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The antioxidant activity of wild medlar (*Mespilus germanica L.*) fruit, stem bark and leaf

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The medlar is an edible fruit. Modern medicine has recognized its healing properties in the treatment of some diseases. There is no scientific data in the literature about the antioxidant activity of methanol or aqueous extract of medlar fruit, leaf or stem bark. Antioxidant activities of these parts were evaluated by employing six test systems. Stem bark extract (aqueous and methanol) showed best activity in 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity with IC_{50} = 10.7 ± 0.6 and 11.4 ± 0.8 μgml^{-1}, respectively. All extracts showed weak Fe^{2+} chelating ability. Methanol extract of fruit had better activity in nitric oxide scavenging model than others (IC_{50} = 247 ± 12.2 μgml^{-1}). The leaves and bark extracts showed good reducing power than fruit extract. In reducing powers, there were no significant differences ($p > 0.05$) among the stem bark and leaves extracts that were comparable with vitamin C ($p < 0.05$). Extracts exhibited good antioxidant activity in the ferric thiocyanate (FTC) method. They manifested almost the same pattern of activity as vitamin C and butylated hydroxyanisole (BHA) at different incubation times (until 72nd hour) but stem bark extract showed higher peroxidation inhibition than vitamin C and BHA at the 96th hour ($p < 0.05$). The extracts were capable of scavenging H_2O_2 in a concentration-dependent manner. Leaves methanol extract showed good activity that was comparable with quercetin ($p > 0.05$). Bark and leaf extracts had higher total phenolic and flavonoid contents than fruit.

Key words: Antioxidant activity, free radical scavenging, *Mespilus germanica*, medlar, phenolic contents, flavonoids contents.

INTRODUCTION

Reactive oxygen species (ROS) have been found to play an important role in the initiation and/or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular disease (Halliwell,

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Abbreviations: ROS, Reactive oxygen species; **DPPH,** 1,1-diphenyl-2-picryl hydrazyl; **TCA,** trichloroacetic acid; **BHA,** butylated hydroxyanisole; **EDTA,** ethylenediaminetetraacetic acid; **WB,** aqueous extract of stem bark; **MB,** methanol extract of stem bark; **ML,** methanol extract of leaf; **WL,** aqueous extract of leaf; **MF,** methanol extract of fruit; **WF,** water extract of fruit; **FTC,** ferric thiocyanate.

1997a). Recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals (Hou et al., 2003). It has been determined that the antioxidant effect of plant products is mainly attributed to phenolic compounds such as flavonoids and phenolic acids (Pietta, 2000). Among the various medicinal plants, some endemic and edible species are of particular interest because they may be used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Exarchou et al., 2002). Medlar, *Mespilus germanica* L. (*Rosaceae*) is a spiny shrub that grows to a height of 2 to 3 m in the wild and 4 to 6 m when cultivated. The brown pear- and apple-shaped fruits are subglobose or pyriform and crowned by foliaceous sepals (drupes 5). The fruit ranges in diameter

from 1.5 to 3 cm and weight from very small (about 10 g) to big (more than 80 g). The plant is native in Europe and Asia (Glew et al. 2003a). Its fruit is well known for its nutritive value especially by the people of Southeastern Europe, Turkey and Iran (Glew et al., 2003c). The pome-like fruit originates from the inferior ovary, generally with five stony seeds. The skin color is brown, sometimes tinged reddish. The harvest of fruit bletted on the plant in late autumn or the harvest of fruits at physiological ripening and their storage in straw until over-ripening are well known traditions. The medlar is a typical climacteric fruit which has gained value in human consumption and commercial importance in recent years, attracting rese-arches to study its chemical or nutritional compositions (Ayaz et al., 2008). Medlar fruit is widely consumed in some countries such as Turkey a unique place where the people grow the wild and alternative cultivars for the consumption of the fruits in different ways. A long list of recipes utilizing medlar fruits such as in jams and jellies are well known (Ayaz et al., 2008). The astringency of the fruit is well known and it has been reported that bletted pulp or syrup of the fruit is a popular remedy against enteritis and has many human healing properties (Ayaz et al., 2008).

The bletted fruits have a sweet and slightly acidic flesh; jams and jellies can be obtained. In cookery, a surprisingly long list of recipes can be found. The astringency of the fruits has been well known since ancient times. The fruit of medlars are used as a nutritional material by the local customer and are consumed by the local people as marmalade. The medlar fruit is also used as treatment of constipation, diuretic, and to rid the kidney and bladder of stones (Haciseferogullari et al., 2005; Glew et al., 2003b). The fruit is consumed as a medicinal remedy in Turkey. In 1964, two new antibiotic cyclopentoid monoterpenes were isolated and identified. These are genipic acid and genipinic acid, which are its carbomethoxy derivative (Haciseferogullari et al., 2005). Recently, the physical, physicochemical and chemical changes during maturation of Spanish medlar have been reported (Romero-Rodriguez et al., 2000; de Pascual-Teresa et al., 2000) and flavanols in medlar fruit have been analyzed. More recently, changes in mineral com-position at different stages of maturity of medlar (Glew et al., 2003c), fatty acid composition during ripening of medlar (Ayaz et al., 2002), fatty acid composition during development and maturation of medlar fruit (Glew et al., 2003b), volatile components of its seeds (Pourmortazavi et al., 2005) and polyphenoloxidase from medlar fruits (Dincer et al., 2002) have been reported. Besides these studies, data on the nutritional value of medlars are still scarce.

Limited studies on physical and chemical properties of medlar fruits have been performed hitherto. As far as we know, there is no scientific data in literature about the antioxidant activity of methanol or aqueous extract of medlar fruits, leaves or stem barks. The determination of antioxidant activity and phytochemical content of medlar may be useful in order to know how important medlar fruit

is, nutritionally. The objective of this study is to examine the antioxidant activity of methanol and aqueous extracts of medlar fruit, stem bark and leaves employing six various *in vitro* assay systems, that is, hydrogen pe-roxide, 1,1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide radical scavenging, reducing power, linoleic acid and iron ion chelating power, in order to understand the usefulness of this plant as a foodstuff as well as in medicine.

MATERIALS AND METHODS

Chemicals

Ferrozine, linoleic acid, trichloroacetic acid (TCA), DPPH and potassium ferricyanide were purchased from Sigma Chemicals Co. (USA). Butylated hydroxyanisole (BHA), ascorbic acid, sulfanila-mide, N-(1-naphthyl) ethylenediamine dihydrochloride, ethylenedia-minetetraacetic acid (EDTA) and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Plant material

Medlar (*Mespilus germanica* L., wild type) fruit at the ripening stage in December 2008, leaves and stem bark (in September 2008) were randomly harvested from 3 trees of various single genotypes of bulk populations in the native habitat of the species located in the Panbe Choole village, Sari, Iran and then identified by Dr. Bahman Eslami. A voucher (No. E1250-2) has been deposited in the Sari School of Pharmacy herbarium. Materials were dried at room temperature and coarsely ground before extraction.

Solvent extraction

A known amount of samples (250 g) were extracted at room temperature for 24 h by percolation method using methanol and water, separately. The extracts were then separated from the sample residues by filtration through Whatman No.1 filter paper. This procedure was repeated thrice. The resulting extracts were concentrated over a rotary vacuum until a crude solid extract was obtained. The aqueous extracts were then freeze-dried for com-plete solvent removal. Six extracts were obtained: WB (aqueous extract of stem bark), MB (methanol extract of stem bark), ML (methanol extract of leaf), WL (aqueous extract of leaf), MF (methanol extract of fruit) and WF (water extract of fruit).

Determination of total phenolic compounds and flavonoid content

Total phenolic compound contents were determined by the Folin-Ciocalteau method according to the recently published method (Ebrahimzadeh et al., 2009b,d). The extracts samples (0.5 ml) were mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g/l sodium carbonate were then added. The absor-bance of reaction was measured at 760 nm after 2 h of incubation at room temperature. Results were expressed as gallic acid equivalents. Total flavonoids were estimated using the method of Ghasemi et al. (2009) and Nabavi et al. (2009). Briefly, 0.5 ml solution of extract in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer

(Perkin Elmer). Total flavonoid contents were calculated as quercetin from a calibration curve.

DPPH radical-scavenging activity

The stable DPPH was used for determination of free radical scavenging activity of the extracts (Ebrahimzadeh et al., 2009a; Nabavi et al., 2008a). Different concentrations of extracts were added, at an equal volume, to methanol solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Reducing power

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Ebrahimzadeh et al., 2009d). The Fe^{3+}-reducing power of the extract was determined according to Ebrahimzadeh et al. (2009c) and Nabavi et al. (2008a). Different amounts of extracts (50 to 1600 µgml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe (CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm against an appropriate blank solution. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control. All tests were performed in triplicate.

Determination of metal chelating activity

Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell et al., 1997b). The ability of the extracts to chelate ferrous ions was estimated by Ebrahimzadeh et al. (2008, 2009e). Briefly, the extracts (0.2 to 3.2 mg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml), the mixture was shaken vigorously and left to stand at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine- Fe^{2+} complex formation was calculated as $[(A_0 - A_s)/A_s] \times 100$, where A_0 is the absorbance of the control and A_s is the absorbance of the extract/standard. Na_2EDTA was used as positive control.

Assay of nitric oxide-scavenging activity

The extracts ability to scavenge nitric oxide was estimated according to Ebrahimzadeh et al. (2010a). For the experiment, sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of extracts dissolved in water and incubated at room temperature for 150 min. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as positive control (Mahmoudi et al., 2009).

Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to Esmaeili et al. (2009) and Nabavi et al. (2008b). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (0.1 to 3.2 mgml^{-1}) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds were calculated as follows:

% scavenged $[H_2O_2] = [(A_o - A_s)/A_o] \times 100$

Where, A_o is the absorbance of the control and A_s is the absorbance in the presence of the sample of extract and standard.

Determination of antioxidant activity by the ferric thiocyanate (FTC) method

The inhibitory capacity of extracts was tested against oxidation of linoleic acid by FTC method. This method was determined according to Dehpour et al. (2009). 20 mg/ml of samples dissolved in 4 ml of 95% (w/v) ethanol were mixed with linoleic acid (2.51%, v/v) in 99.5% (w/v) ethanol (4.1 ml), 0.05 M phosphate buffer pH 7.0 (8 ml) and distilled water (3.9 ml), and kept in screw cap containers at 40°C in the dark. To 0.1 ml of this solution was then added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured, and it was measured again every 24 h until the day when the absorbance of the control reached the maximum value. The percent inhibition of linoleic acid peroxidation was calculated as:

(%) inhibition = 100 − [(absorbance increase of the sample/ absorbance increase of the control) × 100].

All tests were run in duplicate, and analyses of all samples were run in triplicate and averaged. Vitamin C and BHA were used as positive control.

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by analysis of variance (p < 0.05) and the means were separated by Duncan's multiple range test. The IC_{50} values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

The yield percent, total phenolic and flavonoids contents obtained from medlar different parts are shown in Table 1. The maximum extractable polyphenolic content was recorded in WB and ML with 457.07 ± 22.3 and 368.6 ± 21.6 mg gallic acid equivalent/g of extract, respectively, by reference to standard curve (y = 0.0063x, r^2 = 0.987). Maximum flavonoid content was recorded in ML and WL with 59.92 ± 3.6 and 54.36 ± 3.2 mg quercetin equivalent/g of extract, by reference to standard curve (y= 0.0067x+ 0.0132, r^2 = 0.999). Phenols and polyphenolic compounds, such as flavonoids, are widely

Table 1. Phenol and flavonoid contents and antioxidant activities of medlar, *M. germanica L.* extracts. MF: methanol fruit, WF: aqueous fruit, ML: methanol leaves, WL: aqueous leaves, MB: methanol bark and WB: aqueous bark extracts.

Sample	Yield (%)	Total phenol Contents (mg g^{-1})	Total flavonoid contents (mgg^{-1})	DPPH free radical scavenging, IC$_{50}$ (µgml^{-1})[a]	Nitric oxide scavenging, IC$_{50}$ (µgml^{-1})[b]	H$_2$O$_2$ scavenging activity, IC$_{50}$ (µgml^{-1})[c]	Fe^{2+} chelating ability (%)[d] or IC$_{50}$ (µgml^{-1})
MF	35	29.35 ± 1.7	14.88 ± 1.2	419 ± 3.2	247 ± 12.2	1138 ± 77.1	23.0 %
MB	10	287.24 ± 14.5	33.73 ± 2.1	11.4 ± 0.8	376 ± 16.5	427 ± 35.1	28.4 %
ML	31	368.6 ± 21.6	59.92 ± 3.6	19.4 ± 1.3	1129 ± 78.6	58.1 ± 2.3	24.6 %
WF	31	7.26 ± 0.4	14.08 ± 1.1	492 ± 33.1	1328 ± 57.4	2333 ± 87.9	31.7 %
WB	8	457.07 ± 22.3	34.12 ± 1.9	10.7 ± 0.6	557.7 ± 25.1	537 ± 23.6	504 ± 34.5
WL	28	337.7 ± 18.9	54.36 ± 3.2	19.8 ± 1.3	280.3 ± 16.8	171 ± 14.1	30.1 %

[a] IC$_{50}$ of BHA was 53.96 ± 3.1, vitamin C, 5.05 ± 0.1 and quercetin 5.28 ± 0.2, respectively. [b] IC$_{50}$ of quercetin was 5.28 ± 0.2. [c] IC$_{50}$ for vitamin C and quercetin were 21.4 ± 1.1 and 52 ± 2.6, respectively. [d] Inhibition at 800 mgml^{-1}. EDTA was used as control (IC$_{50}$ = 18 ± 1.5 µgml^{-1}).

found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Ebrahimzadeh et al., 2010b).

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Nabavi et al., 2009). DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Specific compounds or extracts are allowed to react with the stable radical, DPPH$^•$, in methanol solution. In the presence of hydrogen donors, DPPH$^•$ is reduced and a stable free radical is formed from the scavenger. The reaction of DPPH$^•$ is monitored by the decrease of the absorbance of its radical at 515 nm, but upon reduction by an antioxidant, the absorption disappears (Brand-Williams et al., 1995).

DPPH$^•$ + antioxidant $\rightarrow$ DPPH-H + antioxidant$^•$
Purple color   Yellow color
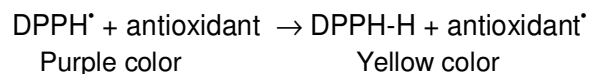
Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Hebbel et al., 1990). It was found that the radical-scavenging activities of all the extracts increased with increasing concentration. High phenol and flavonoid contents of this plant may lead to its good DPPH-scavenging activity. IC$_{50}$ for DPPH radical-scavenging activity exist in Table 1. WB with highest content of phenols showed the highest activity (IC$_{50}$ = 10.7 ± 0.6 µgml^{-1}) that was comparable with vitamin C and quercetin (p > 0.05).

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Dehpour et al., 2009). Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1 shows the dose response curves for the reducing powers of the extracts. It was found that the reducing powers of all the extracts also increased with the increase of their concentrations. There were no significant differences (p > 0.05) among the stem bark and leaves extracts (aqueous and methanol) in the reducing power that were comparable with vitamin C (p > 0.05). Polyphenolic contents of all the sample extracts appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Fruit extracts with lowest phenol and flavonoids contents did not show any activity. Similar observation between the polyphenolic constituents in terms of dose dependent and reducing power activity have been reported for several plant extracts (Ebrahimzadeh et al., 2009a - e, 2010a, b).

Nitric oxide (NO) has been associated with a variety of physiologic processes in the human body since it was identified as a novel signal molecule. It transmits signals from vascular endo-thelial cells to vascular smooth muscle cells and plays an important role in vital physiologic functions in many systems. In the nervous system, NO works as an atypical neural modulator that is involved in neurotransmitter release, neuronal excitability and learning and memory (Aliev et al., 2009; Nath et al., 2006). The scavenging nitric oxide is based on the principle that, sodium
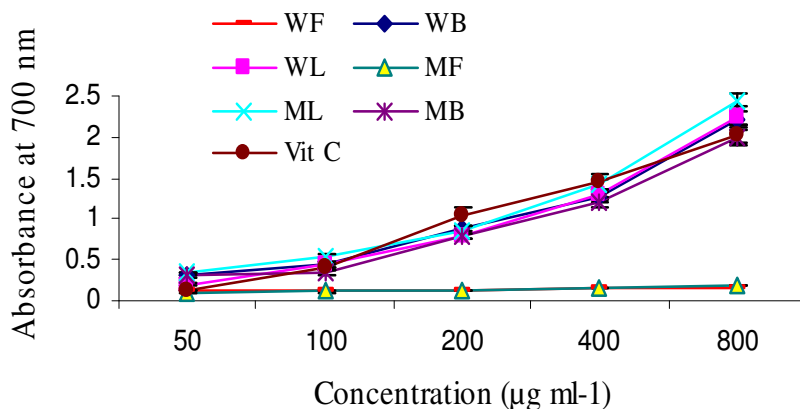
Figure 1. Reducing power of medlar extracts. Vitamin C was used as control. MF: Methanol fruit, WF: aqueous fruit, ML: methanol leaves, WL: aqueous leaves, MB: methanol bark and WB: aqueous bark extracts.

nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Results of assay of nitric oxide-scavenging activity are presented in Table 1. Fruits methanol extract exhibit better activity than the others (IC_{50} = 247 ± 12.2 μgml^{-1}). However, the activity of quercetin was very much pronounced than that of our extracts (5.28 ± 0.2 μgml^{-1}) that was not comparable with them (p < 0.001). Several studies suggest that NO may modulate iron-catalyzed oxidation reactions such as the O^{-2}-driven Fenton reaction, which produces powerful oxidants such as the hydroxyl radical (OH·) and metalloxo complexes. The mechanisms by which NO may inhibit lipid peroxidation are not entirely clear, however, one possible mechanism relates to the ability of NO to terminate propagation of lipid peroxidation reactions. Lipid peroxidation is initiated by the formation of potent oxidants such as OH· or ferryl hemoproteins that produces lipid alkyl radicals (L·) from polyunsaturated fatty acids (LH). This radical is then converted to a hydroperoxyl radical (LOO·) via its interaction with $O^{2.}$ The LOO· can abstract an allylic hydrogen atom from another LH, thereby propagating the free radical reaction. NO will rapidly react with these LOO· and/or lipid alkoxyl radicals (LO·), resulting in LOONO or LONO formation, respectively, which in turn leads to chain termination (Rubbo et al., 1994).

In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other patho-logical conditions (Moncada et al., 1991). The plant/plant products may have the potency to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Further, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

Abundant evidence exists from *in vitro* experiments that iron can catalyze the production of oxyradicals when iron is available in a redox-active form (Halliwell and Gutteridge, 1999). Two important radicals that can result from these reactions are lipid radicals and hydroxyl radicals. One type of reaction promoted by iron that leads to the formation of lipid radicals is the decomposition of preformed lipid hydroperoxides (Britton et al., 2002). Ferrous iron chelates can react with lipid hydroperoxides (LOOH) to form alkoxyl radicals (LO·): LOOH + Fe^{2+} → Fe^{3+} + OH^{-} + LO·

Lipid peroxyl radicals can perpetuate the chain reaction of lipid peroxidation by extracting hydrogen atoms from nearby lipids or can react with other cell constituents. Iron also, can catalyze the production of hydroxyl radicals (OH·) through Fenton chemistry. In this reaction, the OH· is produced from hydrogen peroxide:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$
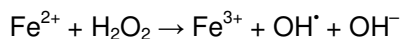
The hydroxyl radical is extremely reactive and can attack many cell constituents, including lipids, nucleic acids and proteins (Welch et al., 2002; Britton et al., 2002). Because the polyunsaturated fatty acids of membrane phospholipids are particularly susceptible to oxidative attack, the process of lipid peroxidation after iron overload has been widely studied.

Iron chelators mobilize tissue iron by forming soluble and stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in human and thereby improves quality of life and overall survival in some diseases such as thalassemia major (Hebbel et al., 1990). In addition, iron chelation could be considered as a rational therapeutic strategy for Alzheimer's disease (Grazul and Budzisz, 2009). Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the
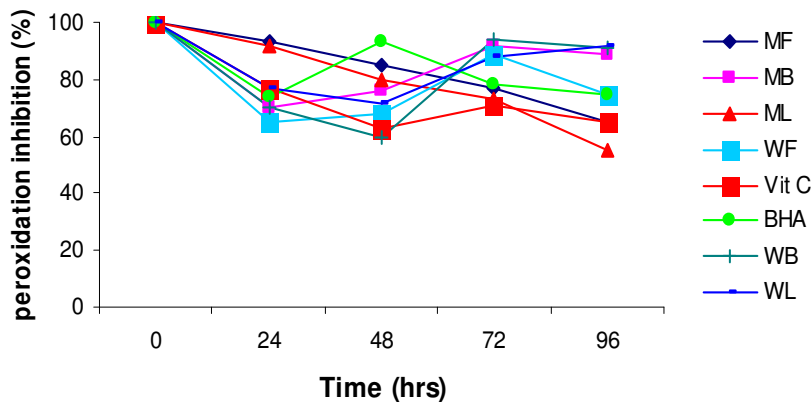
Figure 2. Antioxidant activity of medlar extracts in FTC method at different incubation times. MF: methanol fruit, WF: aqueous fruit, ML: methanol leaves, WL: aqueous leaves, MB: methanol bark and WB: aqueous bark extracts (0.4 mg/ml), vitamin C and BHA (0.2 mg/ml).

formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell, 1997b). These processes can be delayed by iron chelation. Iron can generate free radicals from peroxides and may be implicated in human cardiovascular disease (Halliwell and Gutteridge, 1990). Therefore, minimizing its concentration affords protection against oxidative damage. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the ferrozine complex formation is disrupted with the result that the red color of the complexes decreases (Ebrahimzadeh et al., 2008). The absorbance of Fe^{2+}-ferrozine complex was decreased dose-dependently, that is, the activity was increased on increasing concentration from 0.2 to 3.2 $mgml^{-1}$. Results are presented in Table 1. Only aqueous extract of bark showed weak activity, others did not show any activity.

Scavenging of H_2O_2 by extracts may be attributed to their phenols and other active components such as anthocyanins, tannins and flavonoids which can donate electrons to H_2O_2, thus neutralizing it to water (Ebrahimzadeh et al., 2009f). The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Results are presented in Table 1. Leaves methanol extract (also its aqueous extract) showed good activity that was comparable with quercetin ($p > 0.05$). These two extracts had high phenol and flavonoid contents. Polyphenolic contents of extracts appear to function as good electron donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Extracts with lower contents showed lower activity. Although hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems (Ebrahimzadeh et al., 2010a).

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu, 2001). The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Superoxide indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Ebrahimzadeh et al., 2009a). Hydroxyl radicals eliminate hydrogen atoms from the membrane lipids, which results in lipid peroxidation. Figure 2 shows the time-course plots for the antioxidant activity of the plants extracts using the FTC method. Extracts exhibited good antioxidant activity. There were significant differences among extracts ($p > 0.05$). They manifested almost the same pattern of activity as vitamin C and BHA at different incubation times (until 72nd hour) but methanol and aqueous bark extracts and aqueous leaves extract showed higher peroxidation inhibition than vitamin C and BHA at 96th hour ($p < 0.05$).

Conclusions

The plant extracts exhibited different levels of antioxidant activity in all the models studied. Stem bark extract (both aqueous and methanol) showed the best activities in nearly all tests. Further investigation of individual compounds, their *in vivo* antioxidant activities and different antioxidant mechanisms are needed. Such identified potential and natural constituents could be exploited as cost effective food additives for human and animal health.

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