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# A new flavone from antioxidant extracts of *Pistacia terebinthus*

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**Abstract**

Acetone and methanol extracts of the fruits of *Pistacia terebinthus* L. subsp. *terebinthus* L. were studied for their antioxidant activity by investigating their total phenolic and flavonoid contents, β-carotene bleaching potential, DPPH radical scavenging effect, scavenging activity on superoxide anion radical, reducing power, and metal chelating effect on ferrous ion. Both extracts showed very similar chemical profile by checking on TLC plates, and exhibited high scavenging activity on superoxide anion radical and DPPH radical. Due to these similarities they were combined and fractionated on a silica gel column for their constituents, and the most active three fractions in DPPH assay were purified to afford a new flavone 6′-hydroxyhypolaetin 3′-methyl ether (**1**) besides a group of known flavonoids apigenin, luteolin, luteolin 7-*O*-glucoside, quercetin, quercetagetin 3-methyl ether 7-*O*-glucoside, isoscutellarein 8-*O*-glucoside. Their structures were established by UV, UV shift reagents, and $^1$H NMR spectroscopic techniques. Antioxidant activity of the new flavone was investigated by β-carotene bleaching and DPPH radical scavenging activity methods, and it showed a high activity in the first system, but not so good in the latter.


*Keywords:* *Pistacia terebinthus*; Antioxidant activity; Flavonoids; 6′-Hydroxyhypolaetin 3′-Methyl ether

## 1. Introduction

*Pistacia terebinthus* L. (Anacardiaceae) is one of the 20 *Pistacia* species widely distributed in the Mediterranean region and Asia possessing many biological activities. *Pistacia* species have attracted the attention of researchers because of their antioxidant potential besides antimicrobial, anti-inflammatory and cytotoxic activities, particularly due to flavonoids and other phenolic constituents. In Turkey, the leaves of *P. terebinthus* ssp. *terebinthus* (known as menengiç) are used in the treatment of burns. The resin (*terebinthus* = *Terebenthina chia*, *T. cypria*), locally known as menengiç sakızı, collected from branches of *P. terebinthus* ssp. *terebinthus*, used as an antiseptic for bronchites and other respiratory and urinary system diseases and is given orally in 0.2–0.5 g doses three times daily. It helps to cure asthma, and it also has anti-inflammatory and antipyretic properties (Baytop, 1984). The fruits are consumed as coffee; the fat extracted from its fruits is used as cooking oil as well as in soap production (known as bıttım sabunu) in a certain section of Turkey (Baytop, 1984; Tanker and Tanker, 1998). An investigation showed some hypolipidemic effect of the dried fruits' extract of *P. terebinthus* on the rabbits without toxic effect (Bakirel et al., 2003). Likewise, another study by Edwards, Kwaw, Matud, and Kurtz (1999) showed some reduction of artheriosclerosis and correction of lipid profile. Recent studies indicated that a decrease in blood cholesterol level of about 1% will reduce artheriosclerosis' incidence by about 2%, and antioxidants play an important role to decrease cholesterol level (Riemersma et al., 1991), and another study on antiatherogenic

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effect of *P. lentiscus* extract showed that it restored GSH (intracellular antioxidant glutathione) levels and downregulated CD36 (a class B scavenger receptor) expression (Dedoussis et al., 2004). The bark of *P. lentiscus*is used for the treatment of hypertension in some regions of Spain (Rant Wyllie, Brophy, Sarafis, and Hobbs, 1990). The resin of the same plant exhibited antioxidant and antimicrobial activities (Abdel-Rahman and Soad, 1975; Iauk, Ragusa, Rapisarda, Franco, and Nicolosi, 1996; Magiatis, Melliou, Skaltsounis, Chinou, and Mitaku, 1999). Furthermore, quantitative determination of α-tocopherol in *P. terebinthus, P. lentiscus* and *P. lentiscus* var. *chia* growing in Turkey, was established and the highest amount was found in *P. lentiscus* var. *chia* (Kıvçak and Akay, 2005). Antifungal activity was established in three *Pistacia* species, namely *P. vera, P. terebinthus* and *P. lentiscus* against an agricultural pathogenic fungi (Duru et al., 2003; Kordali, Çakır, Zengin, and Duru, 2003). Lipophylic extracts of different parts of *P. vera* were screened against standard and some isolated bacteria, fungi and viruses and showed noticeable antifungal activity while the kernel and seed extracts exhibited significant antiviral activity (Ozcelik, Aslan, Orhan, and Karaoğlu, 2005). Anti-inflammatory activity (Nishimura, Taki, Takaishi, Iijima, and Akiyama, 2000) was found in triterpenic compounds of *P.terebinthus* (Giner-Larza et al., 2001; Giner-Larza et al., 2002). The chemical constituents of the *Pistacia* genus were studied and monoterpenes (Monaco, Previtera, and Mangoni, 1982), tetracyclic triterpenoids (Ansari, Ali, and Quadry, 1993) besides other triterpenoids (Caputo, Mangoni, Monaco, and Palumbo, 1975; Caputo et al., 1978), flavonoids (Kawashty, Mosharrafa, El-Gibali, and Saleh, 2000) and other phenolics including gallic acid (Shi and Zuo, 1992; Zhao et al., 2005), essential oils (Küsmenoglu, Başer, and Özek, 1995) were found. Procyanidins in a polymeric form were also isolated from *P. lentiscus* with hypotensive activity (Sanz, Terencio, and Payá, 1993, 1992). Six gallotannins and seven flavonoid glycosides were isolated from *P. weinmannifolia* (Hou, Peng, Liu, Lin, and Sun, 2000). Pistafolia A, a novel gallotannin isolated from the leaf extract of *P. weinmannifolia* showed more potent antioxidant capacity than that of Trolox which is water soluble analogue of Vitamin E (Wei et al., 2002). The other two novel gallotannins from *P. weinmannifolia* also showed high protective effects against oxidative damage of biomacromolecules (lipids, proteins and DNA) due to their free radical scavenging ability (Zhao et al., 2005). In a study on four *Pistacia* species growing in Egypt, *P. khinjuk* contained myricetin glycosides while the other three species *P. atlantica, P. lentiscus, P. chinensis* contain quercetin 3-glucoside (Kawashty et al., 2000). In another study from *P. lentiscus* besides gallic acid and galloyl derivatives, anthocyanins, and flavonoid glycosides, namely myricetin and quercetin glycosides, were also obtained (Romani, Pinelli, Galardi, Mulinacci, and Tattini, 2002).

In this work, we aimed to study antioxidant activity guided isolation of the fruits of *P. terebinthus* and investigated active flavonoidal fractions to determine their chemical structures which are responsible for the antioxidant activity.

## 2. Materials and methods

### 2.1. Plant material

*Pistacia terebinthus* L. subsp. *terebinthus* L. was collected from Çanakkale (Dardenelles) Kepez district, it was identified by Dr. Ismet Uysal (University of Çanakkale). A voucher specimen is deposited (COMU-179) in the Herbarium of Biology Department, Faculty of Science and Arts, Canakkale 18 Mart University.

### 2.2. Preparation of the extracts

Air dried and powdered fruit material of the plant (600 g) was sifted using a 10 MESH-sieve (1600 μm, average), then extracted with petroleum ether (40–60 °C), acetone and methanol, respectively. Extractions were carried out by Soxhlet apparatus using 1 L for each solvent to yield 8 g, 10 g and 10 g residues, respectively, upon evaporation under vacuum. The extracts were checked on TLC plates, since the acetone and methanol extracts were found to be similar, they were combined. The combined extract (20 g) was subjected to a silica gel column (5 × 70 cm) (E. Merck, Darmstadt – Germany, 200 mesh) for chromatographic separation. The elution of the column was started with $CH_2Cl_2$ (1.5 L), and followed by gradients of acetone (starting from 5% acetone up to 100%), collecting 40 fraction (each 150 mL), and then gradients of MeOH (starting from 1% MeOH up to 50%) were added, 50 fractions were collected (each 150 mL). The fractions were tested on TLC plates and the similar ones were combined, thus 15 fractions were obtained and were tested on DPPH system. Three fractions [the first one was obtained by the elution of $CH_2Cl_2$:acetone (8:2, v/v), the second $CH_2Cl_2$/acetone (7:3, v/v) and the third acetone/MeOH (9:1, v/v)] were found to be most active ones, and were separated on Sephadex LH-20 columns. The first fraction was eluted with petroleum ether: $CH_2Cl_2$:$CH_3OH$ (7:4:1, v/v/v), and apigenin (8 mg) (Mabry, Markham, and Thomas, 1970), luteolin (9 mg), quercetin (20 mg) were isolated. The second active fraction was eluted with $CH_2Cl_2$:$CH_3OH$ (8:2, v/v) which afforded the new compound 6′-hydroxyhypolaetin 3′-methyl ether (**1**) (15 mg). The third fraction was eluted with only $CH_3OH$ yielding luteolin 7-*O*-glucoside (8.5 mg) (Mabry et al., 1970), quercetagetin 3-methyl ether 7-*O*-glucoside (7 mg) (Ulubelen, Kerr, and Mabry, 1980), and isoscutellarein 8-*O*-glucoside (12 mg) (Markham and Porter, 1975).

### 2.3. Spectral measurements

*Instruments*: UV spectra were recorded on Shimadzu UV–visible 1601 Spectrophotometer, Kyoto, Japan. $^1$H- and $^{13}$C-NMR were run on Varian $^{UNITY}$INOVA, New

York, NY, USA; 500 MHz for proton, 125 MHz for carbon, respectively.

*6′-Hydroxyhypolaetin 3′-methyl ether* (**1**) *(5,7,8,3′,4′,6′-hexahydroxyflavone 3′-methyl ether)*: Pale yellow solid. UV $\lambda_{max}$ (nm) MeOH: 333.5, 271.5; $NaOCH_3$: 397.0, 331.5, 280.5; $AlCl_3$: 397.0, 305.0; $AlCl_3 + HCl$: 394.5, 303.5, 279.0; (NaOAc): 381.0, 280.5; $NaOAc + H_3BO_3$: 322.0, 273.5. $^1$H-NMR ($\delta$, in $CDCl_3$): It was given in the text (see Fig. 1).

### 2.4. Antioxidant activity

Antioxidant activity was carried out using five different test systems (Figs. 2–6) for the extracts while only two of them DPPH scavenging activity and β-carotene-linoleic acid methods were used for the new compound 6′-hydroxyhypolaetin 3′-methyl ether (**1**) due to its small quantity. The concentration of total phenolics and flavonoids was also calculated for the extracts (Table 1).

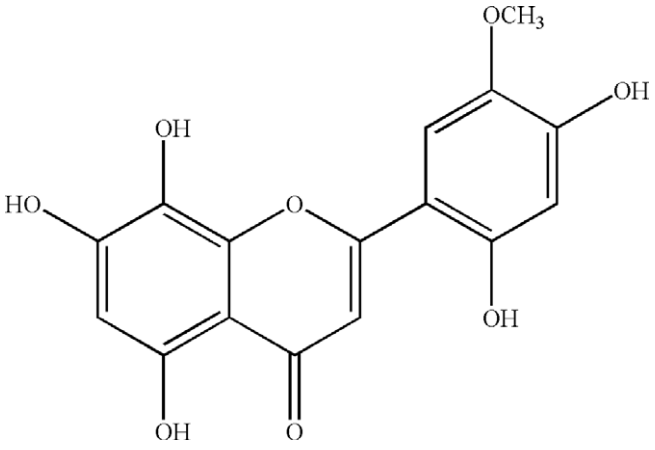


Fig. 1. 6′-Hydroxyhypolaetin 3′-methyl ether (**1**).

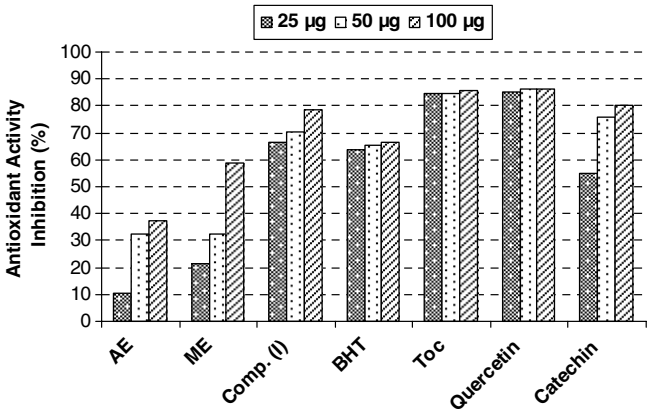


Fig. 2. Inhibition (%) of lipid peroxidation of *P. terebinthus* extracts, Comp. (**1**), BHT, TOC, Quercetin and (+)-Catechin by the β-Carotene bleaching method. [BHT: butylated hydroxytoluene; TOC: α-tocopherol; AE: Acetone extract of *P. terebinthus*; ME: Methanol extract of *P. terebinthus*, Comp. (**1**): 6′-hydroxyhypolaetin 3′-methyl ether (**1**)]. Values are mean ±SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

#### 2.4.1. Chemicals

Ascorbic acid, potassium ferricyanide, ferrous chloride, ferric chloride, Folin–Ciocalteu's reagent (FCR), polyoxyethylenesorbitan monolaurate (Tween 20), methanol, and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT) and α-tocopherol (TOC), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH) were obtained from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). Nitroblue tetrazolium (NBT) and phenazine methosulphate (PMS) were obtained from Fluka Chemical Co. (Fluka GmbH, Steinheim, Germany, packed in Switzerland). All other chemicals and solvents were of analytical grade.

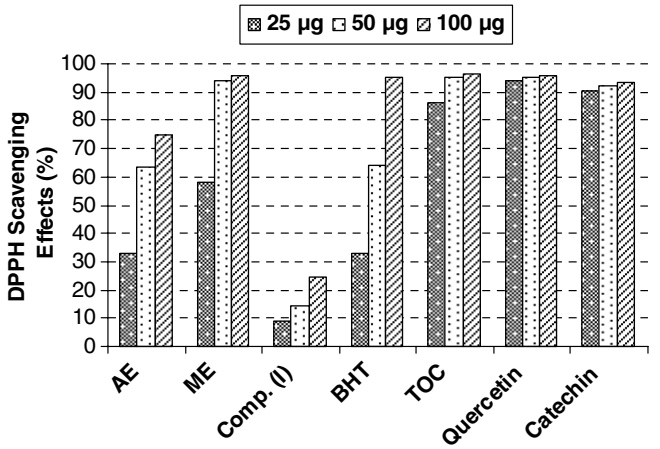


Fig. 3. Free radical scavenging activity of *P. terebinthus* extracts, Comp. (**1**), BHT, TOC, Quercetin and (+)-Catechin by 1,1-diphenyl-2-picrylhydrazyl radical [Comp. (**1**): 6′-hydroxyhypolaetin 3′-methyl ether (**1**)]. Values are mean ±SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

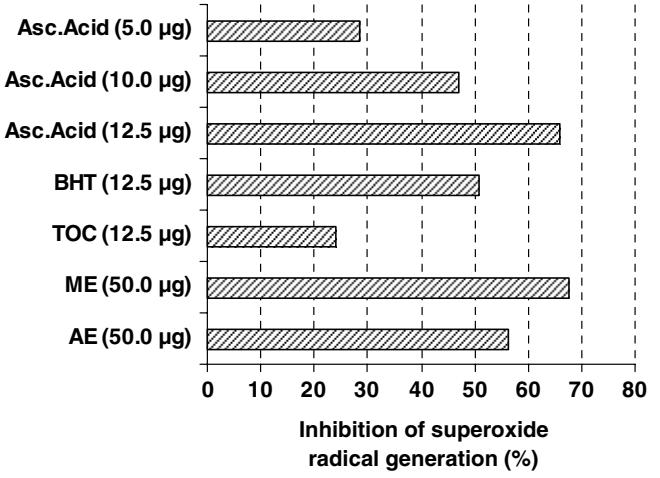


Fig. 4. Superoxide anion radical scavenging activity of *P. terebinthus* extracts, BHT, TOC and Asc.Acid by the PMS-NADH-NBT method. [Asc.Acid: L-ascorbic acid]. Values are mean ±SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

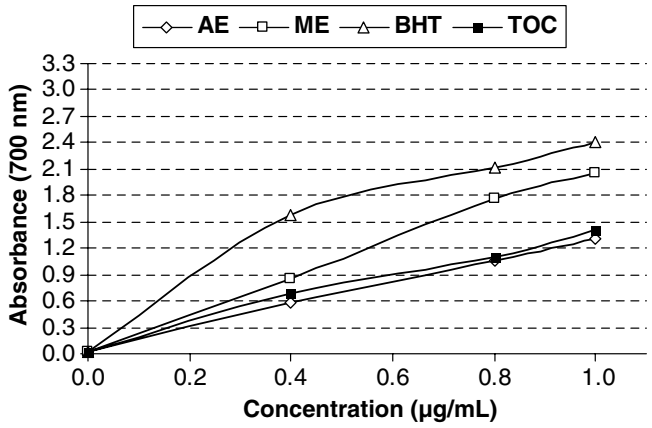


Fig. 5. Reducing power effect of different concentrations of *P. terebinthus* extracts, BHT and TOC by the spectrophotometric detection of the $Fe^{3+}$–$Fe^{2+}$ transformations. Values are mean ±SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

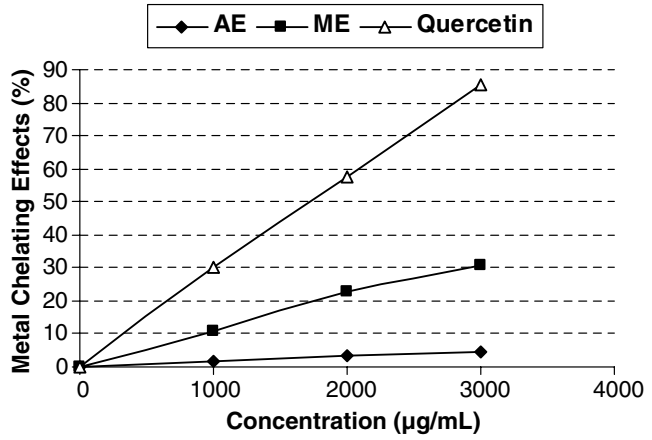


Fig. 6. Metal chelating effect of different concentrations of *P. terebinthus* extracts, and Quercetin by the ferrozine method on ferrous ions. Values are mean ±SD, $n = 3$, $p < 0.05$, significantly different with Student's *t*-test.

### 2.4.2. Determination of the antioxidant activity with the β-carotene bleaching method

The antioxidant activity of *P. terebinthus* extracts and the new compound (**1**) was evaluated in a β-carotene-linoleic acid model system (Miller, 1971). A solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two millilitres of this solution, 40 μL of linoleic acid and 400 μL of Tween 20 emulsifier were pipetted into a 100 mL round-bottomed flask. After chloroform was removed under vacuum, 100 mL of aerated distilled water were added to the flask with vigorous shaking. Aliquots (4 mL) of this emulsion were transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). The tubes were placed in a water bath at 50 °C. Measurement of absorbance was continued until the colour of β-carotene disappeared; a blank, devoid of β-carotene, was prepared for background subtraction. The bleaching rate ($R$) of β-carotene was calculated according to Eq. (1)

$$R = \ln(a/b)/t \quad (1)$$

where ln = natural log, $a$ = absorbance at time 0, $b$ = absorbance at time $t$ (120 min) (Cheung, Cheung, and Ooi, 2003). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using Eq. (2)

$$\mathrm{AA} = [(R_{\mathrm{control}} - R_{\mathrm{sample}})/R_{\mathrm{control}}] \times 100 \quad (2)$$

Table 1
Total phenolic and flavonoid contents of the *P. terebinthus* extracts

| Sample | Phenolic content (μg PEs/mg extract)[b] | Flavonoid content (μg QEs/mg extract)[c] |
|---|---|---|
| Acetone extract | 61.05 ± 3.35[a] | 5.49 ± 0.78[a] |
| Methanol extract | 122.78 ± 0.02[a] | 22.60 ± 0.96[a] |

[a] Values expressed are means ± SD of three parallel measurements ($P < 0.05$).
[b] PEs, pyrocatechol equivalents.
[c] QEs, quercetin equivalents.

### 2.4.3. Determination of total phenolic concentration

The concentrations of phenolic compounds in all extracts were expressed as micrograms of pyrocatechol equivalents (PEs), determined with Folin–Ciocalteu reagent (FCR) according to the method of Slinkard and Singleton (1977). One millilitre of the solution (contains 1 mg) extract in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed throughly. After 3 min, 3 mL of sodium carbonate (2%) were added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated to the following equation that was obtained using standard pyrocatechol curve:

$$\text{Absorbance} = 0.00209\ \text{pyrocatechol}\ (\mu g) + 0.00466 \quad (R^2 = 0.9920)$$

### 2.4.4. Determination of total flavonoid concentration

Measurement of flavonoid concentration in *P. terebinthus* extracts was based on the method described by Moreno, Isla, Sampietro, and Vattuone (2000) with slight modifications. An aliquot of 1 mL of the solution (contains 1 mg) extracts in methanol was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of methanol. After 40 min at room temperature, the absorbance was read spectrophotometrically at 415 nm. Quercetin was used as a standard (Park, Koo, Ikegaki, and Contado, 1997). The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.01626 quercetin (μg) − 0.0062

($R^2 = 0.9970$)

*2.4.5. Free radical scavenging activity*

Radical scavenging activity of *P. terebinthus* extracts and the new compound **1** was determined using DPPH as a reagent (Cuendet, Hostettmann, and Potterat, 1997; Kirby and Schmidt, 1997) with some modifications. Briefly, a 0.004% of DPPH radical solution in methanol was prepared and then, 4 mL of this solution were mixed with 1 mL of sample solution in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm using a spectrophotometer. The scavenging capability of DPPH radical was calculated using the following equation:

$$\text{DPPH}^{\cdot}\text{scavenging effect } (\%) = 100 - [(A_1/A_0) \times 100]$$

where $A_0$ is the absorbance of control and $A_1$ is the absorbance of samples and standards.

*2.4.6. Superoxide anion scavenging activity*

Measurement of superoxide anion scavenging activity of *P. terebinthus* extracts was based on the method described by Liu, Ooi, and Chang (1997) with a slight modification. Superoxide radicals were generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment, superoxide radicals were generated in 3 mL of Tris–HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and sample solutions. The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\text{Inhibition of superoxide generation } (\%) = [(A_0 - A_1)/A_0] \times 100$$

where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of *P. terebinthus* extracts or standards (Gülçin, Oktay, Kireçci, and Küfrevioğlu, 2003; Ye, Wang, Liu, and Ng, 2000).

*2.4.7. Reducing power*

The reducing power of *P. terebinthus* extracts was determined according to the method of Oyaizu (1986). Sample solutions at different amounts (0.4–1 mg) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. Afterwards 2.5 mL of TCA (10%) was added, the mixture was centrifuged for 10 min at 1000*g* (MSE Mistral 2000, London, UK). Supernatant (2.5 mL) was mixed distilled water (2.5 mL) and 0.5 mL of ferric chloride (0.1%), and the absorbance was read at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power (Gülçin et al., 2003).

*2.4.8. Metal chelating activity*

The chelating activity of *P. terebinthus* extracts on $Fe^{2+}$ was measured as reported by Decker and Welch (1990). One millilitre of samples was mixed with 3.7 mL of deionised water, and the mixture was then reacted with $FeCl_2$ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL) for 10 min, and the absorbance at 562 nm determined spectrophotometrically (Gülçin et al., 2003).

Chelating activity of samples on $Fe^{2+}$ was calculated as follows:

$$\text{Metal chelating activity } (\%) = [1 - (\text{absorbance of sample})/(\text{absorbance of control})] \times 100$$

*2.4.9. Statistical analysis*

Experimental results concerning this study were mean ± SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Student's *t*-test, $p$ values $<0.05$ were regarded as significant.

## 3. Results and discussion

In the present study, the extracts (acetone and methanol) prepared from *P. terebinthus* L. subsp. *terebinthus*, collected from Kepez-Çanakkale (Dardanelles), were investigated for their total phenolic and flavonoid contents (Table 1) as well as for their total antioxidant activity in a β-carotene-linoleic acid system (Fig. 2). The scavenging activity of the extracts on DPPH radical (Fig. 3) and superoxide anion radical (Fig. 4) was also measured and found fairly high, however, only the methanol extract was comparable with quercetin in reducing power (Fig. 5) and metal chelating effects (Fig. 6) at over 50 μg/mL. Methanol extract was found to contain higher amounts of phenolics (pyrocatechol equivalents) and flavonoids (quercetin equivalents) than acetone extract as seen in Table 1. However, they seemed to contain similar spots on TLC, they were combined to afford six known and a new flavonoids. The known compounds were determined as apigenine, luteoline, luteoline 7-*O*-glucoside, quercetin, quercetagetin 3-methyl ether 7-*O*-glucoside, isoscutellarein 8-*O*-glucoside and the new compound 6′-hydroxyhypolaetin 3′-methyl ether (**1**). The structures of the isolated flavonoids were established by UV spectra and UV shift reagents as well as $^1$H NMR spectra and by comparison with known compounds on TLC.

The structure of the new compound (**1**) was deduced by spectral data, namely $^1$H NMR (in $CD_3OD$) and UV

spectral data. In MeOH spectrum, the bands A and B were observed at 333.5 and 271.5 nm. A 63.5 nm of shift difference in the NaOMe spectrum compared to MeOH spectrum indicated a free hydroxyl group at 4′ in ring B of a flavone skeleton. Since almost no shift difference was observed by addition of HCl to $AlCl_3$ there was *O*-dihydroxy group was present in ring B. A free 7 hydroxyl group was deduced from its NaOAc spectrum with a 9 nm shift difference compared to the MeOH spectrum (Mabry et al., 1970). The $^1$H NMR spectrum exhibited four singlet signals at $\delta$ 7.60 (1H, s, H-2′), 6.81 (1H, s, H-5′), 6.60 (1H, s, H-3) and 6.08 (1H, s, H-6) besides a methoxy group at $\delta$ 3.80 (3H, s). The singlet appearance of all signals indicated the presence of only one free proton at ring A, and one in ring C. The signal at $\delta$ 6.60 was determined to attribute to H-3 at ring C while the signal at $\delta$ 6.08 could belong to H-6. In case of free hydrogen at C-8, the latter signal would appear around at $\delta$ 6.2–6.6 (Mabry et al., 1970) while a free proton at C-6, its chemical shift appears around $\delta$ 6.00 (Mabry et al., 1970) as seen in compound **1**. The rest two proton singlets which should belong to ring B could only be situated at C-5′ and C-2′. Since the presence of 4′-hydroxyl and the absence of 3′-hydroxyl groups followed by UV spectra with UV shift reagents, the only possibility of the location of the methoxy group should be at C-3′. Therefore, the second hydroxyl of the ring B must be located at C-6′. Thus, the structure of the new compound (**1**) was elucidated as 5,7,8,3′,4′,6′-hexahydroxyflavone 3′-methyl ether corresponding to 6′-hydroxyhypolaetin 3′-methyl ether.

## 4. Conclusions

The studies to date on *Pistacia* species suggested that they might be used as important natural antioxidant sources. Because, they contain phenolics and flavonoids, such as quercetin and α-tocopherol which are used standard antioxidant compounds. As evident from Figs. 2–6, methanol extract was found to have higher antioxidant activity than acetone extract in all test systems. It is worthy that the methanol extract showed as high activity as the standards did, at 50 μg/mL for DPPH scavenging activity. Moreover, the reducing power of the methanol extract was found to be higher than those of α-tocopherol, and the acetone extract reducing power was equal to that of α-tocopherol. Isolation of pure 6′-hydroxyhypolaetin-3′-methyl ether (**1**) in small quantity did allow us to investigate its antioxidant activity in only the two systems. In β-carotene-linoleic acid system, it showed higher activity than both extracts and BHT, while it showed the least activity in DPPH system.

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