EEP were prepared in macro dilution tubes with concentrations ranging from ½ to 1/512. A final inoculum of approximately 10^5 CFU in supplemented Brucella broth was inoculated into tubes of containing EEP dilutions and incubated for 48 h. After incubation, 0.1 ml of diluted cultures were inoculated onto the surface of supplemented Brucella agar and all plates were incubated at 37°C in an anaerobic jar with gas generating kit (90% N_2, 5% CO_2 and 5%H_2) for 48-96 h. MBC was taken as the concentration at which a 99.9% reduction in cfu of the original inoculum occurred. The MBC was defined as the lowest concentration of propolis or honey where no growth was recorded.

Statistical Analysis

All of the statistical analyses were performed by SPSS 11.0 (SPSSFW, SPSS Inc., Chicago, IL. USA) statistical package. Descriptive statistics were given in median (min-max). The values between groups were compared by Mann Whitney U test. P values less than or equal to 0.05 were evaluated as statistically significant [18,19].

RESULTS

The *in vitro* antimicrobial activity of Turkish EEP samples was evaluated against eleven anaerobic bacteria mainly causing periodontal diseases. Each dry propolis samples were dissolved in ethanol as 37.24% for BA, 22.27% for ERI, 32.88% for ERII, 24.76% for AR, 34.57% for TEI, 36.04% for TEII, 43.22% for ZOI, 37.86% for ZOII, 26.34% for ZOIII, 38.68% for BUI *(Table 1).* Organic composition of EEP samples was measured by using the GC-MS system and calculated by using peak area of target compound and the sum peak areas as a percent in the chromatogram of propolis samples. Dominant classes of organic compounds were given in *Table 2.* Turkish EEP samples highly effective against all tested anaerobic bacteria compared with ethanol control, without statistical differences. The control samples (96% aqueous ethanol, v/v) did not effect the growth of bacteria (data not shown). The MIC and MBC of EEP samples ranged from 0.4-0.6 to 108.1-186.2 mg/mL, respectively *(Table 3)*. The microbial susceptibility to the tested EEP samples was variable. *A. odontolyticus* was the most susceptible strain; whereas *P. intermedia* was the least susceptible to all tested propolis samples. ER-I propolis sample was the most effective against all tested anaerobic bacteria; whereas BA propolis samples was the least effective. These values of the EEP samples were given in *Table 3.* It has been shown that Gram-positive anaerobic bacteria were most sensitive to EEP samples; with the MIC values ranging from 0.4 to 6.1 mg/ml compared with Gram-negative anaerobic bacteria to EEP samples with MIC ranging from 5.8 to 108.1 mg/ml (P<0.05).

Table 1. *Geographical origins and other properties of propolis samples*
Tablo 1. *Propolis örneklerinin coğrafik kökenleri ve diğer özellikleri*

Phyto-geographical region	Sample location	Symbol	Concentration in EEP solution (%w/v)	Collection year
Euro-Sibiria	Bartin	BA	37.24	2006
Iran-Turan	Ilic/Erzincan	ERI	22.27	2006
Iran-Turan	Kemaliye/Erzincan	ERII	32.88	2006
Euro-Sibiria	Camili/Artvin	AR	24.76	2006
Euro-Sibiria	Nusratli/Tekirdag	TEI	34.57	2006
Euro-Sibiria	Naip/Tekirdag	TEII	36.04	2006
Euro-Sibiria	Karakavaz/Zonguldak	ZOI	43.22	2006
Euro-Sibiria	Zonguldak	ZOII	37.86	2006
Euro-Sibiria	Zonguldak	ZOIII	26.34	2006
Euro-Sibiria	Bursa	BUI	38.68	2006
Euro-Sibiria	Tahtakopru/Bursa	BUII	34.72	2006
Euro-Sibiria	Yalova	YA	34.33	2006

BA, Bartin; ER-I, Ilic/Erzincan; ER-II, Kemaliye/Erzincan; AR, Camili/Artvin; TE-I, Nusratli/Tekirdag; TE-II, Naip/Tekirdag; ZO-I, Karakavaz/Zonguldak; ZO-II, Zonguldak; ZO-III, Zonguldak; BU-I, Bursa; BU-II, Tahtakopru/Bursa; YA Yalova

Table 2. *Chemical composition of propolis samples*
Tablo 2. *Propolis örneklerinin kimyasal bileşenleri*

Compounds %	Samples											
	BA	ERI	ERII	AR	TEI	TEII	ZOI	ZOII	ZOIII	BUI	BUII	YA
Aromatic alcohol	10.02	3.65	8.06	11.45	1.86	6.8	8.03	6.77	10.01	5.86	5.17	5.57
Alcohols	1.12	-	-	-	1.71	2.24	1.14	-	-	3.14	1.83	1.92
Acids	4.51	-	-	-	-	-	-	-	-	-	1.01	-
Aromatic acid esters	-	-	2.18	-	-	-	-	-	-	1.16		-
Aromatic esters	-	-	-	-	-	-	-	0.61	-	-	-	-
Aromatic acids	8.55	-	-	-	3.35	5.54	-	6.92	-	-	2.74	-
Flavanones	42.64	32.07	40.04	1.34	53.54	57.34	46.01	48.33	64.3	39.05	39.66	36.87
Hydrocarbon	-	-	-	-	-	-	-	-	-	-	1.66	-
Aliphatic esters	0.57	0.82	3.15	33.72	7.09	1.23	0.53	0.92	-	1.04	2.8	1.94
Aliphatic acid esters	-	-	1.9	20.5	0.68	-	-	2.32	-	1.11	1.49	0.57
Aromatic hydrocarbons	1.07	-	-	6.07	0.72	1.71	-	2.27	-	0.75	-	1.13
The others	9.31	10	-	-	-	-	-	1.89	-	0.58	12.36	1.72

BA, Bartin; ER-I, Ilic/Erzincan; ER-II, Kemaliye/Erzincan; AR, Camili/Artvin; TE-I, Nusratli/Tekirdag; TE-II, Naip/Tekirdag; ZO-I, Karakavaz/Zonguldak; ZO-II, Zonguldak; ZO-III, Zonguldak; BU-I, Bursa; BU-II, Tahtakopru/Bursa; YA Yalova

Table 3. *Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of propolis samples*
Tablo 3. *Propolis örneklerinin mi minimal inhibitör konsantrasyonları (MİK) ve minimal bakterisidal konsantrasyonları (MBK)*

Microorganisms [a]	BA [b]		ERI		ERII		AR		TEI		TEII		ZOI		ZOII		ZOIII		BUI		BUII		YA		TOTAL (Mean±SD)	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
Pm (ATCC 29328)	1.4	2.9	1.7	1.7	1.2	2.5	1.9	3.8	2.7	5.4	1.4	2.8	3.3	6.7	1.4	2.9	1.1	2.1	3.1	6.1	2.7	5.4	2.6	5.3	2.0±0.7	3.9±1.7
El (ATCC 43055)	2.9	5.8	0.8	1.7	0.6	1.2	0.9	0.9	1.3	2.7	2.8	5.6	1.6	3.3	2.9	5.9	2.1	4.1	1.5	3.1	1.3	2.7	1.3	2.6	1.6±0.8	3.3±1.7
La (ATCC 4356)	1.4	1.4	1.7	3.4	2.5	5.1	1.9	3.8	2.7	2.7	1.4	2.8	3.3	6.7	5.9	11.8	4.1	8.2	6.1	12.1	5.4	10.8	5.3	10.7	2.2±1.5	6.6±3.9
Ao (ATCC 17929)	5.8	11.6	0.4	0.8	1.2	1.2	0.4	0.9	0.6	1.3	0.7	0.7	0.8	1.6	0.7	1.4	0.5	1.1	0.7	1.5	0.6	1.3	0.6	1.3	1.0±1.5	2.0±3.0
Pi (ATCC 25611)	46.5	93.1	6.9	13.9	41.1	82.2	7.7	7.7	86.4	172.8	90.1	180.2	108.1	108.1	94.6	189.3	65.8	131.7	96.7	193.4	86.8	173.6	85.8	171.6	68.0±34.6	126.4±66.0
Po (ATCC 33269)	23.2	46.5	13.9	27.8	20.5	41.1	61.9	123.8	43.2	86.4	22.5	45.1	13.5	27.0	47.3	47.3	32.9	32.9	24.1	48.3	21.7	43.4	21.4	42.9	28.8±14.7	51.0±27.4
Pm (ATCC 25845)	46.5	93.1	27.8	55.6	10.2	20.5	30.9	61.9	10.8	21.6	45.1	90.1	27.1	54.1	11.8	23.6	32.9	32.9	48.3	96.7	43.4	86.8	42.9	85.8	31.4±14.3	60.2±29.9
Pg (ATCC 33277)	5.8	11.6	27.8	55.6	10.2	10.2	7.7	15.4	43.2	86.4	11.2	11.2	27.1	27.1	47.3	94.6	14.4	32.9	96.7	193.4	43.4	43.4	10.7	21.4	28.7±26.1	50.2±53.3
Fn (ATCC 10953)	93.1	186.2	13.9	27.8	41.1	82.2	15.4	30.9	86.4	86.4	22.5	45.1	54.1	108.1	23.6	23.6	32.9	65.8	24.1	48.3	10.8	21.7	21.4	42.9	36.6±27.6	64.0±47.2
Bw (ATCC 51581)	46.5	93.1	6.9	13.9	20.5	41.1	15.4	15.4	43.2	86.4	5.6	11.2	6.7	13.5	11.8	23.6	8.2	8.2	12.1	24.1	43.4	86.8	42.9	85.8	21.9±16.8	38.3±32.3
Vp (ATCC 10790)	93.1	186.2	27.8	55.6	82.2	82.2	61.9	123.8	21.6	43.2	1.1	22.5	13.5	27.1	5.9	11.8	4.1	8.2	12.1	12.1	5.4	10.8	5.3	10.7	27.8±32.5	49.5±55.6
TOTAL	32.4±3	64.8±6	11.96±	23.8±2	20.9±2	34.2±3	18.4±2	34.9±4	31.0±3	53.21±	18.9±2	38.5±5	26.1±3	40.9±4	23.0±2	40.2±5	17.2±1	28.5±3	28.1±3	54.2±7	22.9±2	42.3±5	21.8±2	43.6±5		
(Mean±SD)	3.4	7.1	10.8	1.7	3.9	2.8	2.1	5.0	0.8	52.2	6.2	1.9	2.0	3.0	7.8	3.7	9.9	7.6	4.6	0.4	6.3	1.0	5.0	0.0		

[a] Pm, Peptostreptococcus magnus; El, Eubacterium lentum; La, Lactobacillus acidophilus; Ao, Actinomyces odontolyticus; Po, Prevotella oralis; Pm, Prevotella melaninogenica; Pi, Prevotella intermadia; Pg, Porphyromonas gingivalis; Fn, Fusobacterium nucleatum; Bw, Bilophila wardsworthia; Vp, Veillonella parvula [b] BA, Bartin; ER-I, Ilic/Erzincan; ER-II, Kemaliye/Erzincan; AR, Camili/Artvin; TE-I, Nusratli/Tekirdag; TE-II, Naip/Tekirdag; ZO-I, Karakavaz/Zonguldak; ZO-II, Zonguldak; ZO-III, Zonguldak; BU-I, Bursa; BU-II, Tahtakopru/Bursa; YA Yalova

DISCUSSION

In the last two decades multi-drug-resistant Gram-positive and Gram-negative bacteria have been emerging rapidly worldwide. The increase in infections caused by these multi-drug- resistant organisms over the past decade poses problems due to the lack of available antimicrobial therapy [9]. Since the anaerobic microflora associated with aggressive oral cavity infections may be resistant to several antibiotics, there is an urgent need for antimicrobial agents which active against these resistant bacteria [20].

Natural products have been used in folk medicine since many years. Among them, propolis has received increased attention due to its antimicrobial activity against a wide range of pathogenic microorganisms including bacteria, fungi, yeasts and viruses [21]. Properties of natural compounds of propolis are known from centuries but they have been only extensively investigated in the last 30-40 years [22]. The chemical composition of propolis as well as its colour and aroma are changed according to the geographical zones. Inhibitory effect of propolis on microorganisms depends on synergism of many compounds [23]. The major bioactive components of propolis are aromatic acids, esters and the flavanoids galangin, quercetin, kaenpferol, acacetin, pinocembrin and pinostobin [24]. It is known that the use of standardized preparations of propolis is safe and less toxic than many other synthetic compounds [21].

Oral cavity infections involve mainly anaerobic bacteria, including *P. gingivalis, Bacteroides forsythus, P. intermedia/ nigrescens, Peptostreptococcus micros, Actinobacillus actinomycetemcomitans, Fusobacterium species, Eubacterium species* and *Campylobacter species* [25]. Santos et al. demonstrated the antibacterial activity of propolis and its fractions against several oral anaerobes, including *A. actinomycetemcomitans, F. nucleatum, P. gingivalis* and *P. intermedia* species frequently associated with destructive periodontitis [26]. The antibacterial activity of propolis and its fractions against several oral anaerobes, including *Peptostreptococcus anaerobius* and *micros, L. acidophilus, Actinomyces neeslundii, P. melaninogenica, P. gingivalis, F. nucleatum* and *V. parvula* species frequently associated with oral cavity infections with MIC ranging from 4 to 512 has been demonstrated [27]. Feres et al.[28] investigated *in vitro* the antimicrobial effect of plant extracts and propolis in chronic periodontitis. They reported that propolis showed significant anti-microbial properties in saliva samples from periodontally healthy and diseased subjects, suggesting that this substance may be used therapeutically in the future to inhibit oral microbial growth [28]. All of the Turkish propolis samples used in this study were highly effective at low concentrations in inhibiting anaerobic bacteria commonly caused oral cavity infections. These results are in similar with the studies testing the antimicrobial activity of various propolis solutions. It can be concluded that Turkish propolis samples have strong antimicrobial effect against anaerobic bacteria causing oral cavity infections without statistical differences. The most susceptible strains to the Turkish propolis were *Lactobacillus acidophilus* and *A. odontolyticus* and the least affected were *F. nucleatum* and *P. intermedia.*

From the literatures, it is well known that propolis samples or some specific compounds in the propolis samples have been shown antibacterial activity of propolis has been attributed to phenolic compounds, especially flavanoids, phenolic acids and their esters [29]. As shown in *Table 2* the falavanones content of the propolis samples are considerably high except for only AR sample. Except for AR propolis samples the other 11 samples were found to be very similiar according to their chemical composition. The AR sample is different from the other 11 samples owing to its low flavanones content and high aliphatic acids and their esters content. Aliphatic acids and their esters ratio in AR sample were found considerably higher than the other 11 propolis samples. Popolis composition varies depending to the region where bees collected the samples. Also some compounds give the synergic effect to the other compound activities in the propolis samples.

P. magnus, E. lentum, L. acidophilus, A. odontolyticus are susceptible to very low propolis content ratios *(Table 3). P. intermedia, P. oralis, P. melaninogenica, P. gingivalis, F. nucleatum, B wardsworthia, V. parvula* are not generally susceptible to very low propolis concentrations, but *V. parvula* is susceptible to TEII propolis sample with a MIC value of 1.1 µg/ml. TEII propolis samples has the most flavanones content compare to the other propolis samples. The effectiveness of TEII propolis sample could be caused from its flavanones content. The AR propolis sample is different from the other 11 samples owing to its low flavanones, high aliphatic acids and their esters content. Total aliphatic acid and their esters percents in AR sample were found considerably higher than the other 11 propolis samples. The effect of AR sample could be resulted from synergic effect of flavanones and aliphatic acids. Popova et al. studied the antibacterial activity of Turkish propolis and their results confirm the importance of phenolics for propolis antibacterial activity. As seen in our results the flavanones compounds that belong to the phenolic group, were observed in high ratios except for only one sample (AR) [30].

In aerobic/anaerobic bacteria, propolis is usually

more active against Gram positive species than against Gram-negative ones. Koru et al. found that MIC values of propolis samples to Gram-positive anaerobic bacteria were lower than MIC values of negative anaerobic bacteria [27]. Similarly, Grange et al. reported that Propolis had antibacterial activity against a range of commonly encountered cocci and Gram-positive rods, including the human tubercle bacillus, but it showed only limited activity against Gram-negative bacilli [31]. In the present study, we found that Gram-positive anaerobic bacteria were most sensitive to EEP samples; with the MIC values ranging from 0.4 to 6.1 mg/ml compared with Gram-negative anaerobic bacteria with MIC ranging from 5.8 to 108.1 mg/ml (P<0.05).

As conclusion, our findings have shown that Turkish propolis samples have higher antibacterial activity against anaerobic bacteria mainly causing oral infections. Because of the high rate of resistance of the anaerobic bacteria isolated from oral cavity infections, standardized preparations of propolis may be used for prevention and treatment of oral cavity diseases. However, further studies are needed to be performed on the clinical applications of propolis in oral cavity infections.

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