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Antioxidative Properties of Decoction of *Pistacia atlantica* Desf. Leaves

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The antioxidative properties of decoction of leaves from *P. atlantica* Desf. were determined as the radical scavenging activity, as the total antioxidant activity, as reducing power, as chelating abilities on metallic ions, as hydrogen peroxide scavenging activity. In addition, preliminary phytochemical screening, total phenolics, anthocyanin, total chlorophyll and total carotenoid contents were also studied. The results were compared with natural and synthetic antioxidants, *e.g.*, α-tocopherol, trolox, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). The decoction prepared from the leaves of *Pistacia atlantica* Desf. is good sources of compounds with antioxidant properties while extract exhibited strong reducing power, free radical scavenging activity and total antioxidant activity.

Key Words: Antioxidant, Decoction, *Pistacia atlantica* Desf.

INTRODUCTION

The genus *Pistacia* belongs to the Anacardiaceae and includes at least eleven species. Turkey has a huge germplasm that mainly consist of *P. atlantica* Desf., *P. vera*, *P. terebinthus* and *P. eurycarpa* Yalt. *P. atlantica* Desf. is a tree located in Marmara, Mediterranean and in Central Anatolia region, which can reach 25 m in height and grows in arid and semi-arid areas[1]. *Pistacia* species were reported to have various biological activities such as antiatherogenic[2], hypoglycemic[3], antiinflammatory[4], antipyretic[5], antifungal, antimicrobial, antiviral[6,7], anti-insecticide[8] and anticancer activities[9]. They are used in eczema treatment, paralysis, diarrohoea, throat infections, renal stones, jaundice, asthma, stomach-ache, as astringent, pectoral and stimulant[10,11]. The mastic obtained from *Pistacia* species is also used as urinary and respiratory antiseptic in Turkish folk medicine[12]. *P. lentiscus* was found as effective as trolox, the water soluble analog of

vitamin E in suppressing iron-induced lipid peroxidation in rat liver homogenates. Iron-induced lipid peroxidation is a well-validated system for generating reactive oxygen species[13]. Previous phytochemical studies on some *Pistacia* species including *P. weinmannifolia*, *P. lentiscus*, *P. terebinthus* and *P. vera* have yielded gallotannins, phenolics, flavonoids, triterpenoids, essential oils and resins[14-16]. These compounds are efficient antioxidants capable of scavenging free radicals and effectively reducing the extent of oxidation. The high ability of phyto-antioxidants to neutralize the active oxygen species is strongly associated with their structure, such as the conjugated double bonds and the number of hydroxyl groups in the aromatic ring mostly attributed flavonoids and phenolics[17]. Therefore, the widespread use of *Pistacia* species in traditional medicine can be attributed to the content of these antioxidant phytochemicals.

Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate, are used to retard lipid peroxidation. However, the use of synthetic antioxidants is under strict regulation due to the potential health hazards caused by such compound[18]. Much attention has been focused on the use of natural antioxidants, to inhibit lipid peroxidation or to protect the human body from the oxidative damage by free radicals.

Antioxidant activity of *P. atlantica* Desf. has not been studied so far. Therefore, in this work, the antioxidative properties of decoction of leaves from *P. atlantica* Desf. were determined as the radical scavenging activity, as the total antioxidant activity, as reducing power, as chelating abilities on metallic ions, as hydrogen peroxide scavenging activity. In addition, preliminary phytochemical screening, total phenolics, anthocyanin, total chlorophyll and total carotenoid contents were also studied.

EXPERIMENTAL

Pistacia atlantica Desf. leaves and preparation of decoction

Fresh leaves from *P. atlantica* Desf. were collected from Istanbul, Turkey during the spring (May). The identity of the leaf samples was confirmed by Prof. Dr. Kerim Alpinar (Department of Botany, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey).

Collected leaf samples were washed with deionized water and dried for 5-7 d in the shade at room temperature. A decoction was prepared, boiling 20 g of dried leaves at 100 °C for 15 min with about 200 mL of twice-distilled water. Extract was rapidly filtered through a linen cloth, the filtrate was evaporated to dryness under vaccum on a rotary evaporator. The crude extract was stored at 4 ºC and dissolved in distilled water. It was used for the assessment of antioxidant activity.

Preliminary phytochemical analysis of decoction of *Pistacia atlantica* Desf. leaves: The decoction of *Pistacia atlantica* Desf. leaves was subjected to preliminary phytochemical testing for the detection of major chemical groups[19]. The details of the tests are as follows: (i) *For phenols:* Decoction was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours. Blue colouration of the spot indicate the presence of phenols. (ii) *Braemer's test for tannins:* To a 2-3 mL of decoction, 10 % (w/v) alcoholic ferric chloride solution was added. (Dark blue or greenish grey colouration of the solution indicate the presence of tannins in the sample). (iii) *Liebermann-Burchardt test for steroids and terpenoids:* To 1 mL of decoction, 1 mL of chloroform, 2-3 mL of acetic anhydride and 1-2 drops of concentrated sulfuric acid were added. (Dark green colouration of the solution indicate the presence of steroids and dark pink or red colouration of the solution indicate the presence of terpenoids). (iv) *Alkaloids:* A drop of sample was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange colouration of the spot indicate the presence of alkaloids). (v) *Borntraeger's test for anthraquinones:* About 50 mg of plant sample was heated with 10 % (w/v) ferric chloride solution and 1 mL of concentrated hydrochloric acid. The sample was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with concentrated ammonia. (Pink or deep red coloration of aqueous layer indicate the presence of anthraquinones). (vi) *Shinoda test for flavonoids:* To 2-3 mL of plant sample, a piece of magnesium ribbon and 1 mL of concentrated hydrochloric acid were added. (Pink red or red colouration of the solution indicate the presence of flavonoids).

Determination of total phenolic compounds: Sample was analyzed spectrophotometrically for total phenolic content with Folin-Ciocalteau reagent according to the method of Slinkard and Singleton[20] using pyrocatechol (20-200 µg/mL) as a standard phenolic compound. Briefly, 1 mL of the sample solution containing 1000 µg extract was mixed with 45 mL distilled water. Folin-Ciocalteau reagent (1 mL) was added and the contents were mixed thoroughly. This mixture was allowed to stand for 3 min before adding 3 mL of 2 % (w/v) Na_2CO_3. The solution was then allowed to stand for 2 h with occasional shaking before reading at 760 nm in a spectrophotometer. The total phenol content was expressed as µg equivalents to the standard used per mg of extract. The equation obtained for the standard curve was y = 0.0028x - 0.0164 for pyrocatechol.

Anthocyanin determination: The anthocyanin content of the dried leaves was determined according to the modified method of Padmavati *et al.*[21]. The dried leaves (25 mg/mL) was mixed with acidified methanol

(1% HCl/methanol) for 24 h at 4°C in the dark and then centrifuged at 1000 × g for 15 min. The anthocyanin concentration in the supernatant was measured spectrophotometrically at 530 and 657 nm and the absorbance values are indicated as A_{530} and A_{657}. The extinction coefficient of 31.6 M^{-1} cm^{-1} was used to convert the absorbance values into anthocyanin concentration. The concentration was calculated using the following equation: anthocyanin concentration (μmol/g) = [(A_{530}–0.33 × A_{657})/31.6] × [volume (mL)/weight (g)]. Results are expressed as the average of triplicates.

Total chlorophyll and carotenoid content assay: Total chlorophyll and total carotenoid content were determined according to the method of Kocacaliskan and Kadioglu[22]. 10 mg of decoction sample of leaves was dissolved with 10 mL distilled water. The absorbance of sample was measured at 450, 645 and 663 nm in the UV-Vis light spectrophotometer. The total chlorophyll and total carotenoid content were calculated using the equations as followed:

Chlorophyll a = $12.7A_{663} – 2.69A_{645}$

Chlorophyll b = $22.9A_{645} – 4.68A_{663}$

Total chlorophyll = $20.2A_{645} + 8.02A_{663}$

Total carotenoid = $4.07A_{450}$ – [(0.0435 × Chlorophyll a) + (0.367 × Chlorophyll b)]

Reducing power: The reducing power of the decoction was measured according to the method of Oyaizu[23]. Various concentrations of decoctions (20-100 μg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide [$K_3Fe(CN)_6$] (1 %, w/v) and then the mixture was incubated at 50 °C for 0.5 h. Afterwards, 2.5 mL of trichloroacetic acid (10 %, w/v) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper-layer solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1 %, w/v) and the absorbance was measured at 700 nm. α-Tocopherol, BHA and BHT were used as standard antioxidants. Higher absorbance of the reaction mixture indicated greater reducing power.

Chelating activity on Fe^{2+}: The chelating activity of the decoction on ferrous ions (Fe^{2+}) was measured according to the method of Decker and Welch[24]. Aliquots of 1 mL of different concentrations (0.25, 0.50, 0.75 and 1.0 mg/mL) of the samples were mixed with 3.7 mL of deionized water. The mixture was incubated with $FeCl_2$ (2 mM, 0.1 mL) for 5, 10, 30 and 60 min. After incubation the reaction was initiated by addition of ferrozine (5 mM and 0.2 mL) for 10 min at room temperature and then the absorbance was measured at 562 nm in a spectrophotometer. A lower absorbance indicates a higher chelating power. The chelating activity of the decoction on Fe^{2+} was compared with that of EDTA at a level of 0.037 mg/mL. Chelating activity was calculated using the following formula:

Chelating activity (%) = [1–(Absorbance of sample/Absorbance of control)] × 100

Control test was performed without addition of the sample.

Free radical-scavenging activity: The free radical scavenging activity of the decoction was measured with 1,1-diphenyl-2-picryl-hydrazil (DPPH·) using the slightly modified methods of Brand-Williams *et al.*[25]. Briefly, 20 mg/L DPPH· solution in methanol was prepared and 1.5 mL of this solution was added to 0.75 mL of the sample, BHA, BHT and ascorbic acid solution (5-25 µg/mL). The mixture was shaken vigorously and the decrease in absorbance at 517 nm was measured at 5, 10, 30 and 60 min. Water (0.75 mL) in place of the plant extract was used as control. The per cent inhibition activity was calculated using the following equation:

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

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the extract sample.

Total antioxidant activity determination: The antioxidant activity was determined according to the thiocyanate method with slight modifications[26]. For the stock solution, 10 mg of extract was dissolved in 10 mL water. Then the solution containing different amounts of stock solution or standards samples (20, 40, 60, 80, 100 µg) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to 2.5 mL of linoleic acid emulsion. 50 mL linoleic acid emulsion contains Tween-20 (175 µg), linoleic acid (155 µL) and potassium phosphate buffer (0.04 M, pH 7.0). On the other hand, 5.0 mL of control contains 2.5 mL of linoleic acid emulsion and 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0). Each solution was then incubated at 37 °C in a glass flask in the dark. At 24 h intervals during incubation, 0.1 mL of this incubation solution was added to 4.7 mL of 75 % (v/v) ethanol and 0.1 mL of 30 % (w/v) ammonium thiocyanate. Precisely 3 min after addition of 0.1 mL of 0.02 M $FeCl_2$ in 3.5 % (w/v) HCl to the reaction mixture, the absorbance of the red colour was measured at 500 nm in a spectrophotometer. The solutions without added extract or standards were used as control. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

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

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the extract sample or standards.

Hydrogen peroxide scavenging activity: The extract (20-100 µg/mL) was dissolved in 3.4 mL of 0.1 M phosphate buffer (pH 7.4) and mixed with 0.6 mL of 43 mM hydrogen peroxide solution. The absorbance value (at 230 nm) of the reaction mixture was recorded after 40 min. For each concentration, a separate blank sample was used for background subtraction[27]. α-Tocopherol, BHA and BHT (20-100 µg/mL) were used as

standard antioxidants. The solutions without added extract or standards were used as control. The percentage of scavenged hydrogen peroxide of sample and standard compounds was calculated using the following equation:

$$\text{Scavenged } H_2O_2\ \% = [(A_0 - A_1)/A_0 \times 100]$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the extract sample and standards.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of decoction of *Pistacia atlantica* Desf. leaves: Decoction of *P. atlantica* leaves were subjected to preliminary phytochemical screening for the presence of different chemical groups (Table-1). Phenols, tannins, flavonoids and antraquinones were found to be major groups in the extract. Phenols and flavonoids are common constituents of foods of plant origin and major antioxidants of human being diet. The total dietary intake is about 1 g/d. It is much higher than that of all other known dietary antioxidants, about 10 times higher than that of vitamin C and 100 times higher than those of vitamin E and carotenoids[28]. As antioxidants, they protect cell constituents against oxidative damage and therefore, limit the risk of various degenerative diseases associated to oxidative stress.

TABLE-1
PRELIMINARY PHYTOCHEMICAL SCREENING OF DECOCTION OF *Pistacia atlantica* Desf. LEAVES

Tested for	Presence/Absence	Test performed
Phenols	+++	Phosphomolybdic acid test
Tannins	+++	Braemer's test
Terpens and steroids	–	Liebermann-Burchardt test
Alkaloids	–	Dragendorff's test
Flavonoids	+++	Borntraeger test
Antraquinones	+++	Shinoda's test

–Absent; +++Abundant

Determination of total phenolic compounds: The amount of total phenolics in *P. atlantica* extract determined using the Folin-Ciocalteau method. Values are expressed as µg pyrocatechol per mg of extract. It was determined that there was 237.15 ± 8.02 µg pyrocatechol equivalent of phenolic compounds in the 1 mg of the extract of *P. atlantica* (Table-2). It may be attributed, in a significant part, to the antioxidant activities of the *P. atlantica*. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. Antioxidant properties of phenolic compounds largely depend on their chemical and physico-chemical environment. Several hundreds of different phenolic compounds have

TABLE-2
TOTAL PHENOLIC AND ANTHOCYANIN CONTENTS OF THE DECOCTION OF *Pistacia atlantica* Desf. LEAVES

Sample	Total phenolic content (µg pyrocatechol/mg of extract)*	Anthocyanin content (µmol/g of extract)*
Extract	237.15 ± 8.02	0.063 ± 0.005

*Values are means ± SD.

been identified in plant. The two main types of phenolics are flavonoids and phenolic acids. Flavonoids are themselves distributed among several classes. One of the most common class is anthocyanins. Numerous studies on animal models have shown that, when added to the diet, they limit the development of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes and osteoporosis[28].

Anthocyanin determination: The anthocyanin content was found to be 0.063 ± 0.005 µmol/g for *P. atlantica* (Table-2). Anthocyanins are antioxidant flavonoids that protect many body systems. They also have anti-inflammatory properties. They support healthy brain function, the peripheral nervous system, the skin and collagen[29,30]. Anthocyanins also provide nutritional support for diabetics. They are hypoglycemic agents which lower blood sugar levels and protect both large blood vessels and capilleries from oxidative damage. They prevent oxidative damage in the capilleries of the eye and extremities, the two most common complications of diabetes[31,32].

Total chlorophyll and carotenoid content assay: Total chlorophyll and carotenoid contents of extract from *P. atlantica* are shown in Table-3. The major group of the pigment, chlorophyll a and b, and carotenoids are the non-polyphenolic compounds of *P. atlantica* leaves. It has been reported that all these pigments exhibit significant antioxidant activities[33]. Combined intake of phenolic compounds and non-polyphenolic compounds as antioxidative pigments of *P. atlantica* may be more efficient to prevent chronic diseases.

TABLE-3
TOTAL CHLOROPHYLL AND TOTAL CAROTENOID CONTENT OF THE DECOCTION OF *Pistacia atlantica* Desf. LEAVES

Sample*	Chlorophyll a (µg/mL)	Chlorophyll b (µg/mL)	Total chlorophyll (µg/mL)	Total carotenoid (µg/mL)
Extract	0.37 ± 0.04	0.60 ± 0.10	0.93 ± 0.16	0.013 ± 0.003

*Concentration of extract: 10 mg/mL.

Reducing power: This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a coloured complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. Fig. 1 shows the reducing power of extract from *P. atlantica*. It was found that the reducing power of the extract increased with the increase of its concentration. From a comparison of the absorbance at 700 nm, the reducing power of *P. atlantica* was found to be significantly higher than those of α-tocopherol and BHT and nearly similar to BHA. These results revealed that the extract of *P. atlantica* was electron donors and also could react with free radicals, converting them to more stable products and terminating the radical chain reaction.

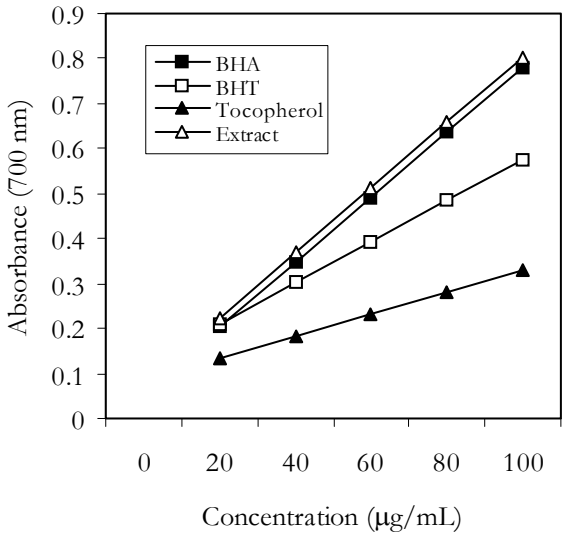

Fig. 1. Reducing power of extract from *Pistacia atlantica* Desf. leaves

Chelating activity on Fe^{2+}: The chelating of ferrous ions by the extract was the estimated by the method of Decker and Welch[24]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red colour of the complexes decreases. Measurement of the rate of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator[34]. In this assay, different concentrations of the sample, interfered with the formation of ferrous and ferrrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. The absorbance of Fe^{2+}-ferrozine complex was decreased dose dependently;

otherwise the activity was increased on increasing concentration. The Fe^{2+} ion chelating effect of *P. atlantica* extract is presented in Fig. 2. The extract at 1.0 mg/mL concentration showed more than 16 % chelating effect on Fe^{2+} ions at an incubation time of 1 h. The chelating activity of samples increased with increasing incubation times with $FeCl_2$. However, the chelating activity of *P. atlantica* extract of 1.0 mg/mL was nearly four fold less than EDTA at 0.037 mg/mL (62 %) for an incubation time of 1 h. The data obtained from present study, reveals that *P. atlantica* extract has slightly effective capacity for iron binding, suggesting that its action as an antioxidant may be related to its iron binding capacity.

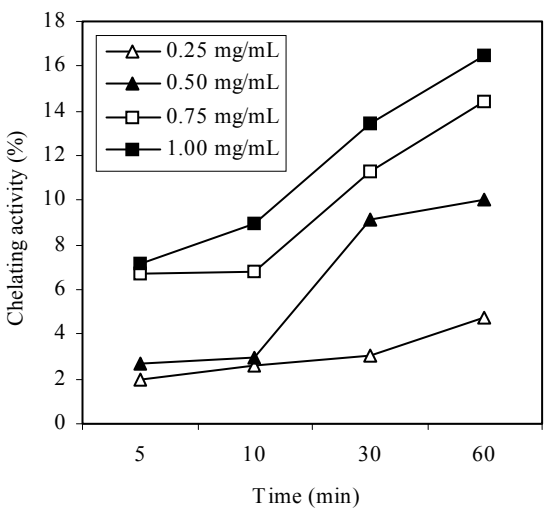

Fig. 2. Chelating effects of extract on Fe^{2+} ions at different incubation times with $FeCl_2$

Free radical-scavenging activity: DPPH radical scavenging activity is the most widely reported method for screening of antioxidant activity of many plant. DPPH assay method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants[23]. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The extract was able to reduce the stable radical DPPH to the yellow coloured diphenylpicrylhydrazine. The DPPH radical scavenging effects of *P. atlantica* extract are presented in Fig. 3. The extract showed antiradical activity by inhibiting DPPH radical with 85 % inhibition rate of 15 µg/mL concentration. It appears that the *P. atlantica* possesses hydrogen donating capabilities and acts as an antioxidant. Scavenging activity of

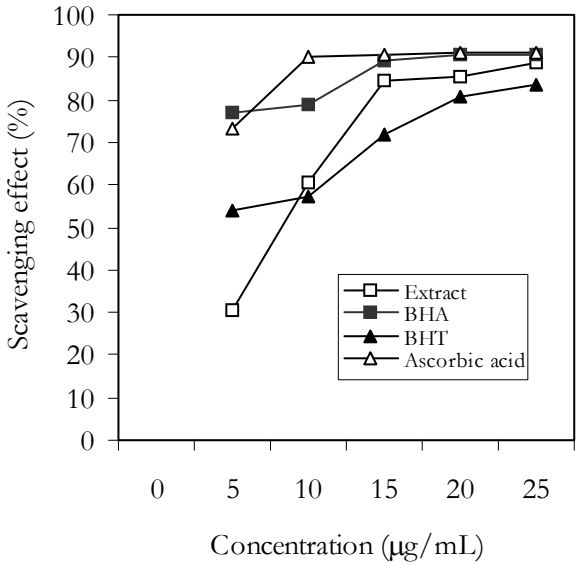

Fig. 3. Scavenging activities of extract against 1,1-diphenyl-2-picryl-hydrazil (DPPH·) radical

ascorbic acid and BHA, known antioxidants, were nearly similar when compared *P. atlantica* extract. However, scavenging activity of BHT was relatively lower than that of *P. atlantica* extract.

Total antioxidant activity determination: There are many different antioxidant components in plants and is relatively difficult to measure each antioxidant component separately. Therefore several different methods have been developed to evaluate the antioxidant activity of plant sample[35]. Total antioxidant activity of *P. atlantica* extract was determined by the thiocyanate method in linoleic acid emulsion. The amount of peroxide in the initial stages of lipid oxidation was measured every 24 h, over a period of 6 d. The effect of *P. atlantica* extract (100 mg/L) on peroxidation of linoleic acid emulsion is shown in Fig. 4. The antioxidative activity of *P. atlantica* extract was compared with commercial antioxidants such as α-tocopherol (Toc), BHT, BHA and trolox. *P. atlantica* extract showed higher antioxidant activity than that of α-tocopherol. The antioxidant activity of *P. atlantica* extract was found to be similar when compared BHA, BHT and trolox.

Hydrogen peroxide scavenging activity: Hydrogen peroxide, a reactive nonradical, is very important as it can penetrate biological membranes. Although H_2O_2 itself is not very reactive, it may convert into more reactive species such as singlet oxygen and hydroxyl radicals[36]. Hydrogen peroxide scavenging activity of *P. atlantica* extract is shown in Fig. 5. The result is compared with BHA, BHT and α-tocopherol as standards.

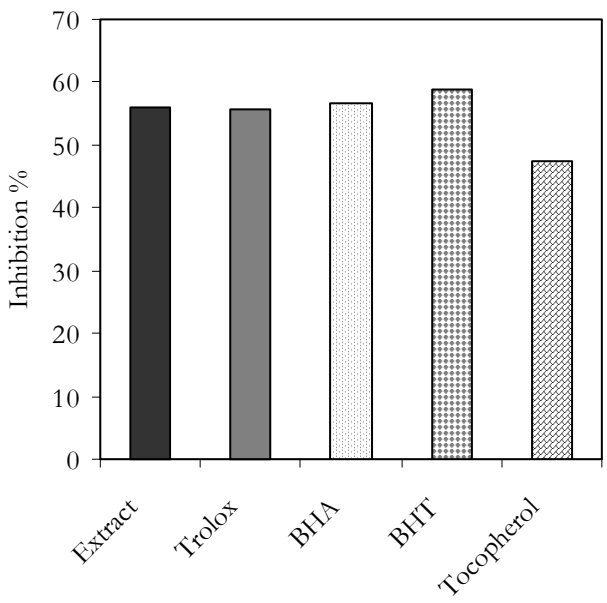

Fig. 4. Total antioxidant activities of extract, trolox, BHA, BHT and α- tocopherol (100 µg/mL concentration)

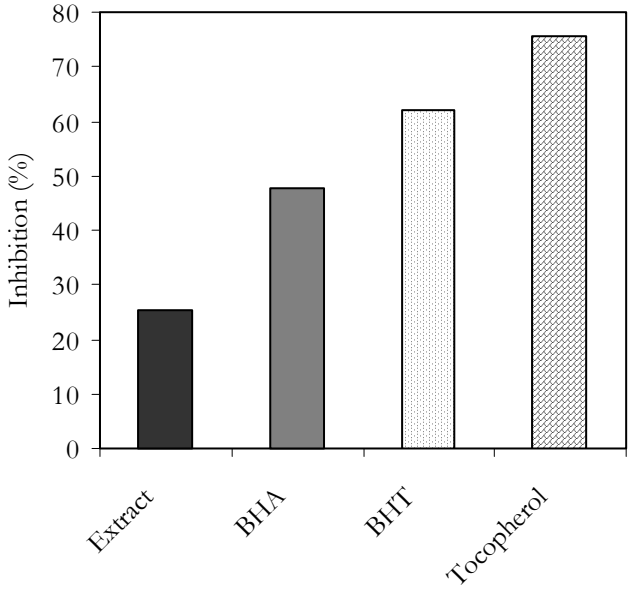

Fig. 5. Hydrogen peroxide scavenging activity of extract, BHA, BHT and α-tocopherol at 100 µg/mL concentration.

P. atlantica extract was capable of scavenging activity in a concentration-dependent manner. At 100 µg/mL, the extract exhibited 25.49 % scavenging activity. On the other hand, BHA, BHT and α-tocopherol exhibited 47.53, 62.17 and 75.54 %, respectively of H_2O_2 scavenging activity at the same concentration. These results showed that *P. atlantica* extract had weak H_2O_2 scavenging activity. At 100 µg/mL concentration, H_2O_2 scavenging activity of extract and both standards decreased in the order of α-tocopherol > BHT > BHA > *P. atlantica* extract.

Conclusion

The decoction prepared from the leaves of *Pistacia atlantica* Desf. is good sources of compounds with antioxidant properties while extract exhibited strong reducing power, free radical scavenging activity and total antioxidant activity. This study suggests that *P. atlantica* leaves are potent antioxidant sources. According to the results, phenolics, flavonoids, tannins and antraquinones were found the main compounds of decoction from *P. atlantica* leaves (Table-1). These fact might explain the stronger scavenging activity and antioxidant activity of *P. atlantica*. More work should be done to characterize individual phenolic compounds and flavonoids of the extract of *P. atlantica* in order to assign particular antioxidant effects to the individual compounds of the extract.

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REFERENCES

1. H.B. Ila, S. Kafkas and M. Topaktas, *J. Horticul. Sci. Biotech.*, **78**, 35 (2003).
2. G.V.Z. Dedoussis, A.C. Kaliora, S. Psarras, A. Chiou, A. Mylona, N.G. Papadapoulos and N.K. Andrikopoulos, *Atherosclerosis*, **174**, 293 (2004).
3. I.I. Hamdan and F.U. Afifi, *J. Ethnopharmacol.*, **93**, 117 (2004).
4. I. Orhan, E. Kupeli, M. Aslan, M. Kartal and E. Yesilada, *J. Ethnopharmacol.*, **105**, 235 (2006).
5. A. Villar, M.J. Sanz and M. Payo, *Int. J. Crude Drug Res.*, **25**, 1 (1987).
6. A. Aksoy, N. Duran and F. Koksal, *Arch. Oral Biol.*, **51**, 476 (2006).
7. A. Kordali, A. Cakir, H. Zengin and M.E. Duru, *Fitoterapia*, **74**, 164 (2003).
8. M.J. Pascual-Villalobos and A. Robledo, *Ind. Crop Prod.*, **8**, 183 (1998).
9. K.V. Balan, J. Prince, Z. Han, K. Dimas, M. Cladaras, J.H. Wyche, N.M. Sitaras and P. Pantazis, *Phytomedicine*, (in press).
10. B. Ozcelik, M. Aslan, I. Orhan and T. Karaoglu, *Microbiol. Res.*, **160**, 159 (2005).
11. A.N. Assimopoulou, S.N. Zlatanos and V.P. Papageorgiou, *Food Chem.*, **92**, 721 (2005).
12. T. Baytop, Therapy with Medicinal Plants in Turkey (past and present), Nobel Tip Kitabevleri, Istanbul, edn. 2 (1999).
13. P. Ljubuncic, H. Azaizeh, I. Portnaya, U. Cogan, O. Said, K.A. Saleh and A. Bomzon, *J. Ethnopharmacol.*, **99**, 43 (2005).

14. X. Zhao, H. Sun, A. Hou, Q. Zhao, T. Wei and W. Xin, *Bio. Chim. Biophys. Acta*, **1725**, 103 (2005).
15. M. Yalpani and J.H.P. Tyman, *Phytochemistry*, **22**, 2263 (1983).
16. S.A. Kawashty, S.A.M. Mosharrafa, M. El-Gibali and N.A.M. Saleh, *Biochem. Syst. Ecol.*, **28**, 915 (2000).
17. M. Leja, A. Mareczek, G. Wyzgolik, J. Klepacz-Baniak and K. Czekonska, *Food Chem.*, **100**, 237 (2007).
18. R. Tripathi, H. Mohan and J.P. Kamat, *Food Chem.*, **100**, 81 (2007).
19. M.N. Ravishankara, N. Shrivastava, H. Padh and M. Rajani, *Phytomedicine*, **9**, 153 (2002).
20. K. Slinkard and V.L Singleton, *Am. J. Enol. Viticul.*, **28**, 49 (1977).
21. M. Padmavati, N. Sakthivel, T.V. Thara and A.R. Reddy, *Phytochemistry*, **46**, 449 (1997).
22. I. Kocacaliskan and A. Kadioglu, Bitki Fizyolojisi Laboratuvar Kilavuzu, Atatürk Universitesi Fen-Edebiyat Fakültesi Yayini S 40 (1990).
23. M. Oyaizu, *Japan J. Nutr.*, **44**, 307 (1986).
24. E.A. Decker and B. Welch, *J. Agric. Food Chem.*, **38**, 674 (1990).
25. W. Brand-Williams, M.E. Cuvelier and C. Berset, *Lebensm.-Wiss. Technol.*, **28**, 25 (1995).
26. T. Osawa and N. Namiki, *Agric. Biol. Chem.*, **45**, 735 (1981).
27. R.T. Ruch, S.J. Cheng and E. Klawnig, *Carcinogenesis*, **10**, 1003 (1989).
28. A. Scalbert, C. Manach, C. Morand and C. Remesy, *Crit. Rev. Food Sci. Nutr.*, **45**, 287 (2005).
29. J.A. Joseph, *J. Neurosci.*, **19**, 8114 (1999).
30. E. Andriambeloson, *J. Nutr.*, **128**, 2324 (1999).
31. F. Cohen-Boulakia, *Metabolism*, **49**, 880 (2000).
32. M. Perossini, *Ann. Ottalmol. Clin. Ocul.*, **12**, 1173 (1987).
33. K. Higashi-Okai, M. Yamazaki, H. Nagamori and Y. Okai, *J. UOEH*, **23**, 335 (2001).
34. F. Yamaguchi, T. Ariga, Y. Yoshimura and H. Nakazawa, *J. Agric. Food Chem.*, **48**, 180 (2000).
35. F. Abas, N.H. Lajis, D.A. Israf, S. Khozirah and Y.U. Kalsom, *Food Chem.*, **95**, 566 (2006).
36. J.Y. Je and S.K. Kim, *Bioorg. Med. Chem. Lett.*, **16**, 1884 (2006).

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