European Journal of Biotechnology and Bioscience
ISSN: 2321-9122
Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 5; Issue 5; September 2017; Page No. 20-24

Comparison of the antimicrobial and antioxidant activity of four different regions of Anatolia

***[1] Dilek Keskin, [2] Nur Ceyhan-Guvensen, [3] Murat Uygun, [4] Deniz Aktaş Uygun**
[1] Çine Vocational High School, Adnan Menderes University, Aydın, Turkey
[2] Biology Department, Faculty of Sciences and Arts, Mugla University, Mugla, Turkey
[3, 4] Biochemistry Department, Faculty of Sciences and Arts, Adnan Menderes University, Aydın, Turkey

Abstract
Propolis is a natural product widely consumed in folk medicine. The present study was carried out to investigate the antimicrobial activity of four different (Zonguldag, Cine, Cankırı and Ardahan) propolis regions of Anatolia. Antimicrobial activities of the propolis samples were investigated by the disc diffusion method. Although Ardahans' propolis is the most effective (14/18), Cankırıs' propolis is the least effective (4/18). The antioxidant activities of the extracts were tested by ferric thiocyanate (FTC) and 2,2-diphenyl-1-picrylrazy (DPPH) free radical scavenging methods. Zonguldag extract exhibited the greatest antioxidant activity with FTC method, whereas Cankiri extract exhibited the lowest IC_{50} value 5.69±0.49 (µg/mL) forDPPH radical scavenging activity. Total phenolic contents of the extracts were estimated by Folin-Ciocaltalteu method and Zonguldag extract was found to contain the highest amount (113.63±5.45 mgGAE/g) extract of phenolics. Ardahan extract contained highest both flavonoid content (20.07±1.59 RtE/g) and reducing power (52.60±0.45). Strong antioxidant, radical-scavenging and antimicrobial activities of Ardahan seemed to relate with high flavonoid contents and total polyphenol.

Keywords: propolis, antimicrobial activity, antioxidant activity, DPPH

Introduction
Resistance to antimicrobial agents has become an increasingly important and pressing global problem [13] Because of this, scientific studies have been carried out to develop new compounds to be beyond conventional antibiotic theraphy. Some natural products have antimicrobial activity, especially propolis and honey. Also propolis has anticancer [27, 33], antioxidative [5] and antitumoral properties [23]. Chemical composition and pharmacological activity might vary widely from region to region [21, 29, 41]. And, it depends on climate, vegetation, season and solvent used for extraction purposes [12, 19]. More than three hundred constituents were identified in different propolis samples by Bankova and colleagues [4]. Flavonoids, aromatic acids, diterpenic acids and phenolic compounds appear to be the principal components responsible for the biological activities of propolis samples. The antioxidant, antibacterial and antifungal properties of propolis make it beneficial in food technology.The substances, which are identified in propolis, generally are typical constituents of food and/or food additives, and are recognized as Gras (Generally Recognized As Safe) [10] substances. As synthetic preservatives are unaccepted, there is a growing interest of introducing natural additives to food, and propolis is an interesting alternative to be considered in new applications of food technology.
The aim of this study is to carry out a comparative analysis of the antimicrobial activity of extracts obtained from four Anatolian propolis samples against seven gram positive, nine gram negative bacteria strains and two yeasts: microorganisms and comparison between antioxidant other bio-chemical properties.

Material and Method
Method
Extraction of propolis samples
The geographical origins and some other properties of standart ethanol extracts of four different Anatolian propolis samples representing the whole country are listed in Table 1. A Hundred grams of frozen propolis is grained and dissolved in 300 ml ethanol (96%). This mixture was kept in the incubator at 30°C for 2 weeks in a bottle closed tightly. After incubation procedure, supernatant was filtered twice with Whatman No. 4 and 1 filter paper. The final filtered concentrated solution was diluted in 1:10 ratio (v/v) with ethanol (96%). A portion of this final solution called ethanol extracts of propolis was evaporated to dryness. Stock solutions of all propolis extracts were prepared in 10% dimethylsulphoxide (DMSO) [58]. The properties of samples are given in table 1.

Table 1: Geographical origins and other properties of four different Anatolian propolis samples.

Sample location	Symbol	Region	Collection Year
Zonguldag	Z	Black Sea	2009
Cine	Ç	Aegean	2009
Cankırı	Ça	Anatolia	2009
Ardahan	A	Eastren Anatolia	2009

Microorganisms, media and growth conditions
In this study, the following microorganisms were used; seven Gram-positive bacteria strains; *Staphylococcus aureus* ATCC 6538P, *S.aureus* ATCC43300 methicillin-oxacillin resistant, *S.aureus* MU 40 methicillin-oxacillin resistant, *Micrococcus luteus* NRRL B-4375, *Streptococcus faecalis* ATCC 4083,

Bacillus subtilis ATCC 6633, *Bacillus cereus* CCM 99, nine Gram-negative bacteria strains; *Escherichia coli* ATCC 29998, *E.coli* ATCC 35218, *Enterobacter aerogenes* ATCC 13048, *Enterobacter cloacae* ATCC 13047, *Salmonella typhimurium* CCM 3819, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* ATCC12843, two specific pathogenic strains (hemorrhagic *E.coli* O157:H7 RSSK 04054, and *Klebsiella pneumoniae* CCM 2318).Yeasts; *Candida albicans* ATCC 10259 and *Candida tropicalis* ATCC 750.

Bacteria were cultured on Trypticasein Soya Agar (TSA), and yeasts on Yeast Extract Agar (YEA). All stock strains were kept at 4°C.

Antimicrobial activities of the propolis extracts

Antimicrobial activities of the propolis samples were investigated by the disc diffusion method [38]. Thirty-microlitre propolis extract were injected into sterilized discs of 6mm in diameter (Schleicher & Shüll no. 2668, Germany). In addition, ethanol (E) and dimethylsulphoxide (DMSO) were used as controllers to determine the sensitivity of the tested strains to solvents. Propolis extracts were suspended into 0.1% bacteriological agar (Merck) in different concentrations and used for the disc diffusion test. 0.1% agar was used as negative control. The test bacteria were incubated at 35 °C for 24 h in nutrient broth (Merck) and the yeasts were incubated in Sabouraud dextrose broth (Merck) for 24 h. One hundred microlitre of suspension containing 10^6cfu/ml of bacteria and 10^5 cfu/ml of yeast were placed into sterile petri dishes (90 mm). Mueller Hinton agar (MHA) (Merck) and Sabouraud dextrose agar (SDA) sterilized in a flask and cooled to 45-50°C were distributed to petri dishes containing bacteria and yeasts respectively. The extracts containing discs were located on the solid agar medium by pressing slightly. The plates were kept at 4°C for 2 h and then the bacterial plates were incubated at 35° C for 24 h while the yeast plates were incubated at 25°C for 72 h. After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times.

Total antioxidant activity determination

Total antioxidant activity was measured by ferric thiocyante (FTC) method described by Saha *et al*., (2004) [44].

DPPH radical scavenginh assay

Radical scavenging activity of propolis extracts was measured by Brand-Williams *et al.*, (1995) [9].

Determination of total phenolic content

Total phenolic contents (TPC) of the extracts were assayed according to Folin-Ciocalteu method) [53].

Determination of flavonoid content

The flavonoid content was measured using the method of Quettier-Deleu *et al*. (2000) [43].

Reducing power of the extracts

Reducing power of the propolis extracts was measured according to the method of Oyaizu (1986) [40].

Results and Discussion

There has been only limited research on antibacterial activity of Turkish propolis [24, 26, 35, 42, 50, 51, 55, 57, 59]. All four Anatolian propolis samples evaluated in this study showed antimicrobial activity against the test microrganisms in Table 2. Among the propolis samples, Zonguldag, Çine, Çankırı and Ardahan have antimicrobial activity with the range of 10-25 mm, 12-27 mm, 12-33 mm and 13-28 mm respectively. When it comes to ratio of microorganisms, 14 from Ardahan, 10 from Zonguldag, 9 from Cine and 4 from Çankırı out of 18 microorganisms. Although Ardahans' propolis is the most effective, Çankırıs' propolis is the least effective. The reason for this is that Ardahan has a natural habitat, a rural area and is distant from industrial center, but Cankiri is near Ankara which has air pollution problem and is center of Turkey. There are a lot of main roads in Cankiri. Many studies have shown that fatty acid esters, phenolic compounds and cinnamic acid were the main propolis constituents and some of them were shown to possess antibacterial activity [20, 30]. Many researchers [1, 6, 31]. have reported that a special substance class or a unique substance is not responsible for the antibacterial activity of propolis instead of these different compounds with different combinations is required for the biological activity of propolis, and that a component of propolis extract cannot have a strong activity as the total propolis extract [50]. However *Bacillus subtilis* ATCC 6633 and *Bacillus cereus* CCM 99 showed antimicrobial activity all propolis samples, none of the propolis samples didn't inhibite *Pseudomonas aeruginosa* ATCC 27853. Many researchers reported that propolis samples active only against Gram positive bacteria and some fungi [31, 32, 39], others found also weak activity against Gram-negative bacteria [16, 49]. It has been suggested that the resistance of Gram-negative bacteria could be due to the presence of efflux pumps preventing intracellular entry of propolis constituents [18]. The weak effect on Gram-negative bacteria may also be explained by the fact that propolis contains mainly plant-derived resin constituents and that resins are sectered by plants to protect mostly from Gram-positive pathogens [15, 18]. In the present study, we could verify that Gram positive and Gram negative bacteria showed inhibition zone 10-33 mm and 11-27 mm respectively.

Table 2: Antimicrobial activity of four different Anatolian propolis samples.

Inhibition zone (mm)* (30µl/disc)							
Microorganisms	**G**	**Samples**					
		Z	**Ç**	**Ça**	**A**	**E**	**DMSO**
S.aureus ATCC 6538P	+	10	-	-	18	-	-
S.aureus ATCC43300 met-oxa res	+	19	-	-	17	-	-
S.aureus MU 40 met-oxa res	+	13	-	-	13	-	-

Micrococcus luteus NRRL B-4375	+	19	-	30	28	-	-
Streptococcus faecalis ATCC 8043	+	-	15	-	-	12	12
B.subtilis ATCC 6633	+	25	20	22	24	12	13
B.cereus CCM 99	+	19	16	33	19	9	13
E.coli ATCC 29998	-	-	-	-	19	10	13
E.coli ATCC35218	-	-	-	12	18	11	-
E.coli O157:H7RSSK 04054	-	-	-	-	21	12	12
*E.aerogenes*ATCC13048	-	-	12	-	-	-	-
E.cloacae ATCC 13047	-	-	13	-	-	10	-
S.typhimurium CCM 3819	-	11	-	-	13	10	11
Klebsiella pneumoniae CCM 2318	-	-	27	-	15	9	-
P.aeruginosa ATCC 27853	-	-	-	-	-	-	-
*P.fluorescens*ATCC 12843	-	13	13	-	15	12	-
C.albicans ATCC 10259	Y	15	18	-	20	10	11
C.tropicalis ATCC 750	Y	12	20	-	24	9	11

Z: Zonguldag Ç:Çine, Ça Çankırı A:Ardahan E:Ethanol; DMSO: Dimethylsulphoxide
*****These results are the means of three experiments. (-): No inhibition G, gram reaction, Y, yeast.

Except from Cankiri samples of propolis, all propolis samples showed anticandidal activity against to the tested *Candida* strains, which corresponds to the literature data [31,45,58]. Consequently, anti-candidal screening indicated clearly that Ardahan propolis samples of propolis had much more effective anticandidal activity when compared with other Anatolian propolis like other bacteria.

It is not always straightforward to compare results obtained from different investigations on the antimicrobial effect of propolis due to the variety of methods used for bioautography, agar dilution, agar diffusion, broth dilution [48]. Most investigations on the antimicrobial activity of propolis carried out so far have used Petri dish methods (i.e. well or disc diffusion and agar dilution [7, 8, 17, 42, 47, 52]. But some reseachers used broth macrodilution method [22, 36, 46, 58] and broth microdilution method [28, 45, 54]. Mayrhofer and colleagues have reported that etest and agar dics diffusion method represents valid methods compared to the broth microdilution method. Therefore, we preferred disc diffusion method [34].

Table 3: TAA, IC_{50} values, total phenolics, total flavonoids and reducing power of propolis samples.

Sample	TAA (% Inhibition)	IC_{50} (µg/mL)	Total Phenolics (mg GAE/g extract)	Total Flavonoids (mg RtE/g extract)	Reducing Power (% Ascorbic Acid)
Zonguldag	93,18 ± 0,52	8,66 ± 1,92	113,63 ± 5,45	18,51 ± 1,66	39,89 ± 1,27
Cine	90,73 ± 0,53	8,67 ± 0,16	80,71 ± 7,22	18,26 ± 0,91	37,05 ± 1,01
Cankiri	92,25 ± 0,56	5,69 ± 0,49	55,71 ± 0,72	8,83 ± 0,61	21,99 ± 1,20
Ardahan	92,06 ± 0,99	9,19 ± 0,23	107,38 ± 9,76	20,07 ± 1,59	52,60 ± 0,45

A variety of tests expressing antioxidant potency of food components have been suggested. These can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions. The features of an oxidation are a substrate, an oxidant and an initiator, intermediates and final products. Measurement of any of one of these can be used to assess antioxidant activity [56]. The ferric thiocyanate test determines the antioxidant activity with the measurement of the amount of peroxides formed in a linoleic acid emulsion of antioxidant during incubation. Zonguldag, Cankiri, Ardahan and Cine caused 93,18 %, 92,25 %, 92,06 % and 90.73 % inhibition, respectively (Table 3). These results suggest that all of Propolis samples have high total antioxidant activity. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenypicrylhydrazine (non radical) with the loss of this violet color [37]. DPPH scavenging activity is best presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50 % of DPPH present in the test solution. Lower IC_{50} value indicates higher antioxidant activity. Cankiri propolis provided the highest radical scavenging activity with the lowest IC_{50} value of 5.69 ± 0.49 µg/mL. Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals [11, 14]. It revealed there is a relationship between the antioxidant ability and total phenol contents. For the determination of total phenolics content generally employs Folin and Ciocalteu's phenol reagent. Gallic acid was used as the standard to represent all phenolics present in Propolis samples. Table 3 exhibits gallic acid equivalents of total phenolic contents of all Propolis samples. As seen in Table 3, total phenolic content of Propolis samples increased in the order of Zonguldag> Ardahan > Cine > Cankiri. Propolis samples of Zonguldag also showed the highest total antioxidant activity in all propolis samples. Flavonoids are a class of secondary plant phenolics found ubiquitously in fruits and vegetables, as well as food products, which act as pharmacological active compounds in many medicinal plants. Many of the biological actions of flavonoids have been attributed to their powerful antioxidant properties [2]. Flavonoid content of the propolis samples was listed in Table 3. However, we didn't found correlation between antioxidant

activity and flavonoid content. The reductive potential of a compound reflects its ability to act as an electron donor. The electron donor reacts with free radicals, converts them to more stable products, and finally terminates radical chain reactions. The reductive capacity of a compound is recognized as a significant indicator of its potential antioxidant activity [11]. Table 3 showed the reducing power of the Propolis samples expressed as % ascorbic acid. Reducing power of Propolis samples increased in the order of Ardahan > Zonguldag >Cine> Cankiri. These results suggested that all the Propolis samples are powerful source of natural antioxidants.

In conclusion, ın this study, the invitro antioxidant and antimicrobial activities of various propolis samples were investigated. Strong antioxidant, radical-scavenging and antimicrobial activities of Ardahan seemed to relate with high flavonoid contents and total polyphenol. Some authors state that the biological activities of propolis are mostly due to the high levels ofphenolic acids [3].

References

1. Amoros M, Simoes CMO, Girre L, Sauvager F, Cormier M. Comparison of the anti *Herpes simplex* virus activities of propolisand 3-methyl-butyl-2-enyl caffeate. Journal of Natural Products. 1994; 57:644-647.
2. Bahramikia S, Ardestani A, Yazadanparast R. Protective effects of four Iranian medicinal plants against free radical-mediated protein oxidation. Food Chemistry, 2009; 115:37-42.
3. Bankova V, Christov R, Kujumgiev A, Marcucci MC, Popov S. Chemical composition and antibacterial activity of Brazilian propolis. Z Naturforsch C. 1995; 50(3-4):167-172.
4. Bankova VS, Castro SL, Marcucci MC. Propolis: Recent advances in chemistry and plant origin. Apidologie, 2000; 31:3-15.
5. Basnet P, Matsuno T, Neidlein R. Potent free radical scavenging activity of propolis isolated from Brazilian propolis Zeitschrift für Naturforsch, 1997; 52C:828-833.
6. Bonvehi JS, Coll FV. Phenolic composition of propolis from China and from South America. Zeitschrift für Naturforchung, 1994; 49c:712-718.
7. Bosio K, Avanzini C, D'avolio A, Ozimo O, Savoia D. In vitro activity of propolis against *Streptococcus pyogenes*. Letters in Applied Microbiology, 2000; 31:174-177.
8. Boyanova L, Gergova G, Nikolov R. Activity of Bulgarian propolis against 94 Helicobacter pylori strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods. Journal of Medical Microbiology, 2005; 54:481-483.
9. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und Technologie, 1995; 26:25-30.
10. Burdock G. Review of the biological properties and toxicity of bee propolis, Food and Chemical Toxicology, 1998; 36**:**347-363.
11. Cao L, Si JY, Liu Y, Sun H, Jin W, Li Z, *et al*. Essential oil composition, antimicrobial and antioxidant properties of Mosla chinensis Maxim. Food Chemistry. 2009; 115(3):801-805.
12. Cheng PC, Wong G. Honey bee propolis: prospect in medicine. Bee World, 1996; 77:8-15.
13. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005; 26:434-356.
14. Decker EA. Phenolics: Prooxidants or antioxidants? Nutrition Reviews, 1997; 55:396-398.
15. Dixon RA. Natural products and plant disease resistance. Nature. 2001; 411:843-847.
16. Dobrowolski JW, Vohora SB, Sharma K, Shah SA, Naqvi SAH, Dandiya P. Cantibacterial, antifungal, antiamobeic, antiinflamatory and antipyretic studies on propolis bee products. Journal of Ethnopharmacology, 1991; 35:77-82.
17. Fernandes Jr A, Sugizaki MF, Fogo ML, Funari SRC, Lopes CAM. In vitro activity of propolis against bacteria and yeast pathogens isolated from human infections. Journal of Venomous Animals and Toxins, 1995; 1:63-69.
18. Garedew A, Schmolz E, Lamprecht I. Microbiological and calorimetric investigations on the antimicrobial actions of different propolis extracts: an in vitro approach. Thermochimica Acta. 2004; 422:115-124.
19. Ghisalberti El. Propolis: a review. Bee world. 1979; 60:59-84.
20. Greenaway W, Scaysbrok T, Whatley FRl. Composition of propolis in Oxfordshire, UK and its relation to poplar exudates. Zeitschrift für Naturforchung. 1998; 43c:301-305.
21. Greenway W, Scaysbrook T, Whatley FR. The composition and plant origin of plant propolis. Bee World, 1990; 71:107-118.
22. Hayacıbara MF, Koo H, Rosalen P. LIn vitro and in vivo effects of isolated fractions of Brazilian propolis on caries development. Journal of Ethnopharmacology. 2003; 101:110-115.
23. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental carries in rats. Caries Research, 1991; 25:347-351.
24. Kartal M, Yıldız S, Kaya S, Kurucu S, Topcu G. Antimicrobial activity of propolis samples from two different regions of Anatolia. Journal of Ethnopharmacology. 2003; 86:69-73.
25. Katırcıoglu H, Mercan N. Antimicrobial activity and chemical compositions of Turkish propolis from different region. African Journal of Biotechnology. 2006; 5(11):1151-1153.
26. Keskin N, Hazir S, Baser SH, Kürkcüoglu M. Antibacterial activity and chemical composition of Turkish propolis. Zeitschrift für Naturforchung, 2001; 56c:1112-1115.
27. Kimoto T, Aga M, Hino K, Koya-Miyata S, Yamamoto Y, Micallef MJ. *et al.* Apopsotosis of human leukemia cells induced by artepillin C, an active ingredient of Brazilian propolis. Anticancer Research, 2001; 21:221-228.
28. Koc AN, Silici S, Ayangil D, Ferahbas A, Cankaya S. Comparison of in vitro activities of antifungal drugs and ethanolic extract of propolis against Trichophyton rubrum and T. mentagrophytes by using a microdilution assay. Mycoses, 2005; 48:205-210.
29. König B. Plant source of propolis. Bee World, 1985; 66:136-139.
30. Kujumgiev A, Bankova V, Ignatova. A &Popov, S.

Antibacterial activity of propolis, some of its components and their analogs. Pharmazie, 1993; 48:785-786.
31. Kujumgiev A, Tsetkova I, Serkedjewa Yu, Bankova VS, Christov R, Popov S. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. Journal of Ethnopharmacology, 1999; 64:235-240.
32. Marcucci MC. Propolis: chemical composition, biological properties and therapeutic activity. Apodologie, 1995; 26:83-89.
33. Matsuno T. A new clerodane diterpenoid isolated from propolis. Zeitschrift für Naturforchung. 1995, 50c:93-97.
34. Mayrhofer S, Domig KJ, Mair C, Zitz U, Huys G, Kneifel W. Comparison of Broth Microdilution, Etest, and Agar Disk Diffusion Methods for Antimicrobial Susceptibility Testing of Lactobacillus acidophilus Group Members. Applied and Environmental Microbiology. 2008; 74(12):3745-3748.
35. Mercan N, Kıvrakİ, Duru ME, KatırcıogluH, Gülcan S, Malcı S, Acar G, Salih B. Chemical composition effects onto antimicrobial and antioxidant activities of propolis collected from different regions of Turkey. Annals of Microbiology. 2006; 56(4):373-378.
36. Miorin PL, Levy Jr NC, Custodio AR, Bretz WA, Marcucci MC. Antibacterial activity of honey and propolis from apismellifera and Tetragonisca angustula against Staphylococcus aureus. Journal of Applied Microbioogy, 2003, 95:913-920.
37. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl DPPH for estimating antioxidant activity. Songklanakarin Journal of Science and Technology, 2004; 26**:**211-219.
38. Murray PR, Baron EF, Pfaller MA, Tenover FC, Yolke RH**.** Manuel of Clinical Microbiology, 6. ASM, Washington DC, 1995.
39. Nieva MMI, Isla MI, Cudmani NG, Vattuone MA, Sampietro AR. Screening of antibacterial activity of Amaicha del Valle Tucuman, Argentina propolis. Journal of Ethnopharmacology, 1999; 68:97-102.
40. Oyaizu M. Studies on products of browning reaction: oxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, 1986; 44:307‑315.
41. Park YK, Alencar SM, Aguiar CI. Botanical origin and chemical composition of Brazilian propolis. Journal of Agricultural and Food Chemistry, 2002; 50:2502-2506.
42. Popova M, Silici S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantativechemical composition. Phytomedicine. 2005; 12:221-228.
43. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M *et al.* Phenolic compounds and antioxidant activities of buckwheat Fagopyrum esculentum Moench hulls and flour. Journal of Ethnopharmacology. 2000; 72:35‑42.
44. Saha K, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S *et al*. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. Journal of Ethnopharmacology. 2004; 92**:**263-267.
45. Salomão K, Dantas AP, Borba CM, Campos LC, Machado DG *et al.* Chemical composition and microbicidal activity of extracts from Brazilian and Bulgarian propolis. Letters in Applied Microbiology. 2004; 38:87-92.
46. Sawaya ACHF, Souza KS, Marcucci MC, Cunha IBS, Shimizu MTAnalysis of the composition of Brazilian propolis extracts by chromatography and evaluationof their in vitro activity against Gram-positive bacteria. Brazilian Journal of Microbiology. 2004; 36:347-363.
47. Sawaya ACHF, Palma AM, Caetano FM. Comparative study of in vitro methods used to analyse the activity of propolis extracts by chromatography and evaluation of their in vitro activity against Gram positive and Gram-negative bacteria. Brazilian Journal of Microbiology. 2002; 36:347-363.
48. Seidel V, Peyfoon E, Watson DG, Fearnley J. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones, Phytotheraphy Research. 2008; 22:1256-1263.
49. Sforcin JM, Fernandes JR A, Lopes CAM, Bankova V, Funari SRC. Seasonal effect on Brazilian propolis antibacterial activity. Journal of Ethnopharmacology, 2000; 73:243-249.
50. Silici S, Kaftanoglu O. Antimicrobial analysis of propolis samples from different regions in Turkey. Uludag Bee Journal. 2003; 8:16-18.
51. Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three races of honeybees in the same region. Journal of Ethnopharmacology, 2005; 99:69-73.
52. Silici S, Koç NA, Ayangil D, Cankaya S. Antifungal activity of propolis collected by different races of honeybees against yeasts isolated from patients with superficial mycoses. Journal of Pharmocological Sciences, 2005; 99:39-44.
53. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin‑Ciocalteu reagent. Methods in Enzymology. 1999; 299:152-178.
54. Sonmez S, Kirilmaz L, Yucesoy M, Banu B, Yilmaz B. The effect of bee propolis on oral pathogens and human gingival fibroblasts. Journal of Ethnopharmacology. 2005; 102:371-376.
55. Sorkun K, Süer B, Salih B. Determination of chemical composition of Turkish propolis. Zeitschrift für Naturforchung, 2001; 56c:666-668.
56. Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis. 2006; 19:531-537.
57. Ugur A, Arslan T. An in vitro study on antimicrobial activity of propolis from Mugla province of Turkey. Journal of Medicinal of Food, 2004; **7:**90-94.
58. Uzel A, Sorkun K, Öncag Ö, Cogulu D, Gencay Ö, Salih B. *et al.* Chemical compositions and antimicrobial activities of four different Anatolian propolis samples, Microbiological Research. 2005; 160(2):189-195.
59. Velikova M, Bankova V, Sorkun K, Popov S, Kujumgiev A. Chemical composition and biological activity of propolis from Turkish and Bulgarian origin. Mellifera, 2001; 1:57-59.