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Original article

Polyphenols analysed by UHPLC-ESI-MS/MS and antioxidant activities of molasses, acorn and leaves of oak (*Quercus robur* subsp. *pedunculiflora*)

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Summary. *Objectives:* The leaves of oak trees have been boiled in water and the extracts have been evaporated to produce oak molasses. Colloidal precipitants have been widely used as dietary molasses since have very effective antiseptic functions as alternative medicine for ages in some parts of the world. Present study aimed to investigate the biological activities of oak products for elucidating the scientific basis of alternative medicine. Thus, antioxidant activities of ethanol and water extracts of acorn, leaves and molasses of oak trees (*Quercus robur* subsp. *pedunculiflora*) were evaluated. *Results:* We determined antioxidant activities of extracts by different in vitro methods including DPPH free radical scavenging, $ABTS^{\bullet+}$ radical scavenging, total antioxidant activity determination by ferric thiocyanate method, reducing power by FRAP methods, separately. The results were compared with BHA, BHT and ascorbic acid as standard antioxidant compounds. The acorn seed extract, which presented the highest antioxidant potential, was the most effective scavenger against DPPH free radical. Also, phenolic compounds of the molasses were identified by UHPLC-ESI-MS/MS. The highest phenolic compound was quinic acid (4556 ± 219 ppb). *Conclusion*: Acorns and leaves of oak trees (*Quercus robur* subsp. *pedunculiflora*), as well as traditional oak molasses" have high antioxidant potential for the food industry as a food ingredient to produce functional food products.

Key words: Antioxidant, phenolic acid, nutrition, acorn, *Quercus,* LC-MS/MS

Abbreviations

BHA: Butylated Hydroxy Anisole, **BHT:** Butylated Hydroxy Toluene, **Vit C:** Vitamin C, **MYE:** ethanol extract of oak leaves, **MYS:** water extract of oak leaves, **PKE:** ethanol extract of acorn shell, **PKS:** water extract of acorn shell, **PÇE:** ethanol extract of acorn seed, **PÇS:** water extract of acorn seed, **GPE:** ethanol extract of oak molasses, **GPS:** water extract of oak molasses

Introduction

Oak is classified in the genus of *Quercus*, which includes more than 300 species. Oak trees are characterized by spirally arranged leaves and acorn fruits. The acorn is the nut of oak that contains a single seed. Some animals such as birds, horses, squirrels, pigs, bears, and small mammals consume large amounts of acorns because of their taste and abundanct. Acorns have been used as a source of food for many cultures around the world especially in famine. Acorns have frequently been used as food substitutes. The nutritional compositions of acorns were determined and large amounts of water, protein, carbohydrates, fats, and some minerals were detected (1). Acorns contain tannins, which are plant polyphenols and also found in several beverages including red wine, beer, coffee, black tea, green tea, and many foodstuffs such as

grapes, pears, bananas, sorghum, black-eyed peas, lentils and chocolate (2).

Reactive oxygen species (ROS) play important roles in many biological processes. Also they can cause significant damages ranging from reversible biochemical modifications to permanent DNA alterations (3, 4). Antioxidants have been suggested to prevent cellular oxidative damage of ROS and to explain the mechanism of numerous diseases that related with oxidative stress. Antioxidants can inhibit the effect of oxidants by donating a hydrogen atom or by chelating metals (5, 6).

Numerous of plants such as herbs, fruits and vegetables have been reported as main sources of natural antioxidants. Thus, consumption of these foods is related to reduce risk of cancers, cardiovascular diseases, cataracts, brain dysfunction (7). The extracts from *Quercus resinosa* leaves presented high phenolic content, antioxidant capacity and cardioprotective effect (8). The phenolic compound and antioxidant activity of *Quercus robur* methanol extracts were investigated previously (9). However, there has been little information regarding the antioxidant activities of acorns and leaves of oak trees (*Quercus robur* subsp. *pedunculiflora*). Therefore, we purposed to investigate the in vitro antioxidant activities of acornsd leaves ano molasses that obtained from thefoak trees (*Quercus robur* subsp. *pedunculiflora*).

Phenolic acids and flavonoids are regularly found in plant extracts (10, 11). Polyphenols play important roles in adsorbing and neutralizing free radicals, or decomposing peroxides. The antioxidant activiyies of phenolic compounds is mainly attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors and quenchers of singlet oxygen (12).

Also, another aim of this work is to evaluate the antioxidant activities and phenolic contents of molasses that obtained from secretion of oak leaves. The oak molasses have been produced and locally named as "gezo molasses" by some farmers of Southeastern Anatolia, Turkey. The molasses have been consumed on diet, as well as used in traditional medicine as anticarcinogenic and antiseptic agents. According to the results, quinic acid and malic acid were identified by UHPLC-ESI-MS/MS, as the main phenolic acids of oak molasses.

Materials and Methods

Plant material

The plant materials, acorns and leaves of oak trees were gathered from Muş, Turkey. Oak molasses obtained from a local farmer. The leaves of plant were dried in air. The air dried leaves and acorns were crumb and collected until used. Dr. Fevzi Özgökçe identified the plant species as *Quercus robur* subsp. *pedunculiflora*

Chemicals

BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), ascorbic acid, ABTS (2, 2-azinobis 3-ethylbenzthiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picryl-hydrazyl), Folin-Ciocalteu's reagent, linoleic acid, polyoxyethylenesorbitan monolaurate (Tween-20), trichloroacetic acid (TCA) and standard phenolic compounds of LC-MS/MS were obtained from Sigma-Aldrich. The other chemicals were obtained from Merck.

Preparation of water and ethanol extracts

For preparation of water extracts, 20 g of each air-dried leaves, acorn seeds and acorn shells were cut into pieces by a blender and added to 200 mL distilled water (1/10: w/v), separately. Mixtures were stirred by a magnetic stirrer during 12 hours at room temperature, and filtered with filter paper. The filtrates were frozen and lyophilised in a lyophiliser (Labconco, Freezone 1L) at 5 mm Hg at -50˚C. The lyophilised samples were stored at -30˚C until analysis.

For preparation of ethanol extracts, 20 g of each air-dried leaves, acorn seed and acorn shell samples were cut into pieces by a blender and added to 200 mL ethanol (1/10: w/v), separately. Mixtures were stirred by a magnetic stirrer during 12 hours at room temperature, and filtered with filter paper. The filtrates were evaporated with a rotary evaporator (Heidolph 94200, Bioblock Scientific). The evaporated samples were stored at -30˚C until analysis.

DPPH• scavenging activity

DPPH· free radical scavenging capabilities of extracts were evaluated according to the method reported by Gülçin (13). This method based on the reaction of the antioxidants with DPPH free radicals. Accord-

ing to this method, different concentrations (10-30 μg/mL) of extracts and standard antioxidants (BHA, BHT, ascorbic acid) were prepared and adjusted to 3 mL with ethanol. Then, 1 mL of ethanolic DPPH radical solution (0.1 mM) was added to the each sample. These samples were vortexed and incubated in the dark at 30°C for 30 minutes. The absorbance was measured at 517 nm by using spectrophotometer (Shimadzu, UV-1800, Japan). Decreasing absorbance indicates DPPH free radical scavenging activity.

$ABTS^{\bullet+}$ scavenging activity

$ABTS^{\bullet+}$ radical scavenging activities of extracts were evaluated by method of Re et al., (14) with a slight modification. First of all, the $ABTS^{\bullet+}$ cation radical was produced by reacting ABTS (2 mM) in H_2O and potassium persulphate (2.45 mM) at room temperature for 12 hours. $ABTS^{\bullet+}$ is dark blue-green in color with a characteristic absorbance at 734 nm. The solution of $ABTS^{\bullet+}$ was diluted with phosphate buffer (0.1 M, pH 7.4) to achieve an absorbance of 0.750±0.025 at 734 nm. Then, 1 mL of $ABTS^{\bullet+}$ solution was added to 3 mL of extract solution in methanol at different concentrations (10-30 mg/mL) of extracts. These samples were vortexed and incubated in the dark for 30 minutes. Finally, absorbances at 734 nm were measured for each sample. Decreasing absorbance of a sample indicates its $ABTS^{\bullet+}$ cation radical scavenging activity.

Ferric ions reducing antioxidant power (FRAP)

Ferric (Fe^{3+}) reducing powers of samples analyzed by FRAP method (15) with a slight modification (16). According to this method, reducing powers of extracts were determined by measuring the reduction of Fe^{3+} to Fe^{2+}. This method is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the antioxidants. For this purpose, different concentrations (10-30 μg/mL) of extracts in 0.75 mL distilled water were prepared. Each preparation was mixed with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL (1%) of potassium ferricyanide [$K_3Fe(CN)_6$], separately. The mixtures were incubated at 50°C for 20 min. After incubation period, the mixtures were acidified with 1 mL of TCA (10%). Finally, 0.25 mL of $FeCl_3$ (0.1%) was added to the each solution. Distilled water was used as blank and for control instead of sample. The absorbance measurements of the mixtures were obtained at 700 nm using a UV spectrophotometer. Increasing absorbance indicates higher ferric reducing power.

Total antioxidant activity determination by ferric thiocyanate method

The total antioxidant activities of samples were determined according to the ferric thiocyanate (TCN) method (17). For this purpose, different concentrations (10-20 μg/mL) of extracts were pipetted into the test tubes and completed to 2.5 mL by sodium phosphate buffer (0.04 M, pH 7.0) and 2.5 mL of linoleic acid emulsion was added to each test tube. The linoleic acid emulsion was prepared by homogenizing 612 mL of linoleic acid, 560 mg of Tween-20 as emulsifier and 100 mL phosphate buffer (0.04 M, pH 7.0). The control was prepared with 2.5 mL of linoleic acid emulsion and 2.5 mL 0.04 M sodium phosphate buffer (pH 7.0). The reaction mixtures (5 mL) were incubated at 37°C in polyethylene flasks. The peroxide levels were determined by reading the absorbance at 500 nm in a spectrophotometer after reactions with $FeCl_2$ and thiocyanate at intervals during incubation. The peroxides formed during linoleic acid peroxidation oxidize Fe^{2+} to Fe^{3+}, and formed a complex with thiocyanate that has a maximum absorbance at 500 nm. So, lower absorbance indicates higher inhibition of peroxidation of linoleic acid emulsion. The assay steps were repeated every 8 hour until maximum absorbance of control was reached.

Identification of phenolics by LC-MS-MS analysis
Preparation of plant extract for LC–MS/MS

The air-dried and powdered plant materials (100 g) were extracted three times with 300 mL of methanol for 24 h at room temperature. The solvent was removed under vacuum at 3°C in a rotary evaporator until dry methanol extracts were obtained (Yield: 15.6%). Dry filtrates were diluted to 1000 mg/L and filtrated with 0.2 μm microfiber filter prior to LC–MS/MS analysis.

LC–MS/MS instrumentation and chromatographic conditions

LC–MS/MS analyzes of the phenolic compounds were performed by using a Nexera model Shimadzu UHPLC coupled to a tandem MS instrument. The

liquid chromatography was equipped with LC-30AD binary pumps, DGU-20A3R degasser, CTO-10ASvp column oven and SIL-30AC autosampler. The chromatographic separation was performed on a C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 μm) analytical column. The column temperature was fixed at 4°C. The elution gradient consisted of mobile phase A (water, 5 mM ammonium formate and 0.1% formic acid) and mobile phase B (methanol, 5 mM ammonium formate and 0.1%formic acid). The gradient program with the following proportions of solvent B was applied t (min), B%: (0, 40), (20, 90), (23.99, 90), (24, 40), (29, 40). The solvent flow rate was maintained at 0.5 mL/min and injection volume was settled as 4 μL.

MS instrumentation

MS detection was performed using Shimadzu LCMS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. LC–MS/MS data was collected and processed by LabSolutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analyzes: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation. The optimum ESI conditions were determined as interface temperature; 35°C, DL temperature; 25°C, heat block temperature; 40°C, nebulizing gas flow (nitrogen); 3 L/min and drying gas flow (nitrogen); 15 L/min (18).

Statistical analysis

The experimental results were performed in triplicate. The data was recorded as mean ± standard deviation and analyzed by SPSS (version 17.0 SPSS Inc.). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's Multiple Range tests. $P < 0.05$ was significant.

Results and Discussion

Antioxidant compounds play important roles to scavenge and inhibie the free radicals. In recent years, investigative studies about new natural sources of antioxidants became more popular (19). This research study is related to radical scavenging, reducing power and inhibition of lipid peroxidation potential of different parts of oak trees (*Quercus robur* subsp. *pedunculiflora*).

Free or ionic radicals are highly reactive species that are responsible for many cell disorders through their action on proteins, lipids and DNA (20). Radical scavenging of samples indicate their antioxidant activities to prevent the oxidation chain initiation. DPPH and ABTS assays have been widely used for determination of the radical scavenging activities of various samples by many researchers (21). Therefore, we used both radical scavenging methods on the present study. The percentages of scavenging capabilities of DPPH free radical and ABTS cation radical were calculated using the following equation:

$$\text{Radical scavenging capability}\ (\%) = \left(1 - \frac{A_{\text{Sample}}}{A_{\text{Control}}}\right) \times 100$$

DPPH• scavenging activity

The DPPH free radical scavenging activities of samples and standard antioxidants (BHA, BHT, vitamin C) were investigated. It was observed that ethanol extract of acorn seed (PÇE) had effective and the most powerful DPPH radical scavenging activity than that of samples. The results indicated that ethanol extract of acorn seed has effective DPPH radical scavenging activities same as standard antioxidants levels (Fig. 1). Also, some extracts (MYE, PÇS and PKE)

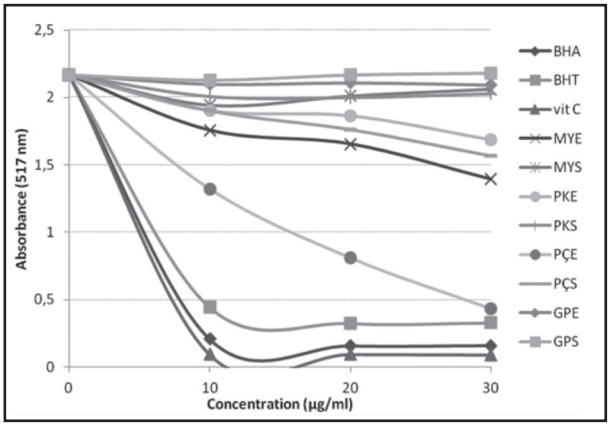

Figure 1. DPPH free radical scavenging activities of samples and standard

demonstrated few free radicals scavenging activity. On the other hand, other extracts (PKS, GPE, MYS and GPS) did not show any scavenging capacity against DPPH• free radical up to the highest tested concentration (30 μg/mL).

DPPH free radical inhibition percentages of extracts and standard antioxidants at the same concentration (30 μg/mL) decreased in the order of vit C (95.8%) > BHA (93.1%) > BHT (84.9%) > PÇE (80.0%) > MYE (35.8%) > PÇS (27.6%) > PKE (22.2%) > PKS (6.5%) > MYS (4.9%) > GPE (3.4%) > GPS (0%).

$ABTS^{\bullet+}$ (cation radical) scavenging activity

ABTS radical cation inhibition of extracts and standard antioxidants at the same concentration (10-30 μg/mL) were determined (Fig. 2). According to the results of the present study GPE, GPS and MYS extracts demonstrated low scavenging capacity as compared to standard antioxidants. On the other hand, other extracts did not show any scavenging capacity against ABTS radical cation, up to the highest tested concentration (30 μg/mL). Gezo molasses exracts showed higher scavenging effects than other extracts.

Reducing power assay (FRAP)

The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species (22). Plants might contain some reductones, which could react with free radicals to stabilize and terminate radical chain reactions (23). In the present study, we investigated reducing powers of different extracts of parts of oak trees by FRAP assay.

Antioxidant compounds cause the reduction of Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) form because of their reductive capabilities. As shown on Fig. 3, ferric reducing powers increased with increasing the concentration of extracts and standard antioxidants. However, increasing potential of extract were not high and significiant as compared to standard antioxidants.

Ferric ion (Fe^{3+}) and cupric ion (Cu^{2+}) reductions are often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (24). Therefore, increasing reduction power indicates increasing antioxidant potential.

Total antioxidant activity

Oxidation of lipids, which is the main cause of quality deterioration in many food systems, may lead to off-flavors and formation of toxic compounds, and may lower the quality and nutritional value of foods. Lipid oxidation is associated with aging, membrane damage, heart disease and cancer. Thus, prevention of lipid peroxidation is connected with antioxidant properties.

Linoleic acid mixture was used as control. The percentage of inhibition was calculated at 48th hour point that the highest absorbance point of control. The percentage of inhibition of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

$$\text{Inhibition of lipid peroxidation } (\%) = \left(1 - \frac{A_{\text{Sample}}}{A_{\text{Control}}}\right) \times 100$$

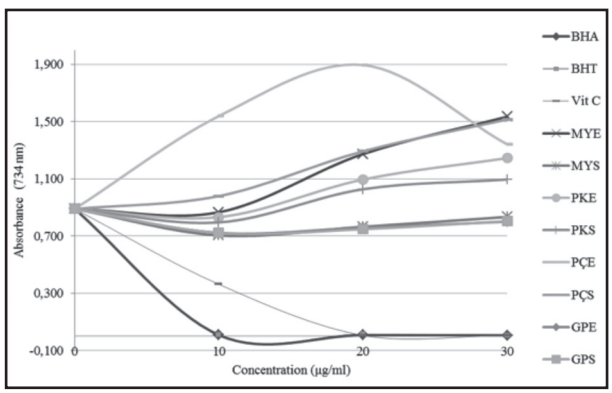

Figure 2. ABTS cation radical scavenging activities of samples and standard

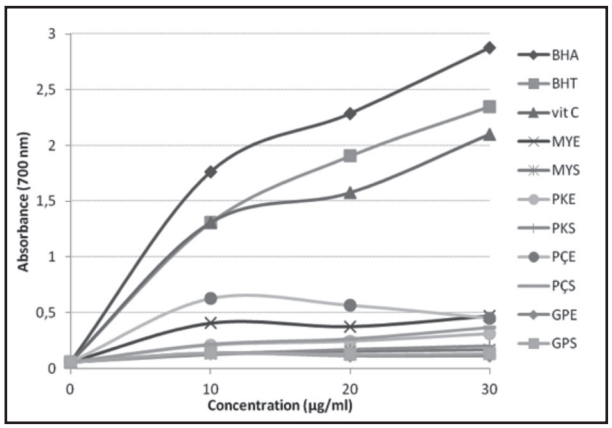

Figure 3. Reducing powers of samples and standard antioxidants by FRAP assay

The inhibition percentages of lipid peroxidation in linoleic acid emulsion of samples and standard antioxidants were determined and the results are given in Fig. 4. The datas are averages of three concentrations (10 μg/mL, 20 μg/mL and 30 μg/mL). The inhibition effect of linoleic acid emulsion peroxidation of samples and standard antioxidants at 48th hour decreased in the order of vit C (59.0%) > BHA (56.9%) > BHT (48.4%) > PKS (31.6%) > GPS (31.1) > PÇS (26.3%) > GPE (14.4%). The inhibition effects of linoleic acid emulsion peroxidation of other samples were detected very low levels.

Identification of phenolics by LC-MS-MS analyses

Phenolic compounds which contain at least one hydroxyl group on an aromatic ring, are present ubiquitously in plants. Phenolic compounds can interrupt chain oxidation reactions by donation of a hydrogen atom or chelating metals. The plants that contain high amounts of phenolic compounds have efficient antioxidant properties. Polyphenols present in fruits can scavenge the free radicals and destroy the oxidation pathways initiated by free radicals like lipid peroxidation and DNA damage in the human body (25).

Many methods such as; spectrophotometric and enzymatic methods have been used for determination of the phenolic acids in plants or foods. Liquid chromatography with various detection techniques has been widely used to determine organic acids because of its reproducibility. UV–Vis spectrophotometry technique has been often used for the detection of organic acids. Because of the high sensitivity and selectivity, liquid chromatography coupled to ESI-MS has received considerable attention for the determination of organic acids (26).

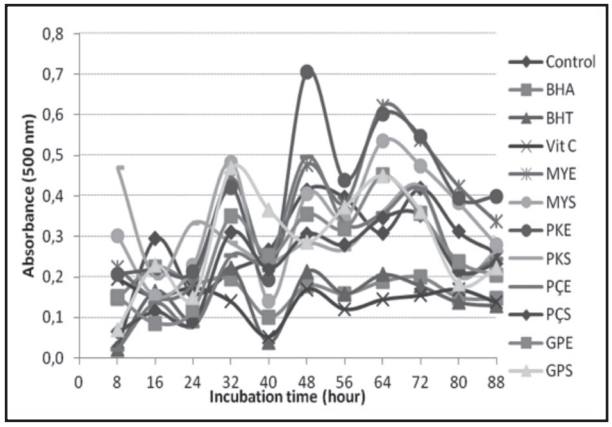

Figure 4. Antioxidant activities of samples and standard antioxidants (by ferric thiocyanate (TCN) method

In this study, the identifications of phenolic acids in oak molasses were analyzed by UHPLC-ESI-MS/MS. Referring to Table 1, Fig. 5 and Fig. 6; quinic acid (4556 ± 219 ppb) and malic acid (421 ± 22 ppb) were identified as the major phenolic compounds, quantitatively. The chromatograms of standards ando molasses extracts are shown in Fig. 5 and Fig. 6, respectively. The peaks belonging to quinic acid and malic acid are clearly visible.

Phenolic compounds contain hydroxyl groups which have significiant roles on antioxidant properties. The ability of the polyphenolic compounds to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups. Also, the structure of aromatic ring and the number of hydroxyl groups are important associated factors on antioxidant abilities. It was reported that compounds with structures containing functional hydroxyl groups can show metal chelating activity (27). It was indicated that many phenolic compounds such as; curcumin, L-adrenaline, L-carnitine and quercetin bounded ferrous ions (Fe^{2+}) through the hydroxyl functional groups and chelated metal ions (13). Recently, Kacem et al. (28) reported the antioxidant activities of *Ruta chalepensis* extracts and attributed these activities to the presence of phenols, flavonoids, ortho-diphenols, tannin and flavonols.

Quinic acid and malic acid were detected from the molasses by UHPLC-ESI-MS/MS method. Both quinic and malic acids contain more than two hydroxyl groups which have significiant roles on antioxidant properties. The chemical structures of quinic and malic acids are shown in Fig. 7a and Fig. 7b. Malic acid is a component of citric acid cycle. Quinic acid is obtained from cinchona bark, coffee beans, and other plant products. It is a constituent of tannins. It was reported that quinic acid had been found on different plant extracts (29).

Conclusion

The antioxidant and antiradical activities of both water and ethanol extracts of oak leaves, oak molas-

Table 1. LC-MS/MS parameters of selected compounds

No	Analytes	RT[a]	Parent ion (m/z)[b]	Ionization Mode	R^{2c}	RSD%[d]	Linearity Range (mg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)	U[f]
1	Quinic acid	3.32	190.95	Neg	0.9927	0.0388	250-10000	22.3 / 74.5	103.3	4.8
2	Malic acid	3.54	133.05	Neg	0.9975	0.1214	250-10000	19.2 / 64.1	101.4	5.3
3	tr-Aconitic acid	4.13	172.85	Neg	0.9933	0.3908	250-10000	15.6 / 51.9	102.8	4.9
4	Gallic acid	4.29	169.05	Neg	0.9901	0.4734	25-1000	4.8 / 15.9	102.3	5.1
5	Chlorogenic acid	5.43	353	Neg	0.9932	0.1882	250-10000	7.3 / 24.3	99.7	4.9
6	Protocatechuic acid	5.63	152.95	Neg	0.9991	0.5958	100-4000	25.8 / 85.9	100.2	5.1
7	Tannic acid	6.46	182.95	Neg	0.9955	0.9075	100-4000	10.2 / 34.2	97.8	5.1
8	tr- caffeic acid	7.37	178.95	Neg	0.9942	1.0080	25-1000	4.4 / 14.7	98.6	5.2
9	Vanillin	8.77	151.05	Neg	0.9995	0.4094	250-10000	10.1 / 33.7	99.2	4.9
10	p-Coumaric acid	9.53	162.95	Neg	0.9909	1.1358	100-4000	15.2 / 50.8	98.4	5.1
11	Rosmarinic acid	9.57	358.9	Neg	0.9992	0.5220	250-10000	10.4 / 34.8	101.7	4.9
12	Rutin	10.18	609.1	Neg	0.9971	0.8146	250-10000	17.0 / 56.6	102.2	5.0
13	Hesperidin	9.69	611.1	Poz	0.9973	0.1363	250-10000	21.6 / 71.9	100.2	4.9
14	Hyperoside	10.43	463.1	Neg	0.9549	0.2135	100-4000	12.4 / 41.4	98.5	4.9
15	4-OH Benzoic acid	11.72	136.95	Neg	0.9925	1.4013	25-1000	3.0 / 10.0	106.2	5.2
16	Salicylic acid	11.72	136.95	Neg	0.9904	0.6619	25-1000	4 / 13.3	106.2	5.0
17	Myricetin	11.94	317	Neg	0.9991	2.8247	100-4000	9.9 / 32.9	106.0	5.9
18	Fisetin	12.61	284.95	Neg	0.9988	2.4262	100-4000	10.7 / 35.6	96.9	5.5
19	Coumarin	12.52	146.95	Poz	0.9924	0.4203	100-4000	9.1 / 30.4	104.4	4.9
20	Quercetin	14.48	300.9	Neg	0.9995	4.3149	25-1000	2.0 / 6.8	98.9	7.1
21	Naringenin	14.66	270.95	Neg	0.9956	2.0200	25-1000	2.6 / 8.8	97.0	5.5
22	Hesperetin	15.29	300.95	Neg	0.9961	1.0164	25-1000	3.3/ 11.0	102.4	5.3
23	Luteolin	15.43	284.95	Neg	0.9992	3.9487	25-1000	5.8 / 19.4	105.4	6.9
24	Kaempferol	15.43	284.95	Neg	0.9917	0.5885	25-1000	2.0 / 6.6	99.1	5.2
25	Apigenin	17.31	268.95	Neg	0.9954	0.6782	25-1000	0.1 / 0.3	98.9	5.3
26	Rhamnetin	18.94	314.95	Neg	0.9994	2.5678	25-1000	0.2 / 0.7	100.8	6.1
27	Chrysin	21.18	253	Neg	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	5.3

[a]RT: Retention time; [b]Parent ion (m/z): Molecular ions of the standard compounds (mass to charge ratio); [c]R^2: coefficient of determination; [d]RSD: relative standard deviation; [e]LOD/LOQ (µg/L): Limit of deteection/Limit of quantification; [f] U (%): Percent relative uncertainty at 95% confidence level (k=2); [g] Values in µg/g (w/w) of plant methanol extract; [h]N.D: not detected.

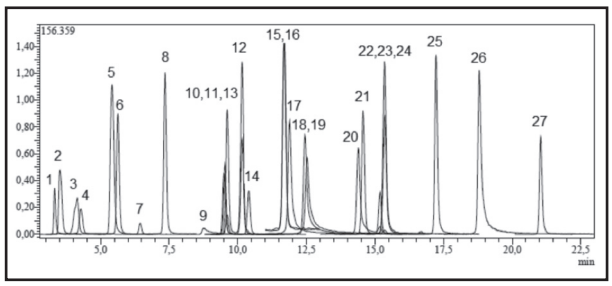

Figure 5. UHPLC-ESI-MS/MS chromatogramsstandard compounds

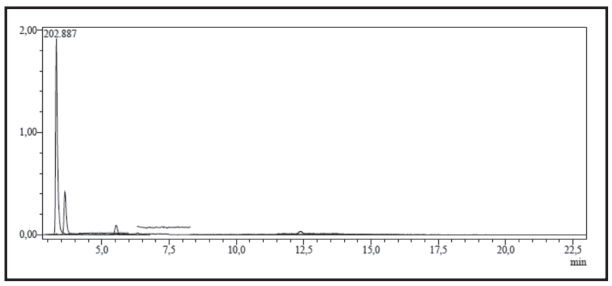

Figure 6. UHPLC-ESI-MS/MS chromatogram of oak molasses

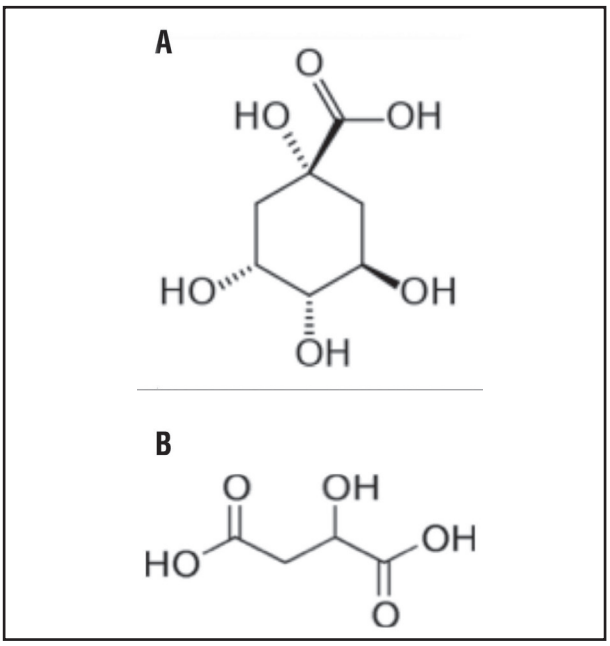

Figure 7. A) Quinic acid; **B)** Malic acid

ses, acorn seeds and shells grown in Turkey were investigated. Also, phenolic composition of molasses that obtained from the leaves of oak trees (*Quercus robur* subsp. *pedunculiflora*) was investigated. The results revealed that both acorn seed and acorn shell extracts exhibited good antioxidant ability on DPPH radical scavenging activity and linoleic acid peroxidation. Antioxidant and antiradical activities of both water and ethanol extracts of acorn seed (PÇE and PÇS) were higher than the other samples in DPPH assay. Also, inhibition of lipid peroxidation in linoleic acid emulsion of PKS, GPS and PÇS were found to be higher than the other samples, that result is consistent with previously mentioned methods. Conversely, ABTS radical scavenging activity and reducing power of ferric ions of samples were found to be low capacities, that result is inconsistent with previously mentioned methods.

In addition, phenolic contents in oak molasses were determined by LC-MS-MS analyses; quinic acid and malic acid had higher quantities among 27 different phenolic acids. The effective antioxidant and antiradical activities of different parts of oak trees might be related with the rich amounts of phenolic compounds. As a result, acorns and leaves of oak trees (*Quercus robur* subsp. *pedunculiflora*) have a good potential to be used in the food industry as a food ingredient to produce functional food products.

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