Turkish Journal of Agriculture and Forestry

Volume 39 | Number 1 Article 2

1-1-2015

Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish Pistacia terebinthus L. extracts

MUHAMMED ZEKİ DURAK

GÜLSÜM UÇAK

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2015) 39: 10-19

doi:10.3906/tar-1403-63

Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish *Pistacia terebinthus* L. extracts

Muhammed Zeki DURAK*, Gülsüm UÇAK
Food Engineering Department, Chemical and Metallurgical Engineering Faculty, Yıldız Technical University, İstanbul, Turkey

Received: 11.03.2014 • **Accepted:** 14.06.2014 • **Published Online:** 02.01.2015 • **Printed:** 30.01.2015

Abstract: The present study investigated 12 different *Pistacia terebinthus* L. (terebinth) fruits, harvested or purchased in Turkey, for fatty acid composition, radical scavenging, antimicrobial activity, and total phenolic content. Solvent optimization for the samples was prepared by using a simplex lattice mixture design prior to the analysis. The optimum solvent mixture was 39% water and 61% acetone, based on the maximization of extraction yield of phenolic compounds. Total phenolic contents (TPCs) of the extracts varied from 12,189 mg GAE/1000 g extract to 36,392 mg GAE/1000 g extract. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of the extracts varied over a wide range (8.86%–64.43%) and were highly correlated with TPCs. Agar diffusion was used to determine the antibacterial activity of the terebinth extracts against 2 gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and 2 gram-negative (*Escherichia coli* O157:H7 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium) bacteria. *L. monocytogenes* and *Salmonella* Typhimurium were more susceptible to the extracts than *E. coli* O157:H7 and *S. aureus*. Considering the fatty acid composition of the extracts, palmitic acid, oleic acid, and linoleic acid were the dominant saturated, monounsaturated, and polyunsaturated fatty acids, respectively. In conclusion, this study confirmed the terebinth fruits' strong bioactive and antimicrobial properties and established their fatty acid profiles, indicating their potential for a variety of applications.

Key words: Antimicrobial, antiradical, fatty acid, mixture design, phenolic, *Pistacia terebinthus* L.

1. Introduction

Extracts from various plants have been used as health remedies and as sources of healthy food since ancient times. It has been demonstrated that consumption of these kinds of plants and their derivatives protects people from the harmful effects of reactive oxygen species (superoxide anion radical, hydroxyl radical, and hydrogen peroxide) that are produced in the body by enzymatic systems through oxygen consumption (Atmani et al., 2009). These harmful species cause cellular aging (Sastre et al., 2000), mutagenesis (Takabe et al., 2001), coronary heart diseases (Khan and Baseer, 2000), and DNA breakage (Takabe et al., 2001) as a result of attacking proteins, lipids, and other molecules found in the body (Halliwell et al., 1992). The most effective prevention against the effects of these reactive species is the consumption of high antioxidant capacity products.

Pistacia terebinthus L. (terebinth) is 1 of 20 *Pistacia* species cultivated in the Mediterranean and Asian parts of Turkey (Topcu et al., 2007). Terebinth fruits have been subject of much research due to their antioxidant, antimicrobial (Topçu et al., 2007), and anti-inflammatory properties (Giner-Larza et al., 2000), and their high oil content (Matthaus and Özcan, 2006). Terebinth fruits are rich in oil (approximately 40%) containing high concentrations of unsaturated fatty acids and carotenoids, phenolic compounds and tocopherols, tannin and resinous substances, and dietary fiber (10%) (Özcan, 2004; Matthäus and Özcan, 2006). Terebinth fruits are widely used in Turkey for various purposes, including the treatment of burns, asthma, and bronchitis, as cooking oil, bread ingredients, soaps, snack foods, and in coffee (after roasting and grounding of the fruits) (Baytop, 1984; Kavak et al., 2010; Durmaz and Gökmen, 2011; Gogus et al., 2011). Bioactive characteristics, fatty acid composition, physicochemical properties, mineral content, volatile compounds, and antimicrobial properties of terebinth fruits have been investigated by different researchers (Kordali et al., 2003; Matthäus and Özcan, 2006; Kavak et al., 2010; Durmaz and Gökmen, 2011; Gogus et al., 2011; Orhan et al., 2012). However, there are no studies (to our knowledge) that compare the characteristics, the antimicrobial and antiradical activities, and the fatty acid profiles of different terebinth fruits. In this study, 12

* Correspondence: mzdurak@yildiz.edu.tr

different terebinth fruits were analyzed. In order to yield the highest amount of phenolic compound, a simplex lattice mixture design was used to determine the best solvent composition as it is well known that solvent type significantly affects extracted phenolic compound quantity (Al-Farsi and Lee, 2008; Karaman et al., 2013; Ozturk et al., 2014). In summary, our study aimed to determine: 1) the optimum solvent mixture of acetone, methanol, and water, based on maximization of phenolic contents, 2) the bioactive properties (total phenolic content and antiradical activity), 3) the antimicrobial characteristics, and 4) the fatty acid compositions of the extracts of 12 different *Pistacia terebinthus* fruits, cultivated in different regions of Turkey.

2. Materials and methods

2.1. Materials

Samples of 12 different *Pistacia terebinthus* L. subsp. *terebinthus* fruits were analyzed in this study. Ten samples were purchased from Siirt, Turkey, and the remaining 2 samples were harvested from *Pistacia terebinthus* L. subsp. *terebinthus* trees found on the campus of Yıldız Technical University in İstanbul, Turkey. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), methanol, and acetone were obtained from Merck (Darmstadt, Germany).

2.2. Solvent optimization for extraction

Prior to the determination of total phenolic contents (TPCs) and DPPH radical scavenging ability, a simplex lattice mixture design was used to determine the optimum solvent combination for methanol, acetone, and distilled water, based on maximization of the TPCs of the extracts. Table 1 presents 14 different solvent combinations used for extraction of phenolic compounds from the terebinth fruits. The 14 points were composed of 6 single-ingredient mixtures, 4 two-ingredient mixtures, 4 three-ingredient mixtures, and 5 four-ingredient mixtures. The coded levels of the solvents were set between 0 and 1 (0%–100% concentration) and the sum of the coded levels of the ingredients equals 1 as shown in the following equation:

$$0 \le X_i \le 1,\ i = 1,2,..,q,\ \text{and} \sum_{i=1}^{q} X_i = 1 \quad (1)$$

where q represents the number of ingredients and X_i represents the proportion of the ith ingredient. The establishment of the mixture design, the analysis of the data set, and the optimization of the solvent combination (for obtaining maximum TPC) were performed using Design-Expert software Version 8.0.5 (Stat-Ease Inc., Minneapolis, MN, USA). Full cubic models were generated to establish the model. Insignificant terms were then removed by the backward elimination method until all the terms in the model were significant ($P < 0.05$) and the most optimal model was obtained.

Using different solvent combinations listed in Table 1, extraction was performed according to the method of Dulger and Gonuz (2004). The dried fruit samples were

Table 1. Simplex lattice mixture design for composition of solvents used for extraction.

Mixtures	Ingredient proportions			Methanol (%)	Acetone (%)	Water (%)	TPC (mg GAE/1000 g extract)
	X_1	X_2	X_3				
1	1.00	0.00	0.00	100.00	0.00	0.00	9488
2	1.00	0.00	0.00	100.00	0.00	0.00	10364
3	0.00	1.00	0.00	0.00	100.00	0.00	15851
4	0.00	1.00	0.00	0.00	100.00	0.00	15594
5	0.00	0.00	1.00	0.00	0.00	100.00	6895
6	0.00	0.00	1.00	0.00	0.00	100.00	6569
7	0.67	0.17	0.17	66.67	16.67	16.67	9693
8	0.50	0.50	0.00	50.00	50.00	0.00	17214
9	0.50	0.50	0.00	50.00	50.00	0.00	17718
10	0.50	0.00	0.50	50.00	0.00	50.00	13174
11	0.33	0.33	0.33	33.33	33.33	33.33	15544
12	0.17	0.67	0.17	16.67	66.67	16.67	13628
13	0.17	0.17	0.67	16.67	16.67	66.67	15300
14	0.00	0.50	0.50	0.00	50.00	50.00	21361

X_1: Methanol, X_2: Acetone, X_3: Water; TPC: Total phenolic content.

finely powdered and the powdered material (20 g) was subjected to extraction using a Soxhlet apparatus with 100 mL of the solvent for 24 h. The extract was filtered through Whatman No. 1 filter paper and the solvent was evaporated under vacuum conditions at 55 °C. Terebinth extracts were stored at –18 °C until use.

2.3. Determination of the total phenolic content (TPC)

TPCs of the terebinth extracts were determined by the Folin–Ciocalteu method, reported by Singleton and Rossi (1965). Specifically, 1 g of the extract was weighed and incorporated with 10 mL of the extraction solvent, following which the mixture was vortexed for 1 min and was kept under ambient conditions for 1 h. It was then centrifuged at 7500 rpm for 5 min and the supernatant was filtered using a 0.45-µm filter. Folin–Ciocalteau's reagent (2.5 mL), diluted 1:10 with distilled water, was incorporated with 50 µL of the extract and vortexed for 1 min. Following this, 2 mL of Na_2CO_3 (7.5%, w/v) was added and the final mixture was incubated at room temperature for 30 min under dark conditions. The absorbance was measured at 765 nm using a spectrophotometer (Shimadzu UV-Visible 1700, Tokyo, Japan) against a blank, consisting of pure solvent. Results were expressed as milligrams of gallic acid equivalent per 1000 grams of sample weight (mg GAE/1000 g extract).

2.4. Determination of antiradical activity

The antiradical activity of the terebinth extracts was determined against DPPH radical according to the method described by Albayrak et al. (2010) with some modifications. Briefly, 0.1 mL of the terebinth extract, obtained with the optimized solvent combination, was mixed with 5 mL of 0.1 mM DPPH solution prepared with methanol and vortexed for 1 min. The mixture was then incubated for 30 min under ambient and dark conditions. Absorbance was measured at 517 nm using a spectrophotometer (Shimadzu UV-Visible 1700, Tokyo, Japan) against methanol as a blank. Distilled water (0.1 mL) was used as a control. Radical scavenging activity was expressed as a percentage of inhibition of the DPPH radical and was calculated by the following equation:

$$\text{Percentage inhibition (I\%)} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

2.5. Microorganisms and growth conditions

Two gram-positive (*Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* ATCC 19118) and 2 gram-negative bacteria (*Escherichia coli* O157:H7 ATCC 33150 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028) were used to assess the antibacterial activity of the terebinth extracts. All bacterial strains were activated twice in Nutrient Broth (Merck, Darmstadt, Germany) and inocula were prepared by 18-h culture in Nutrient Broth at 37 °C.

2.6. Determination of in vitro antibacterial activity

The agar diffusion method was used to determine the antibacterial effect of terebinth extracts based on the method described by Ozkan et al. (2004). Autoclaved Nutrient Agar (Merck, Darmstadt, Germany) was inoculated with each 18-h bacterial strain, targeting a final cell concentration of 10^6–10^7 CFU/mL at 43–45 °C, and was poured in petri dishes. After the solidification of agar, 4 wells were made in the agar using sterile cork pores (4 mm). Three different concentrations (1:10, 1:25, and 1:50 w:v) of the terebinth extracts were prepared using a solvent composed of 61% acetone and 39% distilled water, and 50-µL aliquots of the extracts were pipetted in the wells. A neat solvent was used as a negative control. The petri dishes were incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms in comparison to the negative control.

2.7. Determination of the fatty acid composition

Analysis of the fatty acid compositions of the terebinth extracts was performed with a gas chromatograph (Agilent 6890, AZ, USA), equipped with a flame-ionization detector and a capillary column (100 m × 0.25 mm ID, HP-88). Then 3 mL of η-hexane was added to 0.1 g of the extract, saponified with 0.1 mol/L of KOH. The mixture was vortexed for 1 min and centrifuged at 2516 rpm for 5 min at 25 °C. After centrifugation, 1 mL of the supernatant was put into GC vials. The injection temperature was set at 250 °C. Helium with a flow rate 2 mL/min and split rate 1/50 was used as the carrier gas. The oven temperature was kept at 103 °C for 1 min, then programmed from 103 °C to 170 °C at 6.5 °C/min, from 170 °C to 215 °C for 12 min at 2.75 °C/min, and finally at 230 °C for 5 min.

2.8. Statistical analysis

The results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was conducted using SPSS (SPSS 17.0, Armonk, NY, USA) to determine significant differences among TPCs, antiradical activity, and antimicrobial properties of the terebinth extracts ($P < 0.05$).

3. Results

3.1. Determination of optimum solvent mixture for TPC

Since the type of solvent has a considerable effect on the extraction yield and/or the quality of phenolic compounds from plant materials, it is crucial to select the most favorable solvent/solvent combination in order to achieve successful extraction. In the current study, a simplex lattice mixture design was used to determine the optimum solvent combination among methanol, water, and acetone

in order to maximize the phenolic extraction from the plant materials. One of the terebinth fruit samples was selected and different solvent combinations (presented in Table 1) were tested to obtain the highest phenolic extraction yield. Phenolic contents of the extracts ranged from 6569 mg GAE/1000 g extract to 21,361 mg GAE/1000 g extract (Table 1). Solvent or solvent mixture type significantly ($P < 0.05$) affected the TPC of the extract.

Sample properties such as surface porosity and phenolic profile are among the main factors affecting the selection of optimum solvent combination to be used for extraction purposes. In our study the cubic model was found to be the optimum model describing the solvent effects on the TPC of the terebinth fruits. Table 2 shows the ANOVA results of the established cubic model. It only contains the significant results because the insignificant ones were removed from the model using the backward elimination procedure. The F value of the established model was found to be 8.08 and the cubic model was significant ($P < 0.05$), indicating that this model could be satisfactorily used for optimization of the solvent mixture to maximize the extraction yield from the samples. The TPC of the sample could be predicted as a function of solvent concentration in the mixture by using the following equation:

$$TPC = 95.8X_1 + 152.7X_2 + 71.9X_3 + 1.7X_1X_2 + 2.0X_1X_3 + 4.1X_2X_3 - 0.2X_1X_2X_3 \quad (3)$$

The determination coefficient and the adjusted and predicted R^2 values of the model were 0.9038, 0.7656, and 0.6536, respectively, indicating that the TPC of the sample was very close to the predicted, as seen in Figure 1. In the present study, the ratio of 9.585 indicates that the established model satisfactorily navigates the design space. All of the linear (X_1, X_2, X_3) and interaction terms (X_1X_2, X_1X_3, X_2X_3) of the model were significant ($P < 0.1$). Figure 2 shows the effects of combinations of the solvents on the TPC of the extracts. As seen from the figure, 1:1 methanol:water yielded the highest TPC.

In order to determine phenolic contents of the samples, all solvent types found in the model should be taken into consideration. Figure 3 shows the effects of all solvent concentrations on the TPC of the sample; acetone was found to be the most effective solvent. According to the

Table 2. ANOVA table for cubic models established for effects of the variables on the TPC of the terebinth extracts.

Source of Variance	SS[b]	DF [c]	MS [d]	F value	P value
Model	2.1×10^8	6	3.5×10^7	8.08	0.0072
Linear mixture[a]	1.0×10^8	2	5.1×10^7	11.57	0.0060
X_1X_2	2.4×10^7	1	2.4×10^7	5.52	0.0511
$X_{1X}3$	2.1×10^7	1	2.1×10^7	4.76	0.0654
X_2X_3	8.6×10^7	1	8.6×10^7	19.53	0.0031
$X_1X_2X_3$	2.7×10^7	1	2.7×10^7	6.07	0.0432
Residual	3.1×10^7	7	4.4×10^6		
lack of fit	3.1×10^7	3	1.0×10^7	67.8	0.0007
pure error	6.0×10^5	4	1.5×10^5		
total corrected	2.4×10^8	13			
R^2 [e]	0.9038				
adj-R^2 [f]	0.7656				
pred-R^2 [g]	0.6536				
adequate precision	9.585				

[a] X_1: Methanol; X_2: Acetone; X_3: Water
[b] SS, sum of squares
[c] DF, degree of freedom
[d] MS, mean square
[e] R^2, coefficient of determination
[f] adjusted R^2
[g] predicted R^2
TPC: Total phenolic content.

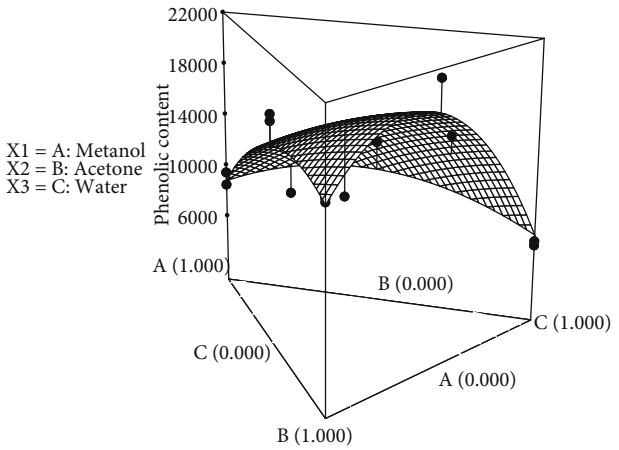

Figure 1. Experimental versus predicted phenolic contents of the terebinth extracts.

results of the simplex lattice mixture design, the optimum solvent combination yielding maximum TPC was found to be 69% acetone and 31% distilled water.

3.2. Total phenolic content and antiradical activity

TPCs and antiradical activity of 12 different terebinth extracts were investigated. The TPCs of the samples ranged from 12,189 mg GAE/1000 g extract to 36,392 mg GAE/1000 g extract (Table 3). Significant ($P < 0.05$) differences among the TPCs of the extracts were observed and the S12 sample had the highest TPC. DPPH radical scavenging activities of the terebinth extracts are presented in Table 3. Significant ($P < 0.05$) differences ranging from 8.86% to 64.43% in antiradical activities among the extracts were observed.

3.3. In vitro antibacterial activity

The in vitro antibacterial activities of the dried extracts of *Pistacia terebinthus* are shown in Table 4. All the terebinth

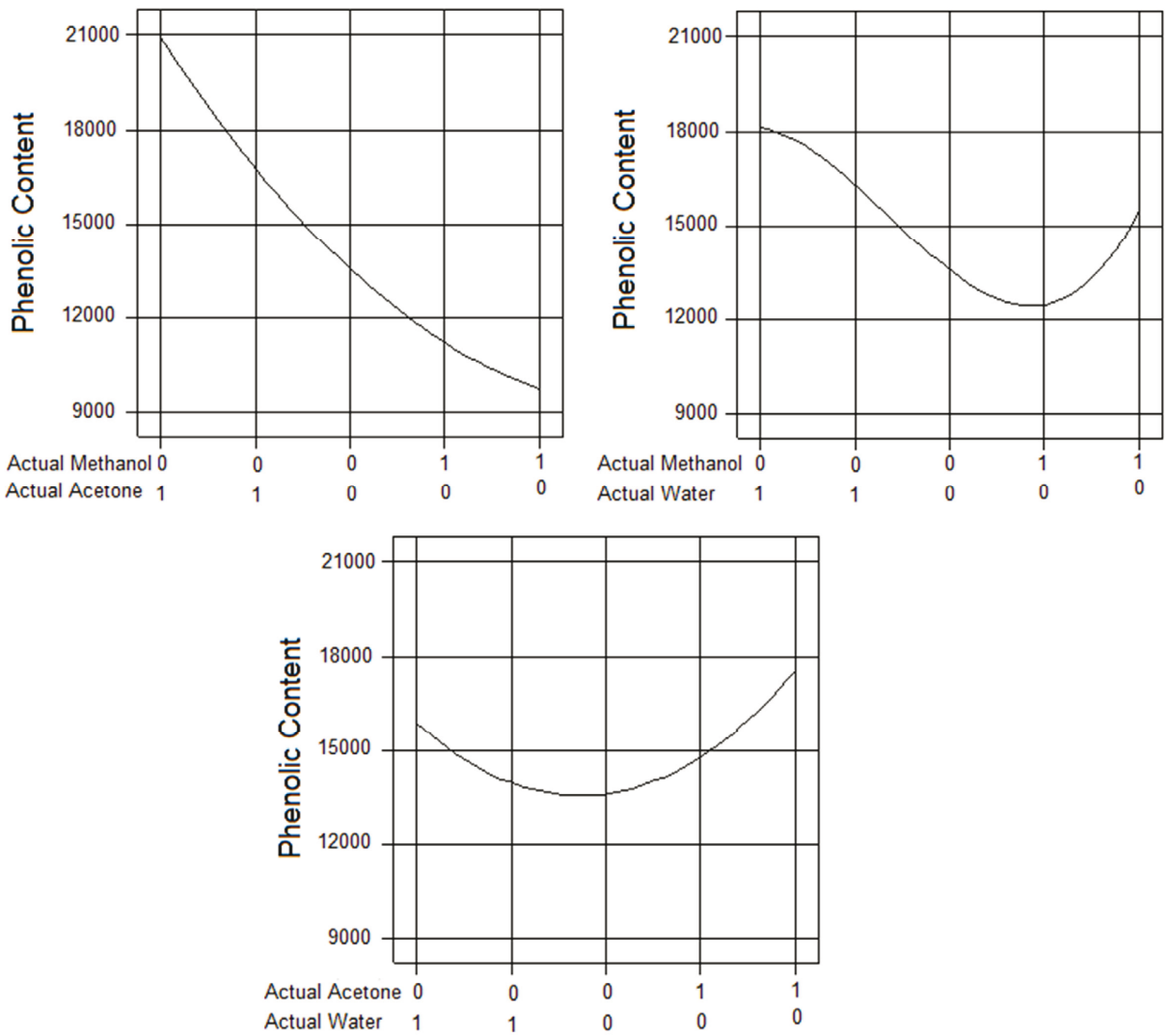

Figure 2. Effect of 2 solvent mixture compositions on phenolic content of the terebinth extracts.

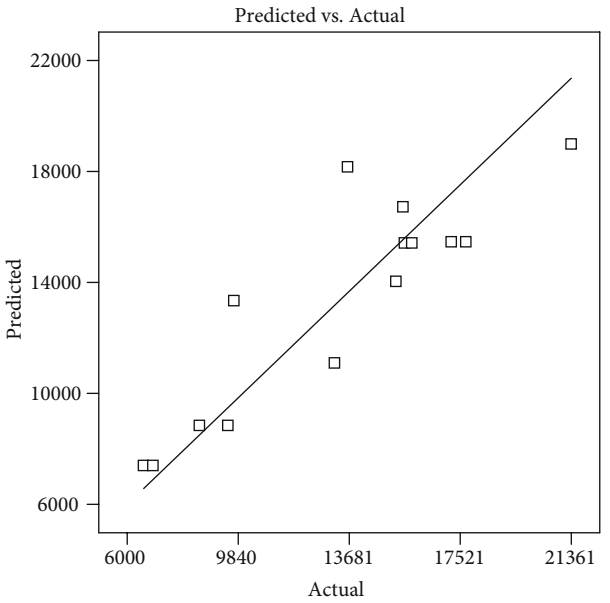

Figure 3. Effect of solvent concentration on phenolic contents of the terebinth extracts.

Table 3. TPCs and antiradical activities of the terebinth extracts.

Samples	TPC (mg GAE/ 1000 g extract)	% Inhibition (DPPH)
S1	17,629 ± 545^{d}	15.68 ± 0.23^{f}
S2	12,564 ± 139^{h}	9.23 ± 0.19^{h}
S3	16,612 ± 115^{ef}	16.83 ± 0.29^{de}
S4	14,184 ± 35^{g}	12.75 ± 0.27^{g}
S5	18,559 ± 38^{c}	14.19 ± 0.54f^{g}
S6	13,841 ± 417^{g}	12.85 ± 0.14^{g}
S7	13,627± 70^{g}	16.20 ± 0.14^{e}
S8	16,222 ± 174^{f}	18.37 ± 0.75^{d}
S9	12,189 ± 73^{h}	8.86 ± 0.34^{h}
S10	17,330 ± 86de	23.36 ± 0.20^{c}
S11	26,118 ± 142^{b}	46.32 ± 0.20^{b}
S12	36,392 ± 167^{a}	64.43 ± 1.09^{a}

TPC: Total phenolic content.

extracts showed antibacterial activity up to 1:25 dilution ratio; no inhibition zone was observed in any of the 1:50 dilutions. As expected, the level of antibacterial activity and the size of the inhibition zones were concentration dependent. The terebinth extracts showed variable inhibitory effects: *L. monocytogenes* and *S.* Typhimurium were more susceptible to the extracts than *S. aureus* and *E. coli* O157:H7. In general, the antibacterial activity of the fifth extract (S5) was lower than those of the other terebinth extracts.

3.4. Fatty acid compositions

The fatty acid compositions of the terebinth extracts are listed in Table 5. Ten different fatty acids were observed in the extracts; 4 of them were saturated and the rest

Table 4. Antimicrobial activity of the terebinth extracts.

Sample No.	*Staphylococcus aureus*		*Listeria monocytogenes*		*Salmonella* Typhimurium		*Escherichia coli* O157:H7	
	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25
S1	10.25 ± 1.26edcA	9.50 ± 0.58aB	12.75 ± 0.96baA	9.75 ± 1.50baB	14.00 ± 1.63baA	12.00 ± 0.00aB	11.00 ± 0.82baA	9.50 ± 0.58bacB
S2	11.25 ± 0.50bacA	9.50 ± 1.29aB	14.25 ± 0.96aA	9.75 ± 0.96baB	12.75 ± 0.96bdacA	10.25 ± 1.71bacB	11.50 ± 0.58aA	9.75 ± 0.50baB
S3	10.25 ± 0.96edcA	9.00 ± 0.00aB	13.00 ± 0.82baA	9.50 ± 0.58bB	15.25 ± 0.96aA	10.00 ± 0.00bacB	11.50 ± 0.58aA	9.25 ± 0.96bacB
S4	11.00 ± 0.82bdcA	8.50 ± 0.58baB	13.75 ± 1.50baA	10.25 ± 0.50baB	13.75 ± 0.96bacA	9.25 ± 0.50cdB	11.50 ± 0.58aA	10.00 ± 0.00aB
S5	9.25 ± 0.50edA	8.25 ± 0.50baB	11.25 ± 0.50bA	9.50 ± 0.58bB	11.75 ± 0.50bdcA	11.75 ± 0.50abA	8.75 ± 0.50cA	7.50 ± 0.58dB
S6	12.75 ± 1.26baA	9.00 ± 0.82aB	12.50 ± 0.58baA	9.50 ± 0.58bB	11.67 ± 0.58dcA	10.25 ± 1.50bacA	11.50 ± 0.58aA	9.00 ± 0.00bdacB
S7	13.00 ± 0.82aA	9.75 ± 0.50aB	14.50 ± 1.73aA	9.25 ± 0.50bB	12.00 ± 1.00dA	9.75 ± 0.50bcdB	11.00 ± 0.82baA	8.25 ± 0.50bdcB
S8	9.00 ± 0.00eA	8.75 ± 0.50baA	14.75 ± 0.50aA	11.50 ± 1.29aB	14.00 ± 0.00baA	11.50 ± 1.00abB	10.50 ± 0.58baA	8.00 ± 0.00dcB
S9	10.00 ± 0.00edcA	8.75 ± 0.50baB	14.00 ± 1.15aA	9.00 ± 0.00bB	11.75 ± 0.50bdc	10.00 ± 0.82bac	11.50 ± 0.58aA	7.50 ± 0.58dB
S10	9.75 ± 0.50edcA	7.25 ± 0.50bB	12.67 ± 0.58baA	9.50 ± 0.58bB	12.00 ± 0.00bcA	7.75 ± 0.50dB	10.25 ± 0.50bacA	8.75 ± 0.50bdacB
S11	10.00 ± 0.00edcA	9.50 ± 1.00aA	14.00 ± 1.63aA	10.25 ± 0.50abB	12.75 ± 0.96bac	10.75 ± 0.50bac	10.25 ± 0.96bacA	9.25 ± 1.26bacA
S12	10.25 ± 0.50edcA	8.75 ± 0.50baB	13.00 ± 0.82baA	9.75 ± 0.50abB	12.75 ± 0.96bac	10.75 ± 1.26bac	9.50 ± 1.00bcA	9.00 ± 1.15bdacA

$^{A–B}$: The same uppercase letters within the same line for each sample show that the results are not significantly different (P > 0.05). $^{a–d}$: The same lowercase letters within the same column for each sample show that the results are not significantly different (P > 0.05).

Table 5. Fatty acid composition of the terebinth extracts (%).

Formula	Fatty acid	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
C14:0	Myristic acid	0.08	0.06	0.00	0.06	0.07	0.00	0.07	0.07	0.07	0.07	0.07	0.00
C16:0	Palmitic acid	20.62	24.04	13.67	21.50	21.09	22.92	21.32	21.94	22.64	21.59	21.60	25.67
C16:1	Palmitoleic acid	1.00	4.75	1.80	2.42	1.15	5.87	3.57	6.35	8.11	1.79	1.79	1.13
C17:1	cis-10 Heptadecanoic acid	0.00	0.12	0.00	0.00	0.00	0.15	0.12	0.14	0.14	0.00	0.00	0.00
C18:0	Stearic acid	3.01	1.58	1.71	2.56	2.91	1.70	1.94	1.78	1.73	3.08	3.08	0.86
C18:1	Oleic acid	55.16	51.96	50.39	51.79	53.04	50.25	54.11	51.15	48.76	52.26	52.26	42.13
C18:2	Linoleic acid	19.00	16.22	29.64	20.62	20.33	17.70	17.51	17.16	17.08	19.92	19.92	28.17
C20:0	Arachidic acid	0.17	0.14	0.18	0.16	0.20	0.12	0.13	0.14	0.19	0.20	0.20	0.08
C18:3 n6	Gamma linolenic acid	0.64	0.93	2.32	0.65	0.93	1.10	1.02	1.10	1.08	0.82	0.81	1.82
C20:1	cis-11 Eicosonoic acid	0.22	0.18	0.22	0.22	0.28	0.18	0.21	0.19	0.19	0.28	0.27	0.12
Saturated fatty acid		23.88	25.82	15.56	24.28	24.27	24.74	23.46	23.93	24.63	24.94	24.95	26.61
Monounsaturated fatty acid		56.38	57.01	52.41	54.43	54.47	56.45	58.01	57.83	57.2	54.33	54.32	43.38
Polyunsaturated fatty acid		19.64	17.15	31.96	21.27	21.26	18.8	18.53	18.26	18.16	20.74	20.73	29.99

were unsaturated. Saturated, monounsaturated, and polyunsaturated fatty acid compositions of the terebinth extracts were 15.56%–26.61%, 43.38%–58.01%, and 17.15%–31.90%, respectively (Table 5). Eight fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, arachidic, γ-linolenic, and cis-11 eicosonoic acids) were common in all the extracts. Oleic acid was the dominant unsaturated fatty acid (42.13%–55.16%) in the extracts. Oleic acid was followed by palmitic acid (a predominantly saturated fatty acid) and linoleic acid (the most abundant polyunsaturated fatty acid), which ranged between 13.67% and 25.67% and 17.16% and 28.17%, respectively. Monounsaturated fatty acids constituted a major part of the total compositions of the extracts.

4. Discussion

4.1. Determination of the optimum solvent mixture for TPC

Considering the TPCs of the extracts obtained from the single solvents, acetone gave the highest TPC. The incorporation of water with acetone further enhanced the extraction yield. Apparently the presence of water improves the permeability of plant cell tissues, and thereby increases the mass transfer by molecular diffusion (Jayaprakasha et al., 2001). Considering the extraction yields using different solvents, however, the TPC results of the present study were not in accordance with those of Topçu et al. (2007), in which methanol extracts of terebinth had higher TPCs than those of acetone extracts. This may be due to the difference in the phenolic profiles of the fruits. Maximum phenolic content was obtained by the extraction using 1:1 acetone/distilled water combination and it was approximately 3 times higher than the minimum phenolic content obtained by the extraction using 100% water.

In the present study the established cubic model had adequate predicting ability because the R^2 values were higher than 0.75 (Henika, 1982). The experimental R^2 and predicted R^2 values of the model were not close to each other, implying that all the terms used in the model were not necessary for the establishment of the model (Cam and Aaby, 2010), which might be due to the X_1X_2 and X_1X_3 terms since they were insignificant ($P < 0.05$). Adequate precision value (representing the signal to noise ratio) of greater than 4 is desirable to navigate the design space (Toker et al., 2013).

In this study, the optimum solvent mixture was found to be 69:31 acetone:distilled water combination, giving the maximum TPC content. Both similar and dissimilar results with respect to optimum solvent mixtures for extraction of phenolics from different products have been reported (Al-Farsi and Lee, 2008; Karaman et al., 2013; Ozturk et al., 2014), which is mainly dependent on the material structure matrix and the phenolic profile found in the material. Flavanol and procyanidin extractions from berries and apples resulted in higher yields compared with methanol, hexane, and water extractions (Kahkonen et al., 2001). It is reported that an acetone/water mixture is a good solvent for the extraction of polar antioxidants (Lu and Foo, 2000). Moreover, this combination is also good for the extraction of phenolics from protein matrices since it is successful in dissolving the phenolic-protein matrices (Kallithraka et al., 1995).

4.2. Total phenolic content and antiradical activity

Flavonoids, apigenin, luteolin, luteolin 7-O glucoside, quercetin, kaempferol, and phenolics (including gallic acid and procyanidins in a polymeric form) have been

found in the genus *Pistacia* (Sanz et al., 1993; Kawashty et al., 2000; Zhao et al., 2005; Kavak et al., 2010). Durmaz and Gökmen (2011) determined tocopherols, lutein, and β-carotene in the terebinth fruit's oil in their respective amounts of 297.82 mg/kg of oil, 14.27 mg/kg of oil, and 8.75 mg/kg of oil. In the same study the TPC was 237.15 mg GAE eq/g. Orhan et al. (2012) investigated extract yields and TPCs of ethyl acetate and methanol extracts from 4 commercial terebinth coffee samples. In that study TPCs of methanol extracts were higher than those of ethyl acetate, while ethyl acetate provided higher extract yields than methanol.

The current and previous studies clearly show that TPCs of terebinth extracts remarkably differ from each other. These differences can be due to factors such as fruit species and collection sites (Farhat et al., 2013). Climate and environmental conditions are also important factors affecting the TPCs of samples. In addition, higher phenolic content was observed in immature fruits than in mature ones; unripe fruits had higher antioxidant activity (Costa et al., 2013). This was also supported in the present study: S11 and S12 fruits had lower maturity than the rest and their TPCs were significantly ($P < 0.05$) higher than those of the other fruit samples. A strong correlation ($r^2 = 0.9713$) was also found between TPCs and antiradical abilities of the extracts as demonstrated by previous studies (Rice-Evans et al., 1997; Silici et al., 2010; Tornuk et al., 2013).

4.3. In vitro antibacterial activity

Pistacia terebinthus L. is a species widely distributed in Turkey and other Mediterranean regions (Kavak et al., 2010). Terebinth extracts have demonstrated antimicrobial activities against a number of bacteria and fungi (Kordali et al., 2003; Alma et al., 2004; Dulger and Gonuz, 2004; Benhammou et al., 2008; Kavak et al., 2010; Djenane et al., 2011; Ulukanli et al., 2012). However, some gram-negative bacteria such as *E. coli* and *Klebsiella pneumoniae* were shown to be resistant to terebinth extracts by some researchers (Dulger and Gonuz, 2004; Benhammou et al., 2008). Similarly, in this study *E. coli* O157:H7 was more resistant to the pistacia extracts. However, *S.* Typhimurium (the other tested gram-negative strain) was the most susceptible one (Table 4). The antimicrobial activity of terebinth extracts can be attributed to their high phenolic contents (Table 3) since organic solvents such as ethanol and acetone allow extraction of phenolic compounds from the plant sources (Garcia-Salas et al., 2010). Some mechanisms have been used to explain the mode of antibacterial action of phenolic compounds; they include a disturbance to the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents (Burt, 2004).

4.4. Fatty acid composition

The fatty acid composition of the terebinth extracts was in accordance with the results reported by several authors (Özcan, 2004; Durmaz and Gökmen, 2011; Orhan et al., 2012). Regarding saturated and unsaturated composition, the fatty acid profiles of the terebinth extracts were more similar to the fatty acid composition of olive oil than those of other vegetable oils (hazelnut, cottonseed, canola, soybean, sunflower, and corn) investigated in previous studies (Yalcin et al., 2012a, 2012b). The fatty acid composition of the terebinth extracts demonstrated that the terebinth fruit has some health benefits because of its high oleic acid and linoleic acid content, and it is known that oleic acid has a positive effect on lowering LDL (Kahndro et al., 2008). Polyunsaturated fatty acids are beneficial for human health in terms of regulation of lipid levels (Mori et al., 2000) as well as cardiovascular (Kris et al., 2002) and immune functions (Hwang, 2000).

In general, slight differences were observed in fatty acid concentrations of the extracts studied. The year of harvest and the location of the fruits could influence the quality of the extracts due to climatic conditions (Tous and Romero, 1994; Beltrán et al., 2004; García-Inza et al., 2014; Rondanini et al., 2014). Moreover, maturity or ripening of the fruit has also been reported as a remarkable factor affecting fatty acid composition. Some studies indicate that oleic acid does not change with ripening, but saturated fatty acids such as palmitic acid might decrease in the process (Poiana and Mincione, 2004; Anastasopoulos et al., 2011). However, controversial results have been also reported: a decrease in oleic acid and an increase in linoleic acid were observed during the ripening period of olives (Arbequina variety) (Cattaneo and Karman de Sutton, 1959).

5. Conclusion

In the present study 12 different *Pistacia terebinthus* (terebinth) fruits were provided and solvent optimization was performed using 2 solvents (methanol and acetone) and distilled water by a mixture design considering the maximization of phenolic contents of the terebinth extracts. Following the optimization, antiradical and antimicrobial activities, total phenolic contents, and fatty acid profiles of the extracts were determined. The optimum solvent mixture was composed of 69% acetone and 31% water. Antiradical activities of the terebinth extracts were significantly dependent on the fruits. *S.* Typhimurium was the most susceptible bacterium against the terebinth extracts while *E. coli* O157:H7 was the most resistant one. Oleic acid was the predominant fatty acid followed by palmitic and linoleic acids. In conclusion, this study has demonstrated that terebinth fruits have the potential for a variety of applications considering their strong bioactive and antimicrobial properties and fatty acid profiles.

References

Alma MH, Nitz S, Kollmannsberger H, Digrak M, Efe FT, Yilmaz N (2004). Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (*Pistacia vera* L.). J Agr Food Chem 52: 3911–3914.

Al-Farsi MA, Lee CY (2008). Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem 108: 977–985.

Anastasopoulos E, Kalogeropoulos N, Koliora AC, Kountouri A (2011). The influence of ripening and crop year on quality indices, polyphenols, terpenic acids, squalene, fatty acid profile, and sterols in virgin olive oil (Koroneiki cv.) produced by organic versus non-organic cultivation method. Int J Food Sci Technol 46: 170–178.

Atmani D, Chaher N, Berboucha M, Ayouni K, Lounis H, Boudaoud H, Debbache N, Atmani D (2009). Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chem 112: 303–309.

Baytop T (1984). Therapy with medicinal plants in Turkey (Past and Present). Nobel Tıp Kitabevleri, İstanbul, Turkey.

Beltrán G, del Rio C, Sánchez S, Martinez L (2004). Influence of harvest date and crop yield on the fatty acid composition of virgin olive oils from cv. Picual. J Agr Food Chem 52: 3434–3440.

Benhammou B, Bekkara FA, Panovska TK (2008). Antioxidant and antimicrobial activities of the *Pistacia lentiscus* and *Pistacia atlantica* extracts. Afr J Pharm Pharmaco 2: 22–28.

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94: 223–253.

Cam M, Aaby K (2010). Optimization of extraction of apple pomace phenolics with water by response surface methodology. J Agr Food Chem 58: 9103–9111.

Cattaneo P, Karman de Sutton G (1959). Composición química de aceites de olive argentinos. Rev. Argent. Grasas Aceites 1: 3–10 (in Spanish).

Costa AGV, Garcia-Diaz DF, Jimenez P, Silva PI (2013). Bioactive compounds and health benefits of exotic tropical red-black berries. J Funct Foods 5: 539–549.

Djenane D, Yanguela J, Montanes L, Djerbal M, Roncales P (2011). Antimicrobial activity of *Pistacia lentiscus* and *Satureja montana* essential oils against *Listeria monocytogenes* CECT 935 using laboratory media: efficacy and synergistic potential in minced beef. Food Contr 22: 1046–1053.

Dulger B, Gonuz A (2004). Antimicrobial activity of certain plants used in traditional Turkish medicine. Asian J Plant Sci 3: 104–107.

Durmaz G, Gökmen V (2011). Changes in oxidative stability, antioxidant capacity, and phytochemical composition of *Pistacia terebinthus* oil with roasting. Food Chem 128: 410–414.

Farhat MB, Landoulsi A, Chaouch-Hamada R, Sotomayor JA, Jordán MJ (2013). Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats. Ind Crop Prod 49: 904–914.

García-Inza GP, Castro DN, Hall AJ, Rousseaux MC (2014). Responses to temperature of fruit dry weight, oil concentration, and oil fatty acid composition in olives (*Olea europaea* L. var. 'Arauco'). Eur J Agron 54: 107–115.

Garcia-Salas P, Morales-Soto A, Segura-Carretero A, Fernandez-Gutierrez A (2010). Phenolic compound extraction systems for fruit and vegetable samples. Molecules 15: 8813–8826.

Giner-Larza, EM, Manez S, Giner-Pons RM, Recio MC, Rios J (2000). On the anti-inflammatory and anti-phospholipase A_2 activity of extracts from lanostane-rich species. J Ethnopharmacol 73: 61–69.

Gogus F, Ozel MZ, Kocak D, Hamilton JF, Lewis AC (2011). Analysis of roasted and unroasted *Pistacia terebinthus* volatiles using direct thermal desorption-GCxGC-TOF/MS. Food Chem 129: 1258–1264.

Halliwell B, Gutteridge J, Cross C (1992). Free radicals, antioxidants, and human disease: how are we now? J Lab Clin Med 119: 598–620.

Henika RG (1982). Use of response surface methodology in sensory evaluation. Food Technol 36: 96–101.

Hwang D (2000). Fatty acids and immune responses—a new perspective in searching for clues to mechanism. Annu Rev Nutr 20: 431–456.

Jayaprakasha GK, Singh RP, Sakariah KK (2001). Antioxidant activities of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chem 73: 285–290.

Kahkonen M, Hopia A, Heinonen M (2001). Berry phenolics and their antioxidant activity. J Agr Food Chem 49: 4076–4082.

Kallithraka S, Garcia-Viguera C, Bridle P, Bakker J (1995). Survey of solvents for the extraction of grape seed phenolics. Phytochem Analysıs 6: 265–267.

Kandhro A, Sherazi STH, Mahesar SA, Bhanger MI, Younis TM, Rauf A (2008). GC-MS quantification of fatty acid profile including trans FA in the locally manufactured margarines of Pakistan. Food Chem 109: 207–211.

Kavak DD, Altiok E, Bayraktar O, Ulku S (2010). *Pistacia terebinthus* extract: as a potential antioxidant, antimicrobial, and possible β-glucuronidase inhibitor. J Mol Catal B-Enzym 64: 167–171.

Kawashty SA, Mosharrafa SAM, El-Gibali M, Saleh NAM (2000). The flavonoids of four *Pistacia* species in Egypt. Biochem Syst Ecol 28: 915–917.

Khan MA, Baseer A (2000). Increased malondialdehyde levels in coronary heart disease. J Pak Med Assoc 50: 261–264.

Kordali S, Cakir A, Zengin H, Duru ME (2003). Antifungal activities of the leaves of three *Pistacia* species grown in Turkey. Fitoterapia 74: 164–167.

Kris-Etherton PM, Harris WS, Appel LJ (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 106: 2747–2757.

Matthäus B, Özcan MM (2006). Quantitation of fatty acids, sterols, and tocopherols in turpentine (*Pistacia terebinthus*) growing wild in Turkey. J Agr Food Chem 54: 7667–7671.

Mori TA, Okamoto T, Aoyama M, Maruyama T, Niiya I, Yanagita T, Sugano M (2002). Trans fatty acids in margarines marketed in eleven countries. J Oleo Sci 51: 555–561.

Orhan IE, Senol FS, Gulpinar AR, Sekeroglu N, Kartal M, Sener B. (2012). Neuroprotective potential of some terebinth coffee brands and the unprocessed fruits of *Pistacia terebinthus* L. and their fatty and essential oil analyses. Food Chem 130: 882–888.

Ozkan G, Sagdic O, Baydar NG, Kurumahmutoglu Z. (2004). Antibacterial activities and total phenolic contents of grape pomace extracts. J Sci Food Agric 84: 1807–1811.

Ozturk I, Karaman S, Baslar M, Cam M, Caliskan O, Sagdic O, Yalcin H (2014). Aroma, sugar, and anthocyanin profile of fruit and seed of mahlab (*Prunus mahaleb* L.): optimization of bioactive compounds extraction by simplex lattice mixture design. Food Anal Method 7: 761–773.

Özcan C (2004). Characteristics of fruit and oil of terebinth (*Pistacia terebinthus* L) growing wild in Turkey. J Sci Food Agric 84: 517–520.

Poiana M, Mincione A (2004). Fatty acid evolution and composition of olive oils extracted from different olive cultivars grown in Calabrian area. Grasas Aceites 55: 282–290.

Rice-Evans C, Miller N, Paganga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci 2: 152–159.

Rondanini DP, Castro DN, Searles PS, Rousseaux MC (2014). Constructing patterns of fatty acid composition and oil accumulation during fruit growth in several olive varieties and locations in a non-Mediterranean region. Eur J Agron 52: 237–246.

Sanz MJ, Terencio MC, Paya M (1993). Pharmacological actions of a new procyanidin polymer from *Pistacia lentiscus* L. Die Pharmazie 48: 152–153.

Sastre J, Pallardo FV, Vina J (2000). Mitochondrial oxidative stress plays a key role in aging and apoptosis. IUBMB Life 49: 427–435.

Silici S, Sagdic O, Ekici L (2010). Total phenolic content, antiradical, antioxidant, and antimicrobial activities of *Rhododendron* honeys. Food Chem 121: 238–243.

Takabe W, Niki E, Uchida K, Yamada S, Satoh K, Noguchi N (2001). Oxidative stress promotes the development of transformation: involvement of a potent mutagenic lipid peroxidation product, acrolein. Carcinogenesis 22: 935–941.

Toker OS, Dogan M, Ersöz NB, Yilmaz MT (2013). Optimization of the content of 5-hydroxymethylfurfural (HMF) formed in some molasses types: HPLC-DAD analysis to determine effect of storage time and temperature levels. Ind Crop Prod 50: 137–144.

Topçu G, Ay M, Bilici A, Sarıürkcü C, Öztürk M, Ulubelen A (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. Food Chem 103: 816–822.

Tornuk F, Karaman S, Ozturk I, Toker OS, Tastemur B, Sagdic O, Dogan M, Kayacier A (2013). Quality characterization of artisanal and retail Turkish blossom honeys: determination of physicochemical, microbiological, bioactive properties and aroma profile. Ind Crop Prod 46: 124–131.

Tous J, Romero A (1994). Cultivar and location effects on olive oil quality in Catalonia (Spain). Acta Hortic 356: 323–326.

Ulukanli Z, Karaborklu S, Ozturk B, Cenet M, Balcilar M (2012). Chemical composition, antibacterial, and insecticidal activities of the essential oil from the *Pistacia terebinthus* L. spp. Palaestina (Boiss) (Anacardiaceae). J Food Process Pres 38: 815–822.

Yalcin H, Toker OS, Dogan M (2012a). Effect of oil type and fatty acid composition on dynamic and steady shear rheology of vegetable oils. J Oleo Sci 61: 181–187.

Yalcin H, Toker OS, Ozturk I, Dogan M, Kisi O (2012b). Prediction of fatty acid composition of vegetable oils based on rheological measurements using nonlinear models. Eur J Lipid Sci Tech 114: 1217–1224.

Zhao X, Sun H, Hou A, Zhao Q, Wei T, Xin W (2005). Antioxidant properties of two gallotannins isolated from the leaves of *Pistacia*. BBA-Gen Subjects 1725: 103–110.