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Volatile composition of Anatolian propolis by headspace-solid-phase microextraction (HS-SPME), antimicrobial activity against food contaminants and antioxidant activity

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Propolis is an important natural resource utilized by humans since ancient times. It is a resinous mixture containing polyphenols, mainly flavonoids and phenolic acids as well as volatile compounds. Propolis has been used for its antibacterial, antifungal, antiviral, antineoplastic, antioxidant, immunomodulating and antiinflammatory properties. Ethanol extracts of three different propolis samples collected from North East Anatolia were chemically and biologically evaluated. Volatile compounds of the samples were analysed by headspace-solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). The main volatile constituents were identified as phenyl ethyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%) and cedrol (4.1%) for the Yesilyurt propolis; cedrol (15.6%) for Sarıcicek propolis and α-bisabolol (14.3%), cedrol (7%), δ-cadinene (5.6%) and α-eudesmol (3.6%) for the propolis from Erzincan, respectively. The best antioxidant activity with IC_{50} value of 4.95 µg/ml was defined using the spectroscopic (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay. The highest antimicrobial activity was determined by agar dilution method against the pathogen *Bacillus cereus* (0.06 to 0.12 mg/ml). Propolis could be a salubrious additive for the production and protection of functional foods having a microbial deterioration potential.

Key words: Propolis, headspace-solid-phase microextraction (HS-SPME), antimicrobial activity, antioxidant activity, functional food, volatile compounds.

INTRODUCTION

Foods, whether fresh, prepared or even preserved, are rarely sterile and may be contaminated with spoilage microorganisms or sometimes with pathogens. Among other factors, microorganisms are important spoilage agents in foods (Gram et al., 2002). Microorganisms that are found on the foods may also cause food poisoning

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(also called food intoxication) or food infections. While food poisoning results from ingestion of foods containing preformed microbial toxins, food infections originate from ingestion of foods containing viable pathogens which cause infection and disease in humans. *Staphylococcus aureus* is one of the major causes of intoxication whereas *Salmonella enterica*, pathogenic *Escherichia coli* strains and *Bacillus cereus* are the other major causes of food infections. Various physical and chemical techniques can be used for protection of foods depending upon the type and amount of the foods (Tosi et al., 2007).

Propolis (from the Greek "*pro*", in defence of, and "*polis*", city) is a complex natural substance produced by honeybees (*Apis mellifera* L.) by using various plant sources and utilised by bees to seal holes in their hives and protect the hive against invaders (Sforcin and Bankova, 2011). Analysis of several samples from different geographic regions showed that both chemistry and biological activity of propolis were highly variable (Bankova, 2005b). Propolis possesses a wide range of biological activities, including anti-cancer, antimicrobial, antioxidant and antiinflammatory among others (Luo et al., 2011).

One of the applications of propolis in the food industry is the production of functional foods that are resistant to spoilage and promote health. Functional foods are an emerging field in food science targeted to provide health benefits through the prevention, management and treatment of diseases and also to extend the shelf life of food by reducing the existence of spoilage microorganisms (Milner, 2000).

The chemical composition of propolis has been extensively studied by using different methods, mainly such as gas chromatography-mass spectrometry (GC–MS) and high performance liquid chromatography mass spectrometry (HPLC–MS) (Alencar et al., 2007; Luo et al., 2011; Popova et al., 2005; Righi et al., 2011).

Headspace-solid-phase microextraction (HS-SPME) offers an efficient alternative sampling and extraction technique for the analysis of volatile organic compounds. The adsorption is performed by immersing the pre-coated fibre in a liquid sample or exposing it to the headspace above a liquid or solid sample. In the last two decades, HS-SPME has found applications in environmental, biological, food and pharmaceutical analyses (Cserháti, 2010; Tasdemir et al., 2003). To the best of our knowledge, there is only one previous study on the volatiles of propolis using HS-SPME (Yildirim et al., 2004).

The aim of this study was to evaluate the antioxidant and antimicrobial activities of three Anatolian propolis extracts against important food spoilage and/or pathogenic microorganisms and to characterise their volatile constituents by the combined techniques of HS-SPME and GC-MS.

MATERIALS AND METHODS

Propolis samples

Crude propolis samples were collected from Gumushane, Yesilyurt village (40° 30' N; 39° 28' E) (Propolis Y), Sarıcicek village (39° 38' N; 42° 19' E) (Propolis S) and Erzincan, Camlica village (39° 36' N; 39° 50' E) (propolis E) from North East Anatolia in the spring period of 2011 from *Apis mellifera* hives. All samples had a pleasant odour and were light yellow to dark brown in colour. Samples were stored at +4°C in the dark until processing. Voucher specimens were deposited at the department.

Preparation of ethanol extracts

Eight (8) grams of each propolis sample were coarsely grained and mixed with 40 ml ethanol (96%) in a 100 ml size Erlenmeyer's flask for extraction at room temperature at 150 rpm for 18 h using an orbital shaker. The extract was filtered twice using Whatman No.1 filter paper to filter the solid material. The solvent was removed using a vacuum evaporator (< 40°C).

HS-SPME of the volatile fraction

The manual SPME device (Supelco, Bellafonte, PA, USA) with a fibre pre-coated with a 65 µm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the volatiles. The vial containing the sample was sealed with parafilm. The fibre was pushed through the film layer for exposure to the headspace of the extract for 15 min at 50°C. The fibre was then inserted immediately into the injection port of the GC-MS for the desorption of the adsorbed volatile compounds for analysis.

GC-MS analysis

The GC-MS analysis was carried out using an Agilent 5975 GC-MSD system (SEM Ltd. Istanbul, Turkey). An Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as the carrier gas (0.8 ml/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min.

Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

Identification of components

Identification of the volatile components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to a series of *n*-alkanes (C9-20). Computer matching against commercial libraries (Adams Library, Wiley GC/MS Library, MassFinder 3 Library) and in-house "Baser Library of Essential Oil Constituents" built up of genuine compounds and components of known oils, as well as MS literature data was used for the identification (Tasdemir et al., 2003; Demirci et al., 2007; Polatoglu et al., 2012).

DPPH radical scavenging activity

Free radical scavenging capacity of ethanol extracts was determined using DPPH (Sigma-Aldrich, Steinheim, Germany) (Choi et al., 2006). Reaction mixtures containing 0.5 ml of 0.3 mM DPPH in ethanol and different dilutions of propolis extracts (15.6 to 500 µg/ml) in 1 ml ethanol were shaken and left in a dark place at room temperature for 20 min. The absorbance was measured at 514 nm with disposable plastic half-micro cuvettes using a Ultraviolet and visible absorption spectroscopy (UV-VIS) spectrophotometer (Jenway 6400 Bibby Scientific, UK). Results were expressed as a percentage of radical scavenging activity using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = 100\ [(A_{sample} - A_{blank}) \times 100] / A_{control}]$$

Ethanol was used to adjust the spectrophotometer, ethanol (0.5 ml) plus propolis extract solution (1 ml) was used as the blank and DPPH solution (0.5 ml) plus ethanol (1 ml) was used as the negative control. Experiments were carried out in duplicate and results were reported as IC_{50} (required concentration to scavenge 50% of DPPH radicals). Ascorbic acid and quercetin were used as reference standard compounds (0.4 to 250 µg/ml).

Minimum inhibitory concentration (MIC)

An agar dilution susceptibility assay was performed according to Clinical and Laboratory Standards (CLSI, 2007) for the determination of minimum inhibitory concentration (MIC) of the propolis extracts. Six bacteria and four yeasts were used as test microorganisms: methicillin resistant *S. aureus* (MRSA) (ATCC 43300), vancomycin resistant *Enterococcus faecium* (VREF) (DSM 13590), *B. cereus* (CCM 99), *E. coli* O157:H7 (RSKK 234), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (CCM 5445), *Candida albicans* (DSM 5817 and ATCC 10231), *C. krusei* (ATCC 6258) and *C. tropicalis* (RSKK 665). Bacterial strains were cultured using Mueller Hinton agar (MHA, Becton Dickinson, Le pont-de-Claix, France) at 37°C for 24 h and the yeasts were cultured in Saboraud dextrose agar (SDA, Merck, Darmstadt, Germany) at 30°C for 48 h for activation. Test media were prepared containing different concentrations of each propolis extract ranging from 0.001 to 1.0 mg/ml. The suspension of the test microorganisms were adjusted according to McFarland 0.5. Finally, 1×10^4 cfu of microorganisms were spotted onto agar plate. Plates were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for yeasts.

The same test was carried out using gentamicin sulphate and nystatin (Sigma-Aldrich, Saint Louis, USA) as a positive control for bacteria and yeasts, respectively. Minimum bactericidal concentration (MBC) was determined by re-inoculating the cultures onto propolis free agar plates. MIC and MBC were defined as the lowest concentration that allowed no visible growth on the agar plates. Two replicates were made for each concentration of all propolis extracts.

Statistical analysis

The data obtained from the radical scavenging assay of the different propolis samples were compared by one-way analysis of variance (ANOVA) using statistical package for social sciences (SPSS) software (New York, USA) 10.0. The level of significance for statistical tests was $p \leq 0.05$.

RESULTS

Volatile constituents of the ethanolic extracts of propolis samples

In the present study, propolis S exhibited the best extractive yield of 53.7% followed by propolis Y (51.2%) and propolis E (44.6%). All samples were stored at +4°C in amber bottles until further use. HS-SPME coupled with GC-MS was used for the characterisation of the volatile compounds of propolis extracts. The profile of volatile compounds is shown comparatively in Table 1. A total of 118 volatile components were identified from the ethanol extracts of three propolis samples originated from North Eastern Anatolia and Black Sea Region of Turkey. In particular, the HS-SPME of the ethanol extract of propolis Y contained 54 volatile compounds, accounting for 73.7% of the whole of the volatiles. The principal constituents of this extract were identified as phenyl ethyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%) and cedrol (4.1%), having a pleasant odour.

Seventy four individual volatile compounds were also identified for propolis S. The sole major volatile constituent of this extract was cedrol (15.6%), having a woody and spicy characteristic smell. The third propolis sample, namely propolis E, contained 72 volatile compounds, accounting for 89.2% of the whole of the volatiles. α-Bisabolol (14.3%), cedrol (7%), δ-cadinene (5.6%) and α-eudesmol (3.6%) comprised the major volatile constituents of this extract, resulting in a characteristic odour. According to the chemical class, the volatile constituents of propolis S and E were mainly composed of oxygenated monoterpenes, sesquiterpene hydrocarbons and oxygenated sesquiterpenes accounting for 68.0 and 81.8% of the whole of the volatiles, respectively. Propolis Y included 24.8% of these volatile compounds (Figure 1).

Radical scavenging activity

The free radical scavenging activity of propolis extracts was photometrically evaluated based on the reduction of DPPH in the presence of different extract concentrations. The IC_{50} values calculated by linear regression of plots are presented in Figure 2. The highest radical scavenging activity was obtained with propolis Y, with IC_{50} values of 4.95 µg/ml. This was followed by propolis S with IC_{50} values of 20.78 µg/ml and propolis E with IC_{50} values of 37.09 µg/ml.

The IC_{50} values of ascorbic acid and quercetin were determined as 4 and 2.9 µg/ml, respectively. Antioxidant activity of propolis Y was slightly lower than that of ascorbic acid.

Table 1. Volatile components of three propolis samples as determined by HS-SPME and GC-MS.

RRI	Compound	Y%	S%	E%
1296	Octanal	1.6	-	-
1327	3-Methyl-2-butenol	1.3	-	-
1400	Nonanal	5.0	0.3	-
1450	*trans*-Linalool oxide (*Furanoid*)	2.1	-	-
1452	α,*p*-Dimethylstyrene	-	0.7	-
1463	1-Heptanol	0.4	-	-
1465	Eucarvone	0.2	-	-
1466	α-Cubebene	-	0.3	0.2
1478	*cis*-Linalool oxide (Furanoid)	0.3	-	-
1496	2-Ethyl hexanol	0.8	-	-
1497	α-Copaene	-	0.3	0.7
1499	α-Campholene aldehyde	0.4	-	-
1506	Decanal	6.7	0.6	-
1519	1,7-Di-epi-α-Cedrene (=α-Funebrene)	0.2	-	-
1532	Camphor	1.0	-	0.4
1535	β-Bourbonene	-	1.2	-
1541	Benzaldehyde	2.0	-	-
1544	Ethyl nonanoate	0.4	0.2	-
1545	*cis*-α-Bergamotene	-	-	0.5
1553	Linalool	tr	0.4	0.5
1562	Octanol	1.1	-	-
1577	α-Cedrene	0.5	0.7	0.5
1586	Pinocarvone	0.6	-	-
1587	β-Funebrene	-	0.8	0.7
1591	Bornyl acetate	0.9	1.0	0.5
1594	*trans*-β-Bergamotene	-	-	1.2
1598	Camphene hydrate	-	0.2	-
1601	Nopinone	0.3	-	-
1612	β-Caryophyllene	-	0.8	1.2
1613	β-Cedrene	1.3	1.0	0.5
1625	4,4-Dimethyl but-2-enolide	-	-	0.5
1644	Widdrene (=Thujopsene)	-	0.6	0.5
1645	*cis*-Verbenyl acetate	-	0.3	-
1647	Ethyl decanoate	-	0.7	0.6
1648	Myrtenal	1.2	-	-
1654	1-Hexadecene	0.4	0.6	0.5
1661	*trans*-Pinocarvyl acetate	-	0.2	-
1661	Alloaromadendrene	-	-	0.6
1663	*cis*-Verbenol	-	-	0.3
1664	Nonanol	2.0	-	-
1670	*trans*-Pinocarveol	2.0	3.6	1.1
1671	Acetophenone	0.2	-	-
1677	epi-Zonarene	-	0.7	1.1
1683	*trans*-Verbenol	0.6	3.2	2.8
1685	Ethyl benzoate	6.5	0.1	0.2
1687	α-Humulene	-	0.4	1.3
1693	β-Acoradiene	-	0.2	0.4
1695	(E)-β-Farnesene	-	-	0.7

Table 1. cont'd

1704	γ-Muurolene	0.2	1.2	2.4
1706	α-Terpineol	0.1	2.2	0.7
1719	Borneol	0.3	1.4	0.4
1722	Dodecanal	0.9	-	-
1729	Zonarene	-	-	1.0
1725	Verbenone	3.5	1.8	-
1740	α-Muurolene	0.3	2.4	2.0
1744	α-Selinene	-	-	0.5
1747	Benzyl acetate	0.7	-	0.3
1747	*trans*-Carvyl acetate	-	0.2	-
1751	Carvone	-	0.4	-
1755	β-Curcumene	-	-	1.2
1758	*cis*-Piperitol	-	0.2	-
1773	δ-Cadinene	0.2	2.7	5.6
1776	γ-Cadinene	0.3	2.3	2.9
1783	β-Sesquiphellandrene	-	-	0.4
1786	*ar*-Curcumene	-	0.9	2.1
1799	Cadina-1,4-diene (=*Cubenene*)	-	-	0.5
1804	Myrtenol	0.5	2.4	0.6
1807	α-Cadinene	-	0.2	0.5
1838	2-Phenylethyl acetate	0.5	0.3	0.3
1845	*trans*-Carveol	0.5	2.4	1.0
1849	Cuparene	0.4	0.5	-
1849	Calamenene	0.7	2.1	2.8
1864	*p*-Cymen-8-ol	0.9	1.7	0.3
1882	*cis*-Carveol	-	0.3	-
1896	Benzyl alcohol	7.4	0.4	0.9
1898	1,11-Oxidocalamenene	-	0.7	-
1900	Nonadecane	-	0.2	0.5
1904	Ethyl-3-phenyl propionate	0.9	0.2	0.4
1912	*p*-Cymen-9-ol	-	0.2	-
1918	β-Calacorene	-	tr	tr
1937	Phenyl ethyl alcohol	7.7	1.1	1.2
1941	α-Calacorene	-	0.8	0.6
1949	Piperitenone	-	0.2	
2008	Caryophyllene oxide		0.4	0.8
2030	Methyl eugenol	-	0.4	
2045	Humulene epoxide-I	-	0.2	tr
2050	(*E*)-Nerolidol	-	0.2	1.3
2056	Ethyl tetradecanoate	-	0.1	-
2071	Humulene epoxide-II	-	0.3	0.8
2073	Cinnamaldehyde	0.9	-	-
2080	Junenol [=Eudesm-4(15)-en-6-ol]	-	0.5	-
2080	1,10-di-*epi*-Cubenol	-	0.2	0.4
2081	Humulene epoxide-III	-	0.2	-
2084	Octanoic acid	-	-	0.3
2088	1-*epi*-Cubenol	-	-	0.4
2092	β-Oplopenone	-	0.9	-
2109	cis-p-Menthan-1,8-diol	-	-	0.2

Table 1. cont'd

2143	Cedrol	4.1	15.6	7.0
2156	α-Bisabolol oxide B	-	-	0.8
2161	Bisabolol oxide	-	-	0.9
2185	γ-Eudesmol	-	0.8	2.1
2187	T-Cadinol	-	0.5	1.0
2192	Nonanoic acid	0.4	0.2	0.4
2204	Eremoligenol	-	0.6	1.6
2200	α-Guaiol	-	-	1.0
2209	T-Muurolol	-	0.2	0.5
2218	4-Vinyl guaiacol	-	-	0.8
2232	α-Bisabolol	-	-	14.3
2250	α-Eudesmol	0.3	1.5	3.6
2255	α-Cadinol	-	-	1.1
2257	β-Eudesmol	1.0	2.0	3.0
2269	Guaia-3,9-dien-11-ol	-	0.5	-
2270	Guaia-6,10(14)-dien-4β-ol		0.3	-
2298	Decanoic acid	0.2	-	-
2308	Cinnamyl alcohol	0.2	-	-
2376	Manoyl oxide	-	0.5	-
2600	Vaniline	0.6	-	-
2655	Benzyl benzoate	-	-	0.3
	Total	73.7	74.6	89.2

Y; Yesilyurt, S; Sarıcicek, E; Erzincan. RRI; Relative retention indices calculated against *n*-alkanes % calculated from flame ionization detector data. tr; Trace (< 0.1%).

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the tested propolis extracts.

Microorganisms - source	MIC-MBC (mg/ml)						MIC-MBC (µg/ml)	
	Y		S		E		ST[a]	
S. aureus (MRSA) ATCC 43300	0.25	0.25	0.25	0.5	0.25	1	0.31	0.31
E. faecium (VREF) DSM 13590	1	>1	0.5	>1	0.5	>1	>20	NA
B. cereus CCM99	0.06	0.12	0.06	0.06	0.06	0.06	0.62	0.62
E. coli O157:H7 RSKK234	>1	NA	>1	NA	>1	NA	0.62	0.62
P. aeruginosa ATCC 27853	>1	NA	>1	NA	>1	NA	1.25	1.25
S. Typhimurium CCM5445	1	>1	1	>1	>1	NA	>20	NA
C. albicans DSM 5817	0.25	0.5	0.25	1	>1	NA	0.31	0.31
C. albicans ATCC 10231	0.5	1	0.5	0.5	1	>1	0.31	0.31
C. krusei ATCC 6258	0.5	0.5	1	1	>1	NA	0.31	0.31
C. tropicalis RSSK 665	0.5	1	1	>1	>1	NA	0.31	0.31

[a]ST; Standarts (gentamicin sulphate was used for bacteria and nystatin for yeasts). Y; Yesilyurt, S; Sarıcicek, E; Erzincan, NA; not applicable.

Antimicrobial activity

As shown in Table 2, the highest MIC and MBC values were against endospore forming species, as *B. cereus* (0.06 to 0.12 mg/ml). An MIC value of 0.25 mg/ml, indicative of a considerable antimicrobial activity, was obtained against Methicillin-resistant *Staphylococcus aureus* (MRSA) for propolis Y, S and E. Among the samples, propolis Y exhibited the most efficient antimicrobial profile towards the tested microorganisms, followed by propolis S and E. Generally, ethanol extracts of propolis were determined as more active against Gram

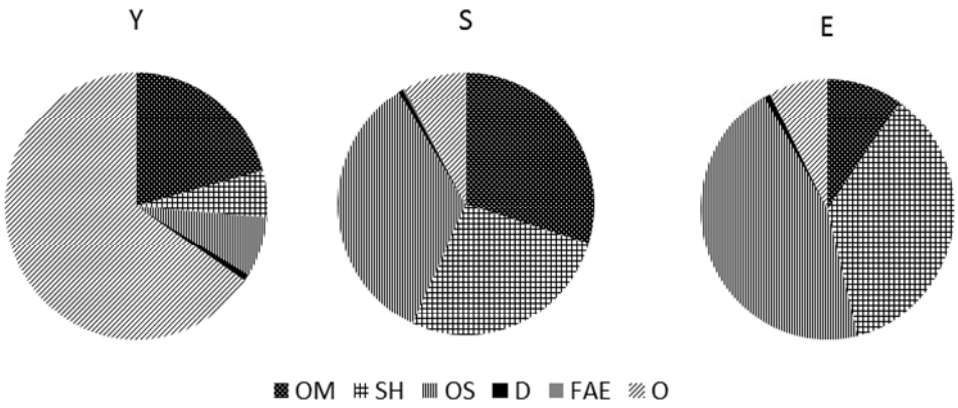

Figure 1. Distribution of the volatile components of propolis samples in terms of chemical class. Yesilyurt; Y, Sarıcicek; S, Erzincan; E, oxygenated monoterpenes; OM, sesquiterpene hydrocarbons; SH, oxygenated sesquiterpenes; OS, diterpenes; D, fatty acids + esters; FAE, others; O.

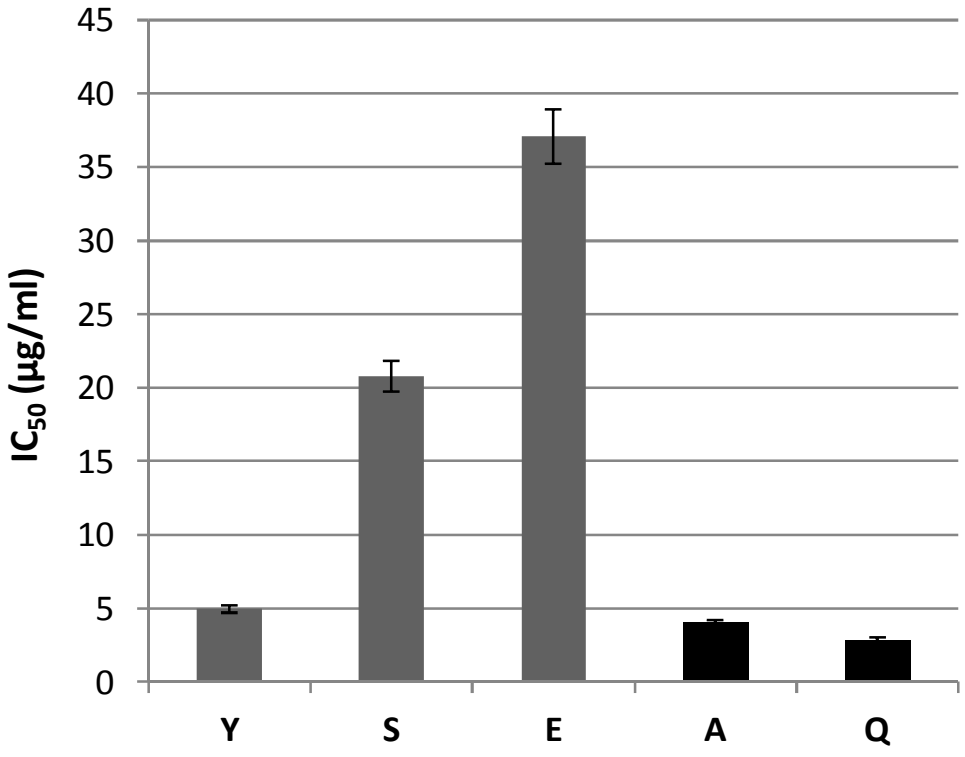

Figure 2. DPPH radical scavenging activity of propolis extracts (Y; Yesilyurt, S; Sarıcicek, E; Erzincan) and reference compounds (A; Ascorbic acid and Q; Quercetin). Values were obtained from regression lines with 95 % confidence level ($p \leq 0.05$).

positive bacteria. Propolis Y has shown an inhibitory and bactericide activity against all yeast strains with concentration range of 0.25 to 1 mg/ml. Propolis S, a similar inhibitory effect against yeasts whereas propolis E

has only shown inhibitory effect against *C. albicans* ATCC 10231.

DISCUSSION

In recent years, the diverse bioactivity and efficiency of propolis has encouraged research and further analysis of propolis to determine potential new applications. The chemical composition of propolis samples depends on the local plant flora, and geographic and climatic characteristics of the area where the sample was collected. Different propolis samples could be completely different in their chemistry and biological activity (Bankova, 2005b). In the current study, this chemical variation can also account for the variety of volatile compounds in the three evaluated samples. Propolis samples have different plant origin due to different geographical position.

Propolis Y was collected from the Eastern Black Sea Region and possess highly diverse flora consisting spruce, beech, fir, mountain alder, chestnut, hornbeam, oak, silver birch, ash, trembling poplar and willow. Main constituents of propolis Y were detected as phenyl ethyl alcohol (7.7%), benzyl alcohol (7.4%), decanal (6.7%), ethyl benzoate (6.5%), nonanal (5%) and cedrol (4.1%). Chemical constituent, antimicrobial and antioxidant activity of propolis Y was significantly different from propolis S and E.

Plant origin of propolis S is composed mainly of pine coniferous tree such as spruce and yellow pine. The major volatile constituent of its extract was cedrol. In accordance with this, cedrol is a sesquiterpene alcohol found generally in cedar wood and other conifer essential oils. Cedrol was also reported by Melliou et al. (2007) as a major component (4.3 to 6.3%) of Greek propolis samples. Cedrol (6.3%), δ-cadinene (8.4%) and α-eudesmol (12.1%) were also reported previously as major components of Greek propolis (Preveza, Arta and Agrinio, respectively) (Melliou et al., 2007).

Propolis E was collected from Eastern Anatolia Region that has cypress, olive tree, oleander and eucalyptus in its flora. The major volatile constituents of propolis E was α-bisabolol (14.3%), also known as levomenol, is a monocyclic sesquiterpenoid alcohol found in German chamomile and *Myoporum crassifolium.* Other components of this extract comprised cedrol (7%), δ-cadinene (5.6%) and α-eudesmol (3.6%) relative similarity with Greek propolis (Melliou et al., 2007).

Different propolis samples have been used and analysed to determine their constituents. The most often reported techniques for chemical analyses of propolis samples are GC-MS and HPLC (Bankova, 2005b; Sforcin and Bankova, 2011). A literature survey showed that the GC and GC-MS methods were generally used for the analysis of non-volatile compounds by derivatisation of propolis samples from various origins (Alcenar et al., 2007; Bankova et al., 2002; Popova et al., 2005; Sorkun et al., 2001; Uzel et al., 2005). There are inconsistencies and discrepancies in the reported analytical results, which are obtained after derivatisation, where many reported components are not possible to trace. In this present study, we could not trace any of the previous reported derivatised products using the same techniques.

HS-SPME-GCMS has been utilised efficiently in the analysis of volatile compounds from various matrixes in the past (Cserháti, 2010; Michel and Buszewski, 2008). The technique is fast, handy and efficient to perform a realistic analysis of the volatiles of the test samples in a non-destructive manner. Consequently, in the current study, HS-SPME-GC-MS was implemented successfully for the analysis of volatiles of Anatolian propolis samples. There is one previous study on the characterisation of propolis volatiles using HS-SPME-GC-MS. α-Terpinene (21.8%), α-terpineol (12.3%), junipene (9.1%), cinnamyl alcohol (8.7%) and β-caryophyllene (8.1%) have been reported as main constituents of propolis from Malatya (South Eastern Anatolia). This study focused on the effect of propolis extract on tuberculosis infection in guinea-pigs more than chemical constituents of the sample (Yildirim et al., 2004).

The results indicate that propolis samples exhibited strong free radical scavenging activity. In a previous study, Sawaya et al. (2009) evaluated the antioxidant activity of three different *Scaptotrigona* propolis samples. Their findings showed that the antioxidant activities of the samples ranged between 43 and 1000 µg/ml. The authors mentioned that *Scaptotrigona* propolis present a much lower antioxidant activity than samples of *Apis mellifera* propolis. The antioxidant activity of propolis from Argentina was reported with IC_{50} values of 25 to 37.5 µg/ml (Isla et al., 2009). These results are in accordance with our experimental data. The propolis from Korea also had strong DPPH free radical-scavenging activity (Choi et al., 2006). Propolis contains a wide variety of phenolic compounds, mainly phenolic acids and flavonoids. Indeed, flavonoids and various phenolic compounds have already been studied as antioxidants and demonstrated to be very active (Isla et al., 2009; Righi et al., 2011). A correlation between the antioxidant activity and total polyphenol and flavonoid content of propolis was also reported (Choi et al., 2006).

Microorganisms are the major deteriorating agents of food and also causative agents of severe food-borne illness (Tosi et al., 2007). In the field of functional food, some additives such as flavonoids can be used against food-borne pathogens and also increase the potential positive effect of the food on health (Luo et al., 2011). Therefore, the inhibitory potential of propolis against a set of pathogenic microorganisms was evaluated. The

representative major food spoilage and/or pathogen microorganism of three gram positive, three gram negative bacteria and four yeast species were used (Table 2). Among the test organisms, two of them were selected from antibiotic resistant species including MRSA and VREF.

It is well-known that *B. cereus* is adapted for growth in the intestinal tract of insects and mammals. From these habitats, it is easily spread to foods, where it may cause food-associated illness by the production of diarrhoeal or emetic enterotoxins (Stenfors, et al., 2008). In addition, endospores of the *Bacillus* sp. have extreme resistance to heat and other preservation treatments (Caspers et al., 2011). The most important bacterial pathogen of the honey bee is endospore forming *Paenibacillus larvae*, which causes severe brood diseases. Therefore, bees need to be protected primarily against the invasion of this pathogen. Probably due to the strategy developed by bees against endospores of *P. larvae*, propolis can be effective in a similar way on *Bacillus* endospores. In a recent study, Mihai et al. (2012) reported the significant inhibition of *P. larvae* treated with ethanol extracts from different propolis *in vitro* using solid agar diffusion technique. In the current study, we observed significant antimicrobial effect of the ethanol extract of propolis against endospore forming *B. cereus*. The lower efficiency of the propolis extracts against gram negative bacteria may be dependent on the membrane structure of gram negative bacteria (Probst et al., 2011). Reports showed that propolis has previously shown no or limited activity against gram negative bacteria (Popova et al., 2005; Righi et al., 2011).

The MIC values of the ethanol extract of Brazilian propolis were reported as 1.54 ± 0.62 mg/ml for *S. aureus* and 19.24 ± 2.2 for *E. coli* (Probst et al., 2011). A previous study reported that the ethanol extract of red propolis had antibacterial activity against *S. aureus* ATCC 25923 with MIC ranging from 100 to 200 µg/ml (Alencar et al., 2007). This result is close to our findings for *S. aureus* (MIC 0.25 mg/ml). Determination of antimicrobial activity by disc diffusion in mm or using extracts solution (%) versus µg/ml or mg/ml values complicated the comparability of results (Choi et al., 2006; Mihai et al., 2012; Popova et al., 2005). Moderate antimicrobial activity of the propolis, especially against gram positive bacteria can be seen in several reports.

The antimicrobial efficiency of propolis is not a surprising phenomenon considering that this natural compound is produced by bees to defend against bacterial and fungal infections (Bankova, 2005a). The antimicrobial efficiency of propolis samples was attributed to their high content of flavones and flavanones (Choi et al., 2006; Righi et al., 2011). Propolis, a defence and protection shield produced by bees against their pathogen *P. larvae* and other invaders, can be a powerful antimicrobial candidate against *B. cereus* strains, which are highly problematic foodborne pathogens and/or spoilage microorganisms. Considering additionally the significant antioxidant activity of propolis, this natural product could be a useful additive for the production of functional foods, especially where foods have the potential for *B. cereus* contamination.

REFERENCES

Alencar SM, Oldoni TL, Castro ML, Cabral IS, Costa-Neto CM, Cury JA, Rosalen PL, Ikegaki M (2007). Chemical composition and biological activity of a new type of Brazilian propolis: Red propolis. J. Ethnopharm. 113:278-283.

Bankova V (2005a). Chemical diversity of propolis and the problem of standardization. J. Ethnopharm. 100:114-117.

Bankova V (2005b). Recent trends and important developments in propolis research. J. Evid. Based Complementary Altern. Med. 2:29-32.

Bankova V, Popova M, Bogdanov S, Sabatini A-G (2002). Chemical Composition of European Propolis: Expected and Unexpected Results. Z. Naturforsch C. 57:530-533.

Caspers MPM, Schuren FHJ, van Zuijlen ACM, Brul S, Montijn RC, Abee T, Kort,R (2011). A mixed-species microarray for identification of food spoilage bacilli. Food Microbiol. 28:245-251.

Choi YM, Noh DO, Cho SY, Suh HJ, Kim KM, Kim JM (2006). Antioxidant and antimicrobial activities of propolis from several regions of Korea. Food Sci. Technol.-LEB. 39:756-761.

CLSI (Clinical and Laboratory Standards) (2007). Performance standards for antimicrobial susceptibility testing. 17th Informational Supplement M100-S17, 27:1.

Cserháti T (2010). Chromatography of Aroma Compounds and Fragrances, Springer Verlag, Heildelberg. P. 392.

Demirci B, Kosar M, Demirci F, Dinc M, Baser KHC (2007). Antimicrobial and antioxidant activities of the essential oil of *Chaerophyllum libanoticum* Boiss. et Kotschy. Food Chem. 105:1512-1517.

Gram L, Ravn L, Rasch M, Bruhn JB, Christensen A B, Givskov M (2002). Food spoilage-interactions between food spoilage bacteria. Int. J. Food Microbiol. 78:79-97.

Isla MI, Zampini IC, Ordonez RM, Cuello S, Juarez BC, Sayago JE, Moreno MIN, Alberto M R, Vera NR, Bedascarrasbure E, Alvarez A, Cioccini F, Maldonado LM (2009). Effect of Seasonal Variations and Collection Form on Antioxidant Activity of Propolis from San Juan, Argentina. J. Med. Food. 12:1334-1342.

Luo C, Zou X, Li Y, Sun C, Jiang Y, Wu Z (2011). Determination of flavonoids in propolis-rich functional foods by reversed phase high performance liquid chromatography with diode array detection. Food Chem. 127:314-320.

Melliou E, Stratis E, Chinou I (2007). Volatile constituents of propolis from various regions of Greece - Antimicrobial activity. Food Chem. 103:375-380.

Michel M, Buszewski B (2008). Isolation, Determination and Sorption Modelling of Xenobiotics in Plant Materials. Pol. J. Environ. Stud. 17:305-319.

Mihai CM, Marghitas LA, Dezmirean DS, Chirila F, Moritz RFA, Schluns H (2012). Interactions among flavonoids of propolis affect antibacterial activity against the honeybee pathogen *Paenibacillus larvae*. J. Invertebr. Pathol. 110:68-72.

Milner JA (2000). Functional foods: the US perspective1–3. Am. J. Clin. Nutr. 71:1654S-9S.

Polatoglu K, Demirci B, Demirci F, Gören N, Başer KHC (2012). Biological activity and essential oil composition of two new *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz Bip. var. chiliophyllum chemotypes from Turkey. Ind. Crop. Prod. 39:97-105.

Popova M, Silici S, Kaftanoglu O, Bankova V (2005). Antibacterial

activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine 12:221-228.
Probst IS, Sforcin JM, Rall VLM, Fernandes AAH, Fernandes Júnior A (2011). Antimicrobial activity of propolis and essential oils and synergism between these natural products. J. Venom. Anim. Toxins 17:159-167.
Righi AA, Alves TR, Negri G, Marques LM, Breyer H, Salatino A (2011). Brazilian red propolis: unreported substances, antioxidant and antimicrobial activities. J. Sci. Food Agr. 91:2363-2370.
Sawaya ACHF, Calado JCP, dos Santos LC, Marcucci MC, Akatsu IP, Soares AEE, Abdelnur PV, Cunha IBS, Eberlin MN (2009). Composition and antioxidant activity of propolis from three species of *Scaptotrigona* stingless bees. JAAS. 1(2):37-42.
Sforcin JM, Bankova V (2011). Propolis: Is there a potential for thedevelopment of new drugs? J. Ethnopharm. 133:253-260.
Sorkun K, Suer B, Salih B (2001). Determination of chemical composition of Turkish propolis. Z. Naturforsch C. 56:666-668.
Stenfors ALP, Fagerlund A, Granum PE (2008). From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Rev. 32:579-606.
Tasdemir D, Demirci B, Demirci F, Dönmez AA, Baser KHC, Ruedi P (2003). Analysis of the volatile components of five Turkish *Rhododendron* species by headspace solid-phase microextraction and GC-MS (HS-SPME-GC-MS). Z. Naturforsch C. 58:797-803.
Tosi E A, Re E, Ortega ME, Cazzoli A F (2007). Food preservative based on propolis: Bacteriostatic activity of propolis polyphenols and flavonoids upon *Escherichia coli*. Food Chem. 104:1025-1029.
Uzel A, Sorkun K, Oncag O, Cogulu D, Gencay O, Salih B (2005). Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol. Res. 160:189-195.
Yildirim Z, Hacievliyagil S, Kutlu NO, Aydin NE, Kurkcuoglu M, Iraz M, Durmaz R (2004). Effect of water extract of Turkish propolis on tuberculosis infection in guinea-pigs. Pharmacol. Res. 49:287-292.