

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

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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

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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:

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

where q represents the number of ingredients and X_i represents the proportion of the i th 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- μ m filter. Folin-Ciocalteu's reagent (2.5 mL), diluted 1:10 with distilled water, was incorporated with 50 μ L of the extract and vortexed for 1 min. Following this, 2 mL of Na₂CO₃ (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:

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 10⁶-10⁷ 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- μ L 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 \times 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 \pm 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:

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

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

^bSS, sum of squares

^cDF, degree of freedom

^dMS, mean square

^e R^2 , coefficient of determination

^fadjusted R^2

^gpredicted R^2

TPC: Total phenolic content.

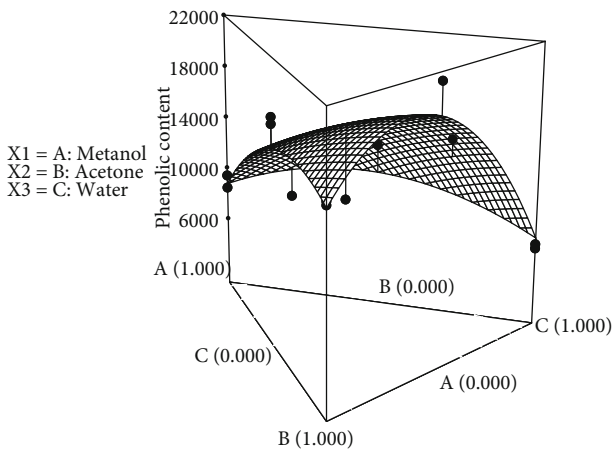


Figure 1. Experimental versus predicted phenolic contents of the terebinth extracts.

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

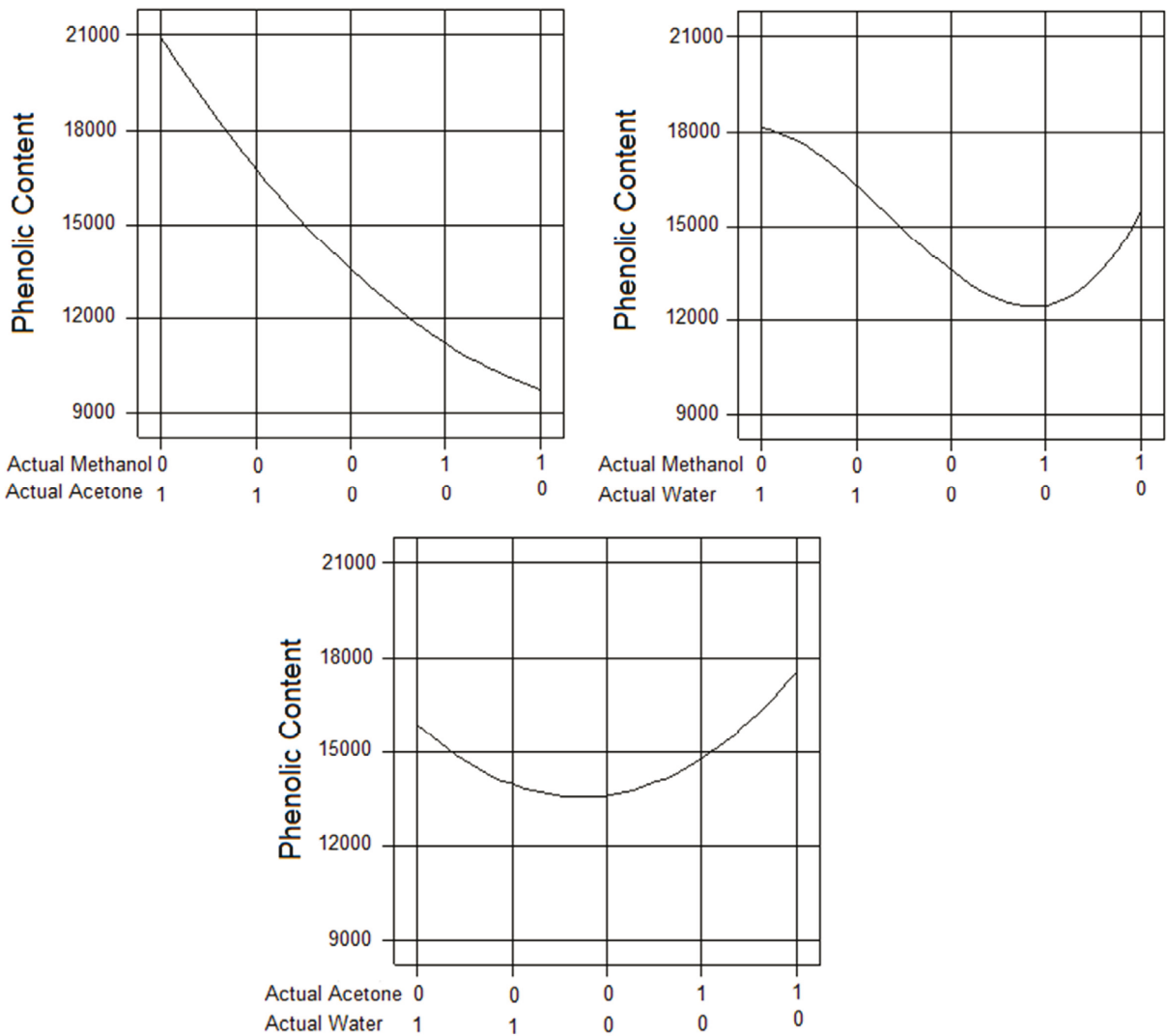


Figure 2. Effect of 2 solvent mixture compositions on phenolic content of the terebinth extracts.

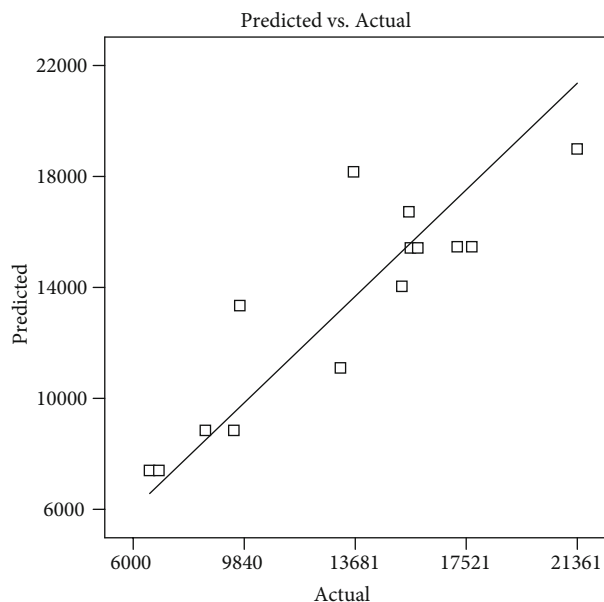


Figure 3. Effect of solvent concentration on phenolic contents of the terebinth extracts.

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

Table 4. Antimicrobial activity of the terebinth extracts.

| Sample No. | <i>Staphylococcus aureus</i> | | <i>Listeria monocytogenes</i> | | <i>Salmonella Typhimurium</i> | | <i>Escherichia coli</i> 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 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 ± 0.54 ^{fg} |
| 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 ± 86 ^{de} | 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.

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

| Formula | Fatty acid | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
|----------------------------|---------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <i>C14:0</i> | 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 |
| <i>C16:0</i> | 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 |
| <i>C16:1</i> | 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 |
| <i>C17:1</i> | 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 |
| <i>C18:0</i> | 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 |
| <i>C18:1</i> | 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 |
| <i>C18:2</i> | 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 |
| <i>C20:0</i> | 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 |
| <i>C18:3 n6</i> | 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 |
| <i>C20:1</i> | 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.

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