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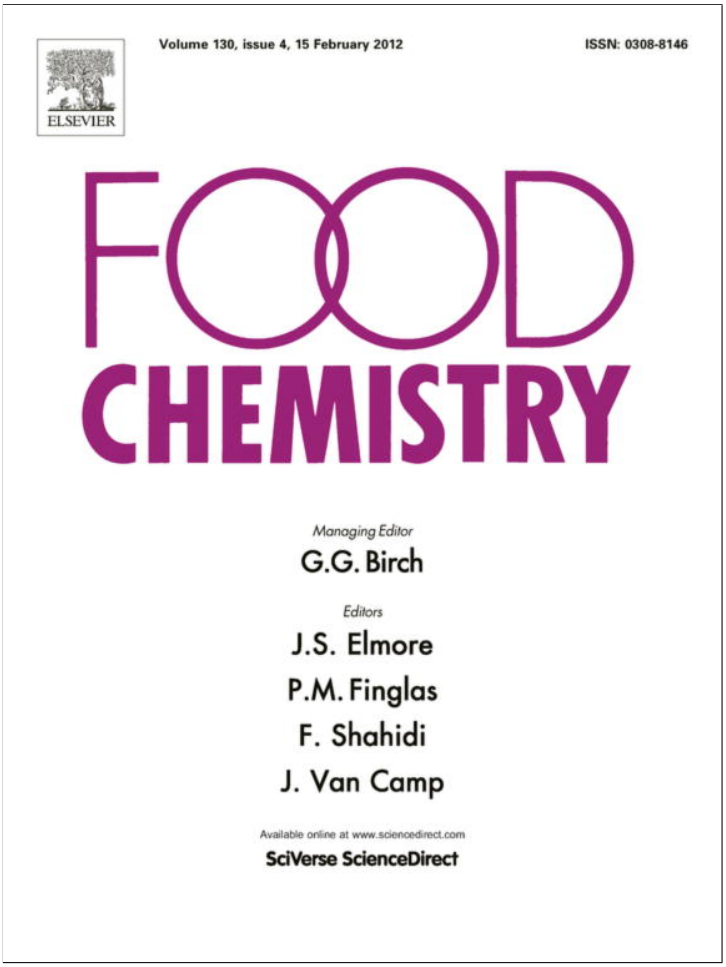

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Neuroprotective potential of some terebinth coffee brands and the unprocessed fruits of *Pistacia terebinthus* L. and their fatty and essential oil analyses

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ABSTRACT

In the current study, neuroprotective effects of the ethyl acetate and methanol extracts of four terebinth coffee brands and the fruits of *Pistacia terebinthus* L. were investigated through enzyme inhibition tests against acetylcholinesterase, butyrylcholinesterase, and tyrosinase as well as antioxidant test systems. Antioxidant activity was measured using radical scavenging activity tests and metal-related tests including metal-chelation capacity, ferric-reducing antioxidant power (FRAP), and phosphomolybdenum reducing power (PRAP). The fatty oils of the coffee brands and the fruits and the fruit essential oil were examined by GC–MS. Total phenol and flavonoid contents were calculated spectrophotometrically. The extracts had moderate inhibition against butyrylcholinesterase (9.78–45.74% at 200 μg mL^{-1}) and potent scavenging activity against DPPH. They exerted strong activity in FRAP and metal-chelation tests and modest activity in PRAP test. Oleic acid was identified as the major fatty acid in the fatty oils, while α-pinene (26.31%) was dominant in the essential oil.

1. Introduction

Consumption of traditional herbal coffees has been widely increased in last decades due to their distinctive aroma. Terebinth coffee, known as "menengic coffee" in Turkish, is one the most consumed herbal coffees in Turkey. It is an oily brown-coloured powder produced from the dried and roasted fruits of terebinth or turpentine tree (*Pistachia terebinthus* L., Anacardiaceae), which is usually cooked in a equal amount milk to that of the menengic coffee (Sekeroglu, Kaya, Inan, Kirpik, 2006). Terebinth plants, native to the Mediterranean region from Morocco and Portugal to Greece, Turkey and Syria, are perennial shrubs or small trees with aromatic dark green leaves. Most of the plant parts including fruits, fruit fatty oil and resin are used as food and traditional medicine in the region (Baytop, 1999). At the end of summer, this plant produces red-green to purple-coloured edible fruits with high fatty oil content. Different parts of *P. terebinthus* have been also reported to have several ethnopharmacological utilisations in Anatolia such as antiseptic for bronchitis, and diuretic, as well as against wounds, burns, and stomache (Cakilcioglu Turkoglu, 2010; Ugulu, Baslar, Yorek, Dogan, 2009; Ugurlu Secmen, 2008).

Terebinth coffee has an appearance of sediment like ground coffee and suggested to drink after all sweet deserts as a perfect aid for digestion, particularly in the southeastern part of Turkey. Since the fruits of *P. terebinthus* have been reported to be used as stimulant (Ozcan, Tzakou, Couladis, 2009) and consumed widely as snack, known as "çedene", and in the form of coffee, we aimed to investigate possible neuroprotective effects related to brain health of the ethyl acetate (EtOAc) and methanol (MeOH) extracts of various commercially available terebinth coffee brands and the unprocessed fruits of *P. terebinthus*, which are traditionally used in production terebinth coffee. Neuroprotective activity of the extracts was assessed through enzyme inhibition assays against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which are linked to Alzheimer's disease as well as tyrosinase related to Parkinson's disease using ELISA microplate reader at 25, 50, 100, and 200 μg mL^{-1} concentrations. Since oxidative damage is one of the major factors contributing to neurodegenaration, antioxidant activity of the extracts was evaluated by a number of *in vitro* test systems including the scavenging activity tests based on radical formation against 2,2-diphenyl-1-picrylhydrazyl (DPPH), *N*,*N*-dimethyl-*p*-phenylendiamine (DMPD), super oxide, and hydrogen peroxide radicals as well as metal-related antioxidant activity tests including metal-chelation capacity, ferric-reducing antioxidant power (FRAP), and phosphomolybdenum reducing power (PRAP) tests. Total phenol and flavonoid contents in the extracts were calculated using Folin–Ciocalteau and $AlCl_3$ reagents,

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respectively. Chemical composition of the fatty oils and essential oil of the fruits along with the fatty oils of the coffee brands was identified by gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

2.1. Plant and coffee materials

The fruits of *Pistacia terebinthus* were collected from Kilis province (Turkey) in September, 2010 and identified by Dr. Nazim Sekeroglu from Department of Biology, Faculty of Art and Sciences, Kilis 7 Aralik University (Turkey). The four terebinth coffee brands produced by different companies were purchased from the local markets in Kilis and Gaziantep provinces in 2010 and were coded as follows: Kaffka-Citlenbik – *A*; Kurkcuoglu – *B*; Tekinoglu – *C*; and Saf – *D*.

2.2. Extraction procedure

The air-dried ripe fruits of *P. terebinthus* were grounded in a mechanic grinder to a fine powder, weighed precisely in a digital balance (Shimadzu). Certain amounts of each coffee brand were weighed accurately and the coffee samples and fruits were sequentially extracted with EtOAc and MeOH during 3 days at room temperature. The organic phases were filtered and evaporated *in vacuo* until dryness to give crude extracts. The extract yields (w/w) are listed in Table 1.

2.3. Fatty oil extraction and isolation of essential oil

The coffee samples and fruits were independently subjected to continuous extraction technique using Soxhlet apparatus, which was extracted with *n*-hexane for 8 h. Then, organic phases were filtrated until dryness to give the fatty oils, whose yields (w/w) were calculated as follows; *A*: 42.95%; *B*: 34.98%; *C*: 40.67%; *D*: 43.99%, fruit: 9.49%.

The air-dried fruits of *P. terebinthus* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce essential oil with 0.07% of yield (v/w).

2.4. Determination of total phenol and flavonoid contents in the extracts

Phenolic compounds were determined in accordance with Folin–Ciocalteau's method (Singleton Rossi, 1965). In brief, a number of dilutions of gallic acid were obtained to prepare a calibration curve. The extracts and gallic acid dilutions were mixed with 750 μL of Folin–Ciocalteau's reagent and 600 μL of sodium carbonate in test tubes. The tubes were then vortexed and incubated at 40 °C for 30 min. Afterward, absorption was measured at 760 nm at a Unico 4802 UV–visible double beam spectrophotometer (USA). Total flavonoid content of the extracts was calculated by aluminium chloride colorimetric method (Woisky Salatino, 1998). To sum up, a number of dilutions of quercetin were obtained to prepare a calibration curve. Then, the extracts and quercetin dilutions were mixed with 95% ethanol, aluminium chloride reagent, 0.1 mL of sodium acetate as well as distilled water. Following incubation for 30 min at room temperature, absorbance of the reaction mixtures was measured at wavelength of 415 nm with a Unico 4802 UV–visible double beam spectrophotometer (USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g^{-1} extract), respectively.

2.5. AChE and BChE inhibitory activity assays

AChE and BChE inhibitory activity was measured by slightly modified spectrophotometric method of Ellman, Courtney, Andres, and Featherstone (1961). Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction, respectively. 5,5′-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents and conditions were same as described in our previous publication (Senol et al., 2010). Hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalysed by enzymes at 412 nm utilising a 96-well microplate reader (VersaMax Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank (ethanol in phosphate buffer pH = 8) using the formula $(E - S)/E \times 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine (Sigma, St. Louis, MO, USA) was used as the reference.

Table 1
Total phenol and flavonoid contents and BChE inhibitory activity of the extracts of terebinth coffee samples and fruits.

Samples	Extract type	Extract yields (w/w%)	Total phenol content[a] ± SEM[b]	Total flavonoid content[c] ± SEM	Inhibitory activity against BChE (Percentage ± SEM)			
					25 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	200 μg mL^{-1}
A	EtOAc	4.06	311.79 ± 4.27	131.64 ± 2.09	21.51 ± 3.01	24.71 ± 3.71	27.91 ± 0.01	34.85 ± 0.77
	MeOH	2.98	518.57 ± 2.63	88.01 ± 1.84	–[d]	11.86 ± 1.17	20.02 ± 0.93	20.68 ± 0.70
B	EtOAc	2.90	377.14 ± 2.60	200.72 ± 3.67	18.15 ± 1.13	20.90 ± 4.61	26.15 ± 0.47	23.06 ± 0.88
	MeOH	2.39	424.29 ± 1.52	106.06 ± 0.60	–	–	8.19 ± 0.36	9.78 ± 0.85
C	EtOAc	2.41	562.14 ± 4.55	260.71 ± 0.87	–	13.73 ± 1.01	16.38 ± 1.38	29.44 ± 0.52
	MeOH	2.17	522.50 ± 4.61	78.70 ± 2.50	–	16.01 ± 3.67	22.80 ± 1.26	31.44 ± 1.68
D	EtOAc	3.58	332.15 ± 4.97	169.71 ± 4.17	12.56 ± 0.54	21.07 ± 0.03	23.36 ± 0.37	25.15 ± 1.82
	MeOH	2.57	593.57 ± 4.89	95.36 ± 3.21	9.13 ± 0.31	19.53 ± 0.38	31.82 ± 1.93	45.74 ± 2.56
Fruit	EtOAc	5.53	237.15 ± 4.55	112.30 ± 1.25	9.77 ± 0.91	19.42 ± 1.54	23.63 ± 2.40	26.99 ± 2.60
	MeOH	3.24	241.07 ± 4.14	47.03 ± 0.59	–	–	9.70 ± 0.65	22.28 ± 2.67
Galanthamine (reference for BChE inhibition)					89.95 ± 0.87 at 100 μg mL^{-1}			

[a] Data expressed in mg equivalent of gallic acid (GAE) to 1 g of extract.
[b] Standard error mean ($n = 3$).
[c] Data expressed in mg equivalent of quercetin to 1 g of extract.
[d] No activity.

2.6. Tyrosinase inhibitory activity assay

Inhibition of tyrosinase (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) was determined using the modified dopachrome method with L-DOPA as substrate (Masuda, Yamashita, Takeda, Yonemori, 2005). Assays were conducted in a 96-well microplate using ELISA microplate reader (VersaMax Molecular Devices, USA) to measure absorbance at 475 nm. An aliquot of the extracts dissolved in DMSO with 80 μL of phosphate buffer (pH 6.8), 40 μL of tyrosinase, and 40 μL of L-DOPA were put in each well. Results were compared with control (DMSO). Baicalein (Sigma, St. Louis, MO, USA) was used as the reference. The percentage tyrosinase inhibition (*I*%) was calculated as follows:

$$I\% = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}})/\text{Absorbance}_{\text{control}} \times 100$$

2.7. Antioxidant activity by radical-formation methods

2.7.1. DPPH radical scavenging activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois (1958). The samples and references dissolved in methanol (75%) were mixed with DPPH solution (1.5×10^{-4} M). Remaining DPPH amount was measured at 520 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). Gallic acid was employed as the reference. Inhibition of DPPH in percent (*I*%) was calculated as given below:

I% = $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the extracts/reference. Analyses were run in triplicates and the results were expressed as average values with SEM (Standard error mean).

2.7.2. DMPD radical scavenging activity

Principal of the assay is based on reduction of the purple-coloured radical $DMPD^+$ (*N*,*N*-dimethyl-*p*-phenylendiamine) (Schlesier, Harvat, Bohm, Bitsch, 2002). According to the method, a reagent comprising of 100 mM DMPD, 0.1 M acetate buffer (pH 5.25), and 0.05 M ferric chloride solution, which led to formation of DMPD radical, was freshly prepared and the reagent was equilibrated to an absorbance of 0.900 ± 0.100 at 505 nm. Then, the reagent was mixed up with 50 μL of the extract dilutions and absorbance was taken at 505 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). Quercetin was employed as the reference and the experiments were done in triplicates. The results were calculated according to the same formula given for DPPH radical scavenging test and expressed as average values with SEM (Standard error mean).

2.7.3. Hydrogen peroxide (H_2O_2) radical scavenging activity

H_2O_2 radical scavenging activity of the extracts was determined according to the method of Long, Evans, and Halliwel (1999). An aliquot of 50 mM H_2O_2 and various concentrations of the extracts were mixed and incubated at room temperature during 30 min. Following incubation, 10 mL of methanol and 0.9 mL of FOX reagent were added to these solutions. After vortexing, the solutions were subjected to incubation again at room temperature for another 30 min. The absorbance of the ferric-xylenol orange complex was read at 560 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). Inhibition of H_2O_2 radical in percent (*I*%) was calculated in accordance with the same formula given for DPPH radical scavenging test. Analyses were run in triplicates and the results were expressed as average values with SEM (Standard error of the mean).

2.8. Antioxidant activity by metal-related methods

2.8.1. Metal-chelation effect

The metal-chelating effect of the extracts *via* ferrous ion was estimated by the method of Chua, Tung, and Chang (2008). Briefly, various dilutions of the extracts were incubated with 2 mM $FeCl_2$ solution. The reaction was initiated by the addition of 5 mM ferrozine into the mixture and left standing at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). The ratio of inhibition of ferrozine–Fe^{2+} complex formation was calculated as follows:

Chelation% = $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$, where A_{blank} is the absorbance of the control reaction (containing only $FeCl_2$ and ferrozine), and A_{sample} is the absorbance of the extracts/reference. Analyses were run in triplicates and the results were expressed as average values with SEM The reference was ethylenediamine tetraacetic acid (EDTA) in this assay.

2.8.2. Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing power of the extracts was tested using the assay of Oyaizu (1986). Different concentrations of the extracts were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide. Later, the mixture was incubated at 50 °C for 20 min and, then, trichloroacetic acid (10%) was added. After the mixture was shaken vigorously, this solution was mixed with distilled water and ferric chloride (0.1%). After 30 min of incubation, absorbance was read at 700 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). Analyses were achieved in triplicates. Increased absorbance of the reaction meant increased reducing power and compared to that of chlorogenic acid as the reference.

2.8.3. Phosphomolybdenum-reducing antioxidant power (PRAP) assay

In order to perform PRAP assays on the extracts, each dilution was mixed 10% phosphomolybdic acid solution in ethanol (w/v) (Falcioni et al., 2002). The solution was subsequently subjected to incubation at 80 °C for 30 min and the absorbance was read at 600 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). Analyses were run in triplicates. Increased absorbance of the reaction meant increased reducing power and compared to that of quercetin as the reference.

2.9. GC and GC–MS conditions for fatty oil analysis

GC analysis was performed on an Agilent 6890N Network GC system under the following conditions; column: HP Innowax Capillary (60.0 m × 0.25 mm × 0.25 μm), oven temperature program: the column held initially at 60 °C for 3 min after injection, then increased to 185 °C with 10 °C min^{-1} heating ramp for 1 min and increased to 200 °C with 5 °C min^{-1} heating ramp for 10 min. Then the final temperature was increased to 220 °C with 5 °C min^{-1} heating ramp for 20 min, injector temperature: 250 °C, detector (FID) temperature: 275 °C, carrier gas: helium, inlet pressure: 40.65 psi, linear gas velocity: 39 cm s^{-1}, column flow rate: 2.7 mL min^{-1}, split ratio: 20:1, injection volume: 1 μL.

GC–MS analysis was performed on an Agilent 6890N Network GC system combined with Agilent 5973 Network Mass Selective Detector. The GC conditions were as follows: column: HP Innowax Capillary (60.0 m × 0.25 mm × 0.25 μm), oven temperature program: the column held initially at 60 °C for 3 min after injection, then increased to 185 °C with 10 °C min^{-1} heating ramp for 1 min and increased to 200 °C with 5 °C min^{-1} heating ramp for 10 min. Then the final temperature was increased to 220 °C with 5 °C min^{-1} heating ramp for 20 min; injector temperature: 250 °C, carrier gas: helium, inlet pressure: 40.65 psi, linear gas

velocity: 44 cm s^{-1}, column flow: 2.9 mL min^{-1}, split ratio: 20:1, injection volume: 1.0 μL. MS conditions were regulated as follows; ionisation energy: 70 eV, ion source temperature: 280 °C, interface temperature: 250 °C, mass range: 35–450 atomic mass units.

Identification of the components was assigned by comparison of their retention times and mass spectra with corresponding data from reference compounds and by comparison of their mass spectra with Wiley and Nist libraries.

2.10. GC and GC–MS conditions for essential oil analysis

GC analysis was performed on an Agilent 6890N Network GC system, under the following conditions; column: HP Innowax Capillary (60.0 m × 0.25 mm × 0.25 μm), oven temperature program: the column held initially at 60 °C for 10 min after injection, then increased to 220 °C with 4 °C min^{-1} heating ramp for 10 min and increased to 240 °C with 1 °C min^{-1} heating ramp for 5 min. Then the final temperature was increased to 240 °C with 10 °C min^{-1} heating ramp without hold, injector temperature: 250 °C, detector (FID) temperature: 250 °C, carrier gas: helium, inlet pressure: 20.93 psi, linear gas velocity: 21 cm s^{-1}, initial flow: 1 mL min^{-1}, split ratio: 30:1, injector volume: 1.0 μL.

The essential oil was analysed by GC–MS using an Agilent 6890N Network GC system combined with Agilent 5973 Network Mass Selective Detector. The GC conditions were: column: HP Innowax Capillary (60.0 m × 0.25 mm × 0.25 μm), oven temperature program: the column held initially at 60 °C for 10 min after injection, then increased to 220 °C with 4 °C min^{-1} heating ramp for 10 min and increased to 240 °C with 1 °C min^{-1} heating ramp for 5 min. Then the final temperature was increased to 240 °C with 10 °C min^{-1} heating ramp without hold, injector temperature: 250 °C; carrier gas: helium, inlet pressure: 20.93 psi, linear gas velocity: 28 cm s^{-1}, initial flow: 1.2 mL min^{-1}, column flow: 1.2 mL min^{-1}, split ratio: 30:1, injection volume: 1.0 μL. MS conditions; ionisation energy: 70 eV, ion source temperature: 230 °C, interface temperature: 280 °C, mass range: 35–450 atomic mass units.

Determination of the components was performed by comparison of their mass spectra with Wiley and Nist GC–MS Libraries and retention indices, relative to *n*-alkanes, with corresponding data from relevant literature. The percentages of the components were calculated from the GC peak areas using the normalisation method.

3. Results

3.1. Enzyme inhibitory activities of the extracts

The EtOAc and MeOH extracts of the coffee brands (*A*–*D*) and fruits of *P. terebinthus* were tested against AChE, BChE, and tyrosinase at 25, 50, 100, and 200 μg mL^{-1}. The extracts did not show inhibition against AChE and tyrosinase, while they selectively inhibited BChE at moderate levels (below 50%) at the tested concentrations (Table 1). A remarkable variation was observed among the extracts and the best BChE inhibition (45.74 ± 2.56%) was caused by the MeOH extract of coffee brand *D* at 200 μg mL^{-1}.

3.2. Total phenol and flavonoid contents of the extracts

Total phenol content of the extracts was calculated according to the equation ($y = 0.6497x + 0.3094$, $r^2 = 0.9852$) as gallic acid equivalent (mg g^{-1} extract), whilst their total flavonoid contents were determined in accordance with the equation ($y = 0.0007x + 0.0185$, $r^2 = 0.9744$) obtained by calibration curves as quercetin equivalent (mg g^{-1} extract). As given in Table 1, the richest extracts in total phenols and flavonoids were found to belong to the MeOH extract of the coffee brand *D* (593.57 ± 4.89 mg g^{-1} extract) and the EtOAc extract of the coffee brand *C* (260.71 ± 0.87 mg g^{-1} extract).

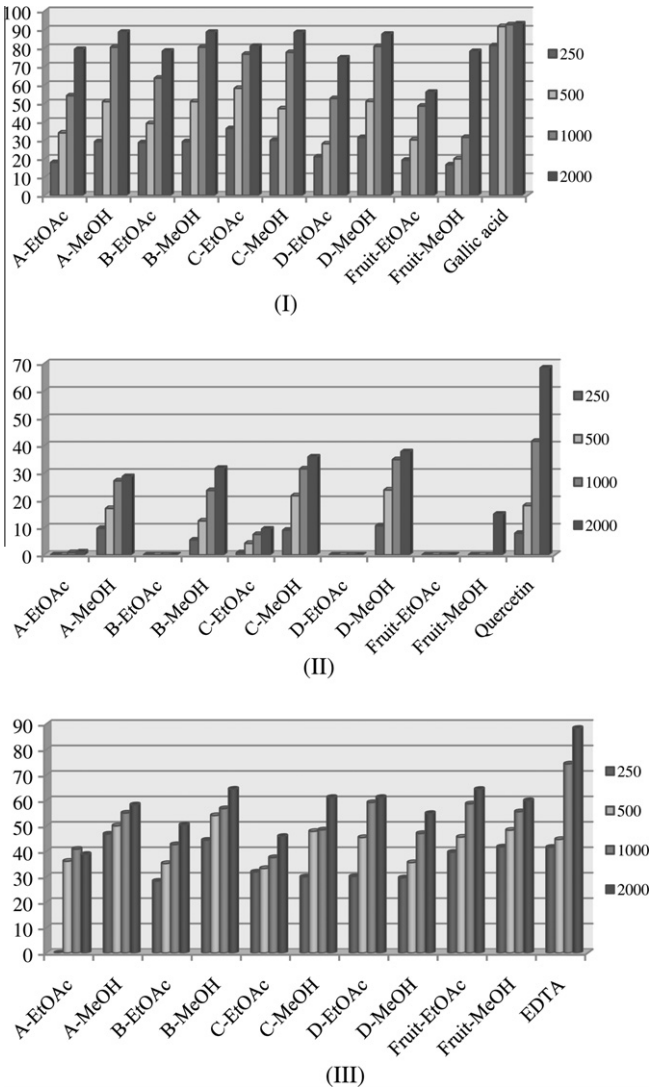

Fig. 1. DPPH (I) and DMPD (II) radical scavenging activity and metal-chelation activity (III) graphs of the extracts of terebinth coffee samples and fruits (Concentrations in μg mL^{-1}).

3.3. Antioxidant activity of the extracts

Radical scavenging activity of the extracts was assessed against DPPH, DMPD, and H_2O_2 radicals. As shown in Fig. 1, all extracts of the terebinth coffee brands displayed a high DPPH scavenging effect, especially at 2000 μg mL^{-1}. Their DMPD scavenging effect was lower as compared to their effect against DPPH. However, all of them were inactive in scavenging H_2O_2 radical at the tested concentrations. The terebinth fruit extracts exerted lower scavenging effect against DPPH and DMPD radicals than those of the terebinth coffee brands.

The extracts of both terebinth coffee brands and fruits showed remarkable metal-chelation properties as compared to that of the reference (EDTA) (Fig. 1). The fruits extracts had a slightly higher effect in this assay than those of the coffee extracts. In the FRAP assay, the extracts exerted high activity, in which the EtOAc extract of the coffee brand *C* (3.267 ± 0.14 at 2000 μg mL^{-1}) was the most potent, while the MeOH extract of the brand *A* (0.536 ± 0.23 at

Table 2
Ferric- (FRAP) and phosphomolybdenum-reducing power (PRAP) of the extracts of terebinth coffee samples and fruits.

Samples	Extract type	Ferric-reducing antioxidant power[a] (FRAP) (Absorbance at 700 nm ± SEM[b])				Phosphomolybdenum-reducing antioxidant power[a] (PRAP) (Absorbance at 600 nm ± SEM)			
		250 μg mL^{-1}	500 μg mL^{-1}	1000 μg mL^{-1}	2000 μg mL^{-1}	250 μg mL^{-1}	500 μg mL^{-1}	1000 μg mL^{-1}	2000 μg mL^{-1}
A	EtOAc	0.434 ± 0.01	0.684 ± 0.02	1.135 ± 0.06	1.549 ± 0.04	0.112 ± 0.01	0.142 ± 0.02	0.145 ± 0.02	0.151 ± 0.04
	MeOH	0.626 ± 0.03	0.990 ± 0.12	1.672 ± 0.11	2.854 ± 0.16	0.138 ± 0.01	0.327 ± 0.03	0.445 ± 0.01	0.536 ± 0.23
B	EtOAc	0.451 ± 0.01	0.802 ± 0.03	1.271 ± 0.02	2.093 ± 0.14	0.088 ± 0.03	0.089 ± 0.01	0.093 ± 0.012	0.095 ± 0.01
	MeOH	0.577 ± 0.02	0.907 ± 0.04	1.407 ± 0.05	3.172 ± 0.01	0.137 ± 0.22	0.187 ± 0.10	0.203 ± 0.02	0.308 ± 0.14
C	EtOAc	0.597 ± 0.03	1.004 ± 0.02	1.525 ± 0.15	3.267 ± 0.14	0.102 ± 0.11	0.120 ± 0.01	0.142 ± 0.05	0.246 ± 0.06
	MeOH	0.631 ± 0.03	1.043 ± 0.11	1.590 ± 0.14	3.155 ± 0.02	0.182 ± 0.01	0.189 ± 0.03	0.268 ± 0.26	0.405 ± 0.01
D	EtOAc	0.444 ± 0.01	0.678 ± 0.12	1.112 ± 0.03	1.784 ± 0.08	0.112 ± 0.01	0.131 ± 0.02	0.137 ± 0.01	0.169 ± 0.07
	MeOH	0.751 ± 0.12	1.079 ± 0.09	1.633 ± 0.01	3.245 ± 0.05	0.194 ± 0.05	0.291 ± 0.03	0.369 ± 0.01	0.523 ± 0.02
Fruit	EtOAc	0.435 ± 0.01	0.627 ± 0.02	0.997 ± 0.03	1.333 ± 0.03	0.092 ± 0.13	0.122 ± 0.01	0.128 ± 0.02	0.139 ± 0.17
	MeOH	0.481 ± 0.01	0.574 ± 0.02	0.964 ± 0.06	1.557 ± 0.16	0.117 ± 0.11	0.131 ± 0.34	0.137 ± 0.02	0.176 ± 0.02
Chlorogenic acid (reference for FRAP)					3.547 ± 0.006 at 1000 μg mL^{-1}				
Quercetin (reference for PRAP)								0.819 ± 0.001 at 1000 μg mL^{-1}	

[a] Higher absorbance indicates greater antioxidant activity.
[b] Standard error mean (n = 3).

Table 3
Fatty acid composition of the fatty oils of the terebinth coffee samples and fruits.

Samples	Fatty acid composition (%[a])			
	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
A	23.93 ± 0.19	1.79 ± 0.06	54.64 ± 0.15	19.64 ± 0.09
B	23.80 ± 0.01	1.82 ± 0.07	50.75 ± 0.05	23.63 ± 0.03
C	23.24 ± 0.01	1.78 ± 0.04	52.10 ± 0.05	22.87 ± 0.05
D	22.54 ± 0.01	1.87 ± 0.01	56.96 ± 0.01	18.63 ± 0.01
Fruit	25.62 ± 0.01	1.70 ± 0.01	53.60 ± 0.07	19.08 ± 0.02

[a] % Calculated from FID data.

Table 4
Essential oil composition of the terebinth fruits.

RRI[a]	Compounds	%[b]
1014	α-Pinene	26.31 ± 0.29
1052	Camphene	1.26 ± 0.07
1103	β-Pinene	4.57 ± 0.08
1112	Sabinene	4.71 ± 0.10
1136	Δ-3-Carene	7.08 ± 0.09
1151	β-Myrcene	3.67 ± 0.10
1184	D,L-Limonene	14.06 ± 0.21
1192	Unknown	1.88 ± 0.25
1219	*cis*-β-Ocimene	9.34 ± 0.03
1229	γ-Terpinene	0.66 ± 0.07
1236	*trans*-β-Ocimene	15.88 ± 0.13
1264	α-Terpinolene	2.08 ± 0.01
1587	Caryophyllene	5.21 ± 0.02
1703	Germacrene D	1.51 ± 0.07
Monoterpene hydrocarbons		89.62
Sesquiterpene hydrocarbons		6.72
Total		98.22

[a] Relative retention indices calculated against n-alkanes.
[b] % Calculated from FID data.

2000 μg mL^{-1}) possessed the highest PRAP value (Table 2). The fruit extracts had the lower FRAP and PRAP values than those of the coffee extracts.

3.4. Fatty and essential oil compositions

The main saturated fatty acid in the coffee brands and fruits was found to be palmitic acid ranging between 22.54 ± 0.01 and 25.62 ± 0.001% (Table 3), while oleic acid was the dominant unsaturated fatty acid (50.75 ± 0.05–56.96 ± 0.01%) in the samples, followed by linoleic acid (18.63 ± 0.01–23.63 ± 0.03%). The fatty oils of the fruits and coffee brands studied herein had a similar composition. The components identified in the essential oils are listed in Table 4 in order of their experimental relative retention indices (RRI) (Fig. 2). According to the results; α-pinene (26.31 ± 0.29%) was detected as the main component in the essential oil of the fruits of *P. terebinthus* (Table 4). *Trans* (15.88 ± 0.13%) and *cis* (9.34 ± 0.03%) isomers of β-ocimene as well as D,L-limonene (14.06 ± 0.21%) were other major constituents in the essential oil.

4. Discussion

Although the fruits of *P. terebinthus* are consumed as snack and used in production of terebinth coffee in Turkey, only a few phytopharmacological studies have been performed on the fruits so far except for some antioxidant activity reports (Bakirel et al., 2003; Durmaz Gokmen, in press; Topcu et al., 2007). According to the results we obtained, the coffee extracts usually exhibited a better activity in the test methods applied for establishment of *in vitro* neuroprotective activity. It is interesting to point out that the terebinth coffees produced after roasting of the powdered fruits have shown greater activity in these tests as compared to the fruits *per se*. In the light of this finding, a suggestion can be made that roasting process may cause an elevation in antioxidant activity of the fruits. In fact, a recent study on *P. terebinthus* oil indicated that roasting process of the fruits caused an increase in the passage of phenolic compounds into the oil whereas the level of tocopherols, lutein and β-carotene was decreased (Durmaz Gokmen, in press). The same researchers also concluded that no variation was observed in fatty acid composition of the oil due to roasting. These findings are totally consistent with our present data. According to our literature survey, there has been no study so far examining neuroprotective effect of *P. terebinthus*. On the other hand, a few studies on antioxidant activity of the terebinth fruits have been reported up to date. In a study on acetone and methanol extracts obtained from the fruits of *P. terebinthus* (Topcu et al., 2007), the extracts were tested for their antioxidant activity and both of the extracts showed a high DPPH and superoxide radical scavenging activity. This report is in accordance with our data as the extracts obtained in the current study were found to have a remarkable scavenging effect against DPPH radical. The authors concluded some flavonoid derivatives to be responsible for high antioxidant activity of the fruit extracts, which can also be applicable for our coffee and fruit extracts. On the other hand, the aqueous extract of *P. terebinthus* growing in Algeria did not show H_2O_2 scavenging effect, which is again compliant with our findings (Atmani et al., 2009).

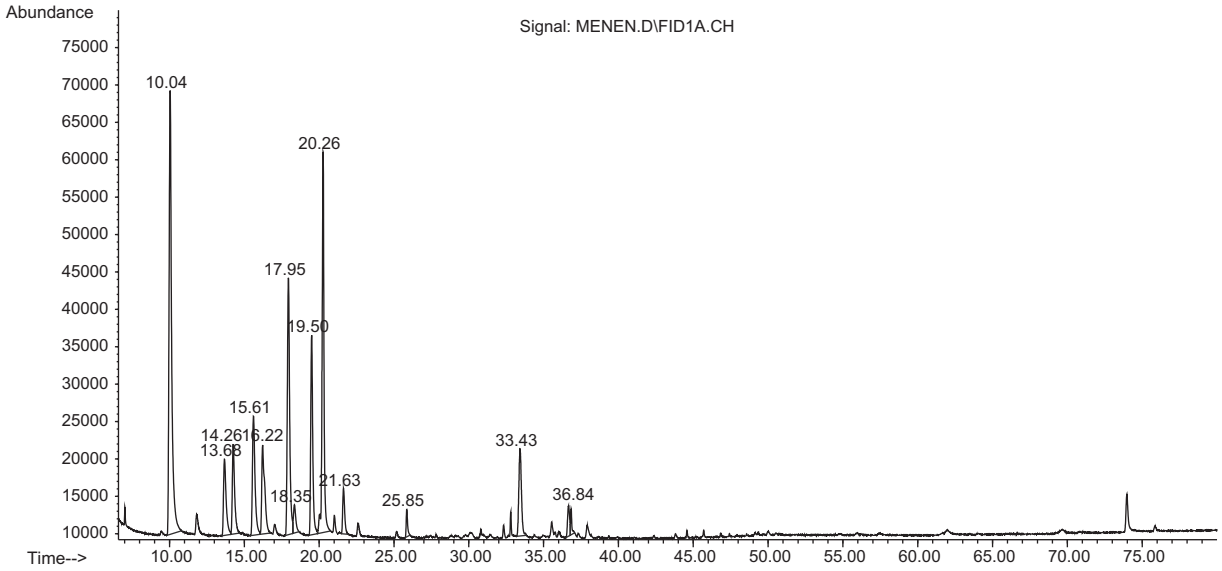

Fig. 2. GC chromatogram of the fruit essential oil of *Pistacia terebinthus*.

The crushed, powdered, and roasted terebinth fruits yield a very oily coffee consumed in southeast Anatolia, whose some commercial brands were analysed in this study. Among these coffee brands, oleic acid was found to be the main fatty acid (50.75 ± 0.05–56.96 ± 0.01%) (Table 3) in accordance with some previous reports (Ciftci, Ozkaya, Kariptas, 2009; Gecgel Arici, 2009; Matthäus Özcan, 2006; Ozcan, 2004). However, former studies performed on the fruit essential oil of terebinth tree underlined existence of some variations between those studies and our findings. For instance; Couladis, Özcan, Tzakou, and Akgül (2003) reported that limonene was the major component in unripe (34.2%) and ripe fruits (32.8%) of *P. terebinthus* of Turkish origin, whereas α-pinene was found to be the main component (26.31 ± 0.29%) in our study. In another study (Usai, Pintore, Chessa, Tirillini, 2006), the fruitful twigs of *P. terebinthus* collected from Sardinia contained α-pinene (54.8%) as the chief component, which is more similar to our results. Doubtlessly, notable variations in the oil compositions might be possibly resulting from locality and climate differences even within the same country, which was also supported by a study investigating effect of locality on oil yields and compositions of terebinth tree (Ozcan et al., 2009).

On the other hand, chelation therapy is important in modern medicine particularly in Alzheimer's disease (AD). Increased localisation of Zn, Fe, Cu and Al within the senile plaques (SP) exacerbates amyloid beta (Aβ)-mediated oxidative damage and acts as catalyst for Aβ aggregation in AD (Bandyopadhyay, Huang, Lahiri, Rogers, 2010). Therefore, chelation therapy constitutes a rational therapeutic strategy against Alzheimer's amyloid pathogenesis. In this regard, it is important to note that the coffee and fruit extracts of *P. terebinthus* were demonstrated to have a quite high metal-chelating effect through iron (Fig. 1). This might be a positive factor for these extracts to contribute to their neuroprotective activity.

5. Conclusion

Our results obtained from this work showed that the terebinth coffee brands had higher antioxidant activity than those of the fruits of the plant *per se*. This may show that roasting procedure applied to the fruits of *P. terebinthus* in order to prepare terebinth coffee might be causing an obvious increase on antioxidant activity. This finding is also in accordance with our other data that the extracts obtained from the terebinth coffee brands have much higher total phenol and flavonoid amounts than those of the fruits. It may be concluded that the terebinth coffee might provide neuroprotection to some extent, based mostly on their moderate BChE inhibitory and strong antioxidant effects *via* metal-chelation. Besides, the production technique especially roasting process of the fruits seems to be a quite important parameter for terebinth coffee to have a good level of phenolics as well as antioxidant activity.

To the best of our knowledge, this is the first evaluation of *in vitro* neuroprotective activity of the coffee and fruit extracts of *P. terebinthus* investigated by inhibition of cholinesterase and tyrosinase enzymes along with antioxidant activity test systems such as FRAP, PRAP, and metal chelation applied for the first time in the current study.

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