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Original article

Screening for antioxidant capacity, pollen types and phytochemical profile by GC/MS and UHPLC from propolis

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Summary. Propolis is a resinous mixture collected by honeybees from different parts of plants such as poplars, birches, alders, conifers, pines, palms and willows. This study aims to determine pollen types (by light microscope), chemical compound profile (by GC-MS), fatty acid composition (by GC-MS), total phenolic content (by the Folin-Ciocalteu method), total flavonoid content (by the aluminium chloride method) and antioxidant capacity (by the CUPRAC, ABTS and CERAC methods) of a propolis sample from the western part of Istanbul, Turkey. As a result of microscopic analysis of the sample, pollen types of taxa belonging to 27 plant families were diagnosed. The GC-MS analysis of propolis revealed the presence of 38 phytochemical constituents that may contribute to its quality. Of these compounds, rates of "4H-1-benzopyran-4-one, 2,3-dihydro-5,7-dihydroxy-2-phenyl-,(S)" were highest. In addition, the concentrations of naringenin, pinocembrin and galangin were determined to be 2.45, 9.92 and 7.06 mg/ml by UHPLC analysis. The extract had significant antioxidant activity in all assays, with values of 282.8±9 mg TE /g in the CUPRAC, 425.7±18 mg TE/g in the CERAC and 186.4±8 mg TE/g in the ABTS assays. Antioxidant capacity of the propolis extract was positively associated with the total phenolic and flavonoid contents of the extract. Moreover, the major fatty acids were C20:1n9 (cis-11-eicosenoic acid), C22:1 (erucic acid) and C24:1 (nervonic acid). The results show that the propolis used in our study has important potential as an alternative food supplement and for cosmetic and therapeutic medicine and it can be used as an active agent in these areas.

Key words: Propolis, pollen content of propolis, fatty acid of propolis, antioxidant activity.

Introduction

Propolis (bee glue) is a natural resin-like substance collected by honeybees. The chemical composition of propolis is very complex. To date, more than 500 chemical components have been identified in its chemical structure (1, 2). The main components of propolis are pollen (5%-10%), oil and wax (30%-50%), resin (50%-70%), and other chemical compounds including: vitamins B, C and E, sugars, minerals, phenols, flavonoids, amino acids, fatty acids, steroids and stilbenes as well as aromatic compounds (3). The diversity and rate of these constituent's change depending on the region of collection, plant source and weather conditions when it was collected.

Propolis has been used by people for therapeutic purposes for centuries. It has antioxidant (4), antibacterial (5) and antifungal (6), antiviral (7), anti-inflammatory (8), antitumor, hepatoprotective, antipsoriatric (9), antihyperalgesic (10) and antigenotoxic (11) effects. In particular, several studies have reported that flavonoids from the main components of propolis are responsible for the majority of these activities (12-14) The degree of these effects varies according to the ac-

tive substances contained in the propolis and the contents of the propolis samples produced in different regions also varies, which makes standardization difficult. Therefore, we evaluated the phenolic and chemical profile in addition to the antioxidant capacity of propolis from İstanbul (Turkey) in order to contribute to standardization efforts in this study. We also examined the fatty acid composition.

Material and Methods

Chemicals

All chemicals used in the experiments were of analytical grade. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman carboxylic acid), 2,20 -Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), gallic acid, quercetin, sodium carbonate, Folin-Ciocalteu's phenol reagent, dry pyridine, bis (trimethylsilyl) trifluoroacetamide (BSTFA), pinocembrin and galangin were purchased from Sigma-Aldrich. Cu (II) chloride, neocuproin, ammonium acetate, cerium sulphate, aluminium chloride, sodium hydroxide, sodium nitrite and ethanol were obtained from Merck. Supelco 37 FAME component mix (47885-U) was purchased from SUPELCO Inc.

Geographical origin of propolis

A propolis sample was collected from an apiary located in the western part of Istanbul, Turkey in 2018. It was then pulverized with a grinder and stored in the freezer (-18^{0}C) until analysis.

Microscopic investigation of propolis

The pollen spectra of the propolis sample was detected according to the methodology of Warakomska and Maciejewicz (1992) (15). 1 g of the propolis sample was mixed with ethanol-ether-acetone (1:1:1) and vortexed. This mixture was filtered through a strainer with about 0.3 mm holes. The suspension was centrifuged for 20 minutes at 3500-4000 rpm. The supernatant liquid was then poured off; glycerin gelatin with basic fuchsine about 1-2 mm3 in width was taken with a sterile needle and transferred onto a slide after imbruing it in the pellet in the bottom of the centrifuge tube. The slide in this form was heated at 30-40°C to allow the dissolution of fuchsine glycerin gelatin; and then covered with a 18x18 lamella. Then, it was examined with a light microscope (16).

Preparation of propolis extract

The process of extraction from propolis by ultrasound treatment was performed using a 25 kHz ultrasonic processor (model VCX 750; Sonics & Materials, Inc., Newtown, CT, USA). Thirty grams of propolis powder was dispersed with 100 mL of ethanol (LC-grade). Sonication was carried out in a double-walled stainless steel chamber using a titanium probe with an emitting face 19 mm in diameter that was kept immersed 2 cm below the surface of the sample during sonication. The temperature was kept constant throughout sonication by circulating water through the jacket of the chamber. The samples were exposed to extract at 40% amplitude for 5 minutes at 30°C. The extract was cooled to room temperature and filtered through Whatman no 1 filter paper and 0.22 μm polypropylene filter, then transferred to amber bottles and stored at -18°C until analysis (5).

Chemical screening of propolis composition by gas chromatography-mass spectroscopy (GC-MS)

The filtered solution was diluted in 1:10 ratio (w/v) with 96% ethanol and evaporated to complete dryness. About 5 mg of the dry matter was mixed with 75 μL of dry pyridine and 50 μL bis (trimethylsilyl) trifluoroacetamide (BSTFA) and heated at 80°C for 20 min. The final supernatant was analyzed by GC-MS. The extract was analyzed using a GC 7890A from Agilent (Palo Alto, CA, USA) coupled with mass detector (MS 5975C, Agilent) equipped with a DB-5 MS capillary column (30 m × 0.25 mm and a film thickness of 0.25 μm). The column oven temperature was initially maintained at 50°C for 1 min, then programmed to rise to 150°C at 10°C/min and maintained for 2 min. Finally, the temperature was increased to 280°C at 20°C/min and maintained at 280°C for 5 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The mass spectra were recorded within 40-550 (m/z), full scan mode, that revealed the total ion current (TIC) chromatograms. MS transfer line temperatures were set at 265° C (2).

The active ingredients (naringenin, pinocembrin and galangin) of propolis by ultra-high performance liquid chromatography (UHPLC)

In the study, double distilled water and formic acid (19: 1, v: v) were used as the mobile phase and the separation of phytochemicals was performed on a reverse phase column Perkin Elmer RP-18 (USA) (12.5 x 0.4 cm, 5 μm particle size). Formic acid and water (A) and methanol (B) have a constant solvent flow rate of 1 mL of min-1, starting isocratically with 30% B in A for up to 15 minutes, then loading a gradient to reach 40% B in 20 minutes, 45% B at 30 minutes, 60% B at 50 minutes, 80% B at 52 minutes and 90% B at 60 minutes, then isocratic for 65 minutes. The propolis extract was analyzed using a UHPLC Flexar using a multi-channel photodetector FX-20 and samples were injected with a Flexar autosampler FX-15 device. The column was maintained at room temperature and the chromatograms were eluted with Chromera Manager software (Perkin Elmer, USA).

Bioactivity tests

Total phenolic content (TPC)

The analysis of total phenolic content was performed to the Folin-Ciocalteu method proposed by Magalhães et al. (2010) using gallic acid as a reference standard (17). 50 μL of sample and 50 μL of Folin-Ciocalteu reagent (1:5, v/v) were placed in each well. After that, 100 μL of sodium hydroxide solution (0.35 M) was added. The absorbance at 760 nm of the blue complex formed was read after 3 minutes. The results were expressed as gallic acid equivalent (mg GAE/g).

Total flavonoid content (TFC)

Total flavonoid analysis of the extract was performed using a modified version of the method Zhishen et al. (1999) used in their work (18). Accordingly, an aliquot (1 mL) extract was mixed with 0.3 mL 10% AlCl3.6H2O solution after the addition of 0.3 mL 5% NaNO2 solution. 2 mL of 1 M NaOH solution was added and 2.4 mL of water was added and the mixture was stirred. At 510 nm the absorbance was measured against the prepared reagent blank by Epoch Multi-Detection Microplate Reader with 96-well plates (BioTek Instruments, Inc., P). Total flavonoid content was expressed as mg quercetin equivalent (mg QE/g).

Determination of antioxidant activity

Cupric ion reducing antioxidant capacity (CUPRAC) assay: To a test tube was added 1 mL of 1.0 x 10-2 M Cu (II) chloride solution, 1 mL of 7.5 x 10-3 M neocuproin solution and 1 mL of 1 M ammonium acetate buffer (pH 7.0), respectively. Then x mL propolis extract was stirred into 95% ethanol-water (1.1-x). The tube was kept at room temperature for 30 minutes. At the end of the period, the absorbance value of the solution at 450 nm was measured against the non-including antioxidant solution by Epoch Multi-Detection Microplate Reader with 96-well plates (BioTek Instruments, Inc., P) (19).

Ceric ion reducing antioxidant capacity (CERAC) assay: The total antioxidant capacity of the tested sample was also determined using the Cerium (IV) assay of Ozyurt et al. (2007). A 1.0 mL 2.0 × 10-3 M Ce(SO4)2 + x mL sample + (9-x) mL H2O solution with a total volume of 10.0 mL is prepared and left at room temperature for 30 minutes. After 320 nm the absorbance is measured against the distilled water and compared to a Trolox standard curve of 1-20 mM (20).

2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay: The ABTS reagent prepared at 7 mM concentration was dissolved in water. The ABTS radical cation was reacted with 2.45 mM potassium persulfate and left in the dark at room temperature for 12-16 hours before use. To study the sample, the ABTS solution was diluted with ethanol to an absorbance of 0.70 at 734 nm and equilibrated at 30°C. 1 ml of sample and 1 ml of ABTS solution, were diluted with methanol to a total volume of 4 ml. After the tube was kept closed at room temperature for 6 minutes, the absorbance value of the sample was read at 734 nm (21).

Fatty acid methyl ester (FAME) analysis of propolis by GC-MS

Lipid extraction

2 g of pulverized raw propolis was weighed into a soxhlet cartridge, which was placed in the extraction chamber in the soxhlet apparatus (Buchi B-811). A 100 mL aliquot of n-hexane was transferred into the solvent cup and placed on the heating plates. The cooling water supply to the condensers was opened to ensure continuous recycling of the solvent and tempera-

ture selected as per the Büchi manual for extraction in the continuous mode with extraction (4 h), rinsing (1 h) and drying (1 h) steps.

Preparation of FAME

A 100 mg sample (propolis oil) was weighed in a 20 mL test tube and dissolved in 10 mL of n-hexane. 0.1 mL of 2N KOH solution was added to tube. This mixture was vortexed for 30 seconds and then centrifuged. 2 mL of saturated NaCl solution was added and the organic phase was separated. 1 µl of the final solution was analyzed in GC-MS (22).

FAME Analysis by GC-MS

Methylated fatty acid samples were analyzed by Agilent 6890 GC gas chromatography and 5973 MSD mass spectrometry. DB-23 60 m x 0.25 mm ID, 0.15 µm (J&W 122-2361) column was used in the analyses. Helium was used as carrier gas. The oven temperature was initially maintained at 50˚C for 1 min and then programmed to rise to 175˚C at 25˚C/min. Finally, the temperature was increased to 230˚C at 4˚C/min and maintained at 280˚C for 5 min. The injection temperature was set to 230˚C. 1 µL injection was made and the split ratio was adjusted to 1/50 (23).

Results and Discussion

The chemical structure of propolis is quite complex and varies considerably depending on environmental factors, flora and vegetation of the region. While bees collect the propolis from different plant sources, they also enrich its chemical structure by adding plant pollen, and the chemical composition of propolis contains around 5% pollen. Pollen types of the Aceraceae (<1%), Apiaceae (7.75%), Asteraceae (16.18%), Boraginaceae (4.55%), Brassicaceae (2.69%), Campanulaceae (2.69%), Caprifoliaceae (<1%), Caryophyllaceae (2.86%), Cyperaceae (<1%), Dipsacaceae (<1%), Fabaceae (19.56%), Fagaceae (7.08%), Geraniaceae (<1%), Lamiaceae (16.52%), Malvaceae (<1%), Onagraceae (<1%), Papaveraceae (1.34%), Plantaginaceae (<1%), Poaceae (4.34%), Polygonaceae (1.01%), Pinaceae (<1%), Ranunculaceae (<1%), Rosaceae (1.34%), Liliaceae (<1%), Rubiaceae (1.01%), Salicaceae (3.70%) and Scrophulariaceae (<1%) families were determined at different percentages in the propolis sample. The pollen types found in the structure of propolis give information about the flora of the region and also contribute to the understanding of the plants visited by bees. The results indicate that plant taxa belonging to Fabaceae, Lamiaceae and Asteraceae families are visited by bees for collecting nectar, propolis or pollen. These families are among the most common plant families in Turkey and their presence in propolis in large amounts is to be expected.

As a result of GC-MS analysis of propolis, benzyl alcohol, phenylethyl alcohol, benzoic acid, catechol, 4-vinyl-phenol, 2-propen-1-ol, 2-methoxy-4-vinylphenol, 4-hydroxy-3-methoxy, trans-cinnamic acid, gamma-muurolene, delta-cadinene, naphthalene, tau-cadinol, alpha-copaene, t-muurolol, (2e)-3-phenylpent-2,4-dienoic acid, benzyl benzoate, (2s,4as,5s,8ar)-perhydro-5,8a-dimethylnaphthalene-2-ol, 3,4-dimethoxycinnamic acid, benzyl cinnamate, p-coumaric acid, n1,n3-dimethyl-8-cyclohexylxanthin, eicosane, 2-propen-1-one, cinnamyl cinnamate, trifluoroacetic acid, 4H-1-benzopyran-4-one, 2,3-dihydro-5,7-d,hydroxy-2-phenyl-,(s), eicosane, 2-methoxymethyl-5-(4-methoxyphenyl)pyrrolo(1,2-c)pyrimidin-1(2h)-one, 4H-1-benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl, 9,10-anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl-, diethyl 2-acetoxy-2-(2-oxocyclohexyl)malonate, chrysin,5,7-dihydroxy-3-methoxy-2-phenyl-4H-1-benzopyran-4-one, estra-4,9,11-trien-3-one, 4',5-dihydroxy-7-methoxyflavanone, 9-nonadecene, docosyl pentafluoropropionate individual compounds were detected in different concentrations (Table 1). These compounds possess many biological properties. For instance, benzoic acid (RT/9.24) has antioxidant (24) and antimicrobial (25) properties. Similarly, chrysin (RT/36.345), is a flavonoid that exhibits many pharmacological activities, including anticancer, anti-inflammatory, antioxidant, and antiviral effects (26). This compound revealed a high peak area in our propolis, which may mean that propolis also exhibits the above-listed activities. In different studies, benzoic acid, 4-vinyl-phenol, eicosane, chrysin, delta-cadinene, phenylethyl alcohol, benzyl alcohol compounds were determined for propolis types from different origins

Table 1. Chemical screening of propolis composition

Retention Time	Compounds	% of total ion current
7.20	Benzyl alcohol	0.56
8.45	Phenylethyl alcohol	0.68
9.24	Benzoic acid	1.71
9.61	Catechol	0.15
9.91	4 - vinyl - phenol	0.54
11.25	2-Propen-1-ol	0.44
11.38	2-Methoxy-4-vinylphenol	1.86
12.80	4-hydroxy-3-methoxy	0.10
13.24	Trans-cinnamic acid	0.36
15.19	Gamma.-muurolene	0.19
15.34	Delta.-cadinene	0.23
15.66	Naphthalene	0.09
17.83	Tau.-cadinol	0.45
17.93	Alpha.-copaene	0.15
18.12	T-muurolol	0.92
19.12	(2E)-3-Phenylpent-2,4-dienoic acid	0.27
20.36	Benzyl benzoate	0.12
21.42	(2S,4as,5S,8ar)-perhydro-5,8a-dimethyl-naphthalen-2-ol	0.42
23.21	3,4-Dimethoxycinnamic acid	0.51
26.71	Benzyl cinnamate	0.22
27.01	P-coumaric acid	0.13
28.77	N1,N3-Dimethyl-8-cyclohexylxanthin	2.38
30.22	Eicosane	0.43
31.99	2-Propen-1-one	7.61
32.12	Cinnamyl cinnamate	1.06
33.05	Trifluoroacetic acid	0.08
33.31	4H-1-benzopyran-4-one, 2,3-dihydro-5,7-d,hydroxy-2-phenyl-,(s)	15.35
33.42	Eicosane	0.31
34.76	2-Methoxymethyl-5-(4-methoxyphenyl) pyrrolo(1,2-c)pyrimidin-1(2H)-one	4.27
34.98	4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl	5.26
35.53	9,10-Anthracenedione, 1,8-dihydroxy-3-methoxy-6-methyl-	0.31
36.18	Diethyl 2-acetoxy-2-(2-oxocyclohexyl) malonate	6.73
36.34	Chrysin	11.85
36.88	5,7-Dihydroxy-3-methoxy-2-phenyl-4H-1-benzopyran-4-one	1.18
37.00	Estra-4,9,11-trien-3-one	2.64
37.47	4',5-Dihydroxy-7-methoxyflavanone	1.40
41.96	9-nonadecene	0.79
46.39	Docosyl pentafluoropropionate	0.75

(2, 27-29). The similarities observed in the samples of propolis from different origins suggest that the same plants are the source of these propolis samples.

In addition to the determination of the general chemical profile by GC-MS of the propolis extract, quantitative detection of the active ingredients of naringenin, pinocembrin and galangin was made using UHPLC (Figure 1). As a result of the investigation, the ratio of the active ingredients of the naringenin, pinocembrin and galangin of the extract was 2.45, 9.92, 7.06 mg/ml, respectively. Naringenin, galangin and pinocembrin are the essential phenolic compounds found in propolis extracts from Turkey and, hence, are most likely to contribute to the antioxidant activity of propolis extracts. These phenolics were also found in propolis by Guzelmeric et al. (2018) and Morlock et al. (2014) (30, 31). Also, Ristivojević et al. (2014) determined pinocembrin, galangin, chrysin and caffeic acid phenethyl ester as specific markers of Populus nigra buds (32). This could indicate that the resin source for the propolis used in the context of our work may be Populus nigra. Similarly, Yang and You (2017) reported that pinocembrin with its antioxidant, antimicrobial, vasorelaxant, anti-inflammatory and neuroprotective effects, etc. was present at the highest concentration in propolis (33). Naringenin, a flavonoid in the class of flavanones, can be extracted from various natural products. Different reports have shown the biological effects of naringenin, including blood lipid- and cholesterol-lowering effects (34), anti-inflammatory (35) and analgesic (35) activities (36). However, it is believed that the degree of such protective activities in propolis varies depending on the synergistic effects of individual compounds in its chemical composition.

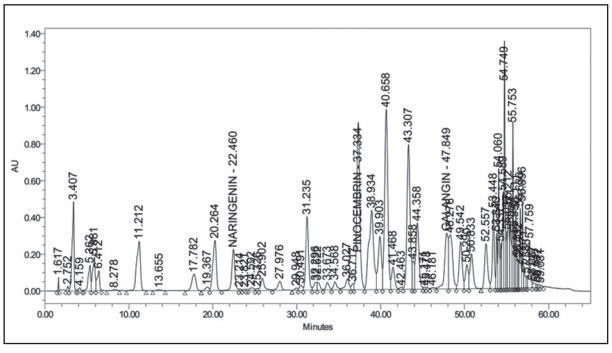

Figure 1. HPLC chromatogram of propolis extract

Phenolic substances are important food components in terms of human health because of their effects on taste and odor formation, their participation in color formation and change, their antimicrobial and antioxidative effects and their enzyme-inhibiting properties (37). Different types of bee products are assumed to be an effective source for antioxidants capable of resisting the impacts of oxidative stress underlying the pathogenesis of many diseases. Generally, compounds exhibiting phenolic characteristics, which express the ability to scavenge free radicals, are principally responsible for the antioxidant effect of bee products (38). For this reason, it is essential to determine the phenolic compositions of the products used as food or food supplements. There are several different techniques for the evaluation of the total phenolic, flavonoid or antioxidant capacity of synthetic antioxidants or bee products. However, the differences in these techniques make comparisons between studies in the literature difficult, and in some cases, conflicting results can be obtained (39). In this study, we used the Folin-Ciocalteu method and the aluminium chloride method to detect the total phenolic and flavonoid contents, respectively. These methods are assays that are widely used for phenolic profile determination in many different samples, including propolis (40, 41). In the literature, it has been stated that the antioxidant capacity of multicomponent mixtures cannot be evaluated satisfactorily using a single antioxidant test due to different variables that may affect the results. Therefore, the application of different test methods simultaneously is recommended for better evaluation of antioxidant activity (42-44). Herewith, the antioxidant activity of propolis was evaluated using three test procedures (CUPRAC, CERAC, ABTS) in our study. The CUPRAC method developed by Apak et al. (2004) was used to evaluate the total antioxidant capacity of biological samples (45). The spectrophotometric CERAC assay is a method based on the determination of Ce (III) ions as a result of the reaction between Ce (IV) ions and antioxidants in the sulfate acid medium (20). In the ABTS method, the preformed radical monocation of ABTS is produced by oxidation of ABTS with potassium persulfate and decreases in the presence of hydrogen-donating antioxidants (21). In this research, total phenolic-flavonoid contents in propolis extract were determined 81.2±3 mg GAE/g and 164.8±5 mg QE/g of ethanol extract of propolis, respectively (Table 2). Antioxidant capacity of extract was detected with a trolox equivalent of 282.8±9, 425.7±18, 186.4±8 mg TE/g according to the methods, CUPRAC, CERAC, and ABTS, respectively (Table 2). Similarly, Moreira et al. (2008) reported that Portugal propolis is an important source of phenols, with antioxidant properties that may be beneficial for human health (46). Ahn et al. (2004) detected total phenolic-flavonoid content ranging from 85±2 - 283±5 and 16±2 -136±9 mg/g for propolis extracts from Korea (47). Kumazawa et al. (2004) obtained the total polyphenol-flavonoid contents in extracts of the propolis samples from Argentina, Brazil, Bulgaria, Chile, China, Hungary, New Zealand, South Africa, Thailand, Ukraine, Uruguay, United States and Uzbekistan as 31.2±0.7 - 299±0.5 and 2.5-176 mg/g, respectively (48). The antioxidant activity of Algerian propolis was found to be greatly affected by TPC and TFC. This observation is consistent with previous studies of propolis. Differences in total phenolic-total flavonoid contents are due to the fact that the phenolic content depends on climate, geography and plant origin (49).

The fatty acids composition of propolis extract is shown in Table 3. Saturated fatty acids were determined as arachidic acid, behenic acid, capric acid, lignoceric acid, palmitic acid and stearic acid. Monounsaturated fatty acids were detected as cis-11-eicosenoic acid, erucic acid, oleic acid, palmitoleic acid, and nervonic acid. In addition, four polyunsaturated fatty acids were detected; linoleic acid, linolenic (ALA) acid, arachidonic acid, cis-13,16-docosadienoic acid. Similarly, it was reported that all propolis samples from Algeria contained oleic, linoleic, stearic, eicosenoic, palmitoleic and palmitic acid, but only two samples did

Table 2. The results of bioactivity tests and extraction yield of propolis as mean±SD

	Extraction Yield (mg /g)	**TPc (mg GAE /g)**	**TFc (mg QUE /g)**	**CUPRAC (mg TE /g)**	**CERAC (mg TE /g)**	**ABTS (mg TE /g)**
Propolis extract	528.8±12	81.2±3	164.8±5	282.8±9	425.7±18	186.4±8

not contain arachidonic acid (3). The propolis oil was found to have cis-11-eicosenoic acid and erucic acid as its major fatty acid. cis-11-eicosenoic acid and erucic acid were present in amounts up to 31.87% and 22.93% respectively. cis-11-eicosenoic acid is beneficial as a raw material for medical supplies and a moisturizing component of cosmetic creams. It is also a precursor of erucic acid, which is beneficial for various applications such as cosmetics, creams, bio-diesel, lubricating oil, and therapeutic medicine (50). Studies on nervonic acid, another fatty acid that is highly (15.14%) detected in our propolis, showed a negative correlation between nervonic acid and obesity-related risk factors (51). This information suggests that our propolis sample has the potential to be used in these areas.

Conclusion

In conclusion, our study provides evidence that extract of propolis sampled from the western part of Istanbul, Turkey has high total phenolic content, and powerful antioxidant activity. Hence, it can be used as a food supplement and a preventive agent for many diseases which are caused by free-radicals. Moreover, due to the specificity of total fatty acid composition, it can be used as a nutritional supplement as well as a potential raw material supply for the cosmetic sector.

Table 3. Fatty acid composition of propolis sample (as a percentage of total fatty acids)

Retention Time	Common Name	Fatty acid	% of total
6.11	Capric acid	C10:0	0.33
10.37	Palmitic acid	C16:0	1.23
10.71	Palmitoleic acid	C16:1	0.4
12.77	Stearic acid	C18:0	0.39
13.10	Oleic acid	Cis- C18:1	1.29
13.77	Linoleic acid	Cis-C18:2	0.29
14.64	Linolenic (ALA) acid	C18:3n3	0.77
15.57	Arachidic acid	C20:0	1.23
15.94	cis-11-Eicosenoic acid	C20:1n9	31.87
17.38	Arachidonic acid	C20:4n6	0.93
18.5	Behenic acid	C22:0	1.19
18.9	Erucic acid	C22:1	22.93
19.70	Cis-13,16-Docosadienoic acid	C22:2	0.57
21.45	Lignoceric acid	C24:0	0.15
21.70	Nervonic acid	C24:1	15.14

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