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Radical Scavenging Activity and Antioxidant Capacity of Bay Leaf Extracts

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Bay leaves (BL) (*Laurus nobilis* L., Family: Lauraceae) are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence. In this study, lyophilized extracts (both water and ethanol) of BL were studied for their antioxidant properties. The antioxidant activity, reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities were evaluated to determine the total antioxidant capacity of both BL extracts. Both extracts exhibited strong total antioxidant activity in linoleic acid emulsion. Concentrations of 20, 40, and 60 μg ml^{-1} showed 84.9, 95.7, 96.8, and 94.2, 97.7, and 98.6% inhibition of lipid peroxidation of linoleic acid emulsion, for water and ethanol extracts, respectively. On the other hand, 60 μg ml^{-1} of the standard antioxidants butylated hydroxyianisole (BHA), butylated hydroxytoluene (BHT), and α-tocopherol exhibited 96.6, 99.1, and 76.9% inhibition of lipid peroxidation in linoleic acid emulsion, respectively. In addition, the both BL extracts had effective reducing power, DPPH· free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging and metal chelating activities at 20, 40, and 60 μg ml^{-1}. The total amount of phenolic compounds in each BL extract was determined as gallic acid equivalents.

Keywords: Antioxidant activity, Bay leaves, *Laurus nobilis*, Linoleic acid emulsion, Free radical scavenging activity

INTRODUCTION

Free radicals can be described as chemical species that have an unpaired electron. The reactivity of free radicals varies from relatively low, as in the case of the oxygen molecule itself, to very high, as in the case of the short-lived and highly reactive hydroxyl radical (OH·) [1]. Fatty acids are susceptible to attack by highly reactive oxygen species (ROS) such as OH·; hence any reaction or process that forms ROS stimulates lipid oxidation. Hydrogen abstraction is easier in unsaturated fatty acids than in their saturated counterparts, thus making them more susceptible to ROS attack. Oxygen and ROS are among the major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro*. The electronic structure of oxygen has two unpaired electrons in its outermost energy level [2]. The reaction of oxygen with ground state molecules of singlet multiplicity such as polyunsaturated fatty acids is spin-forbidden. However, this barrier does not apply to reactions, involving single electrons, hydrogen atoms, and molecules containing unpaired electrons, such as transition metal complexes and free radicals. Therefore, triplet state oxygen can react with other molecules to yield ROS such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot -}$), and hydroxyl radical (OH·) [3-6]. Superoxide radical ($O_2^{\cdot -}$) is generated by the four electron reduction of molecular oxygen into water.

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This radical is also formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide anion radicals ($O_2^{\cdot -}$) are also formed by activated phagocytes such as monocytes, macrophages, eosinophils, and neutrophils and the production of $O_2^{\cdot -}$ is an important factor in the killing of bacteria by phagocytes. In living organisms, $O_2^{\cdot -}$ is removed by the enzymes called superoxide dismutases [7-8].

Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death tissue damage. Oxidative damage plays a significantly pathological role in human disease. Cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage [4]. Also, excessive generation of ROS, which is induced by various stimuli and exceeds the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [9,10]. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutases and catalase or antioxidant compounds such as ascorbic acid, tocopherols, and gluthatione [11]. When the mechanism of antioxidant protection becomes unbalanced by exogenous factors such as tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides and endogenous factors such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes, the result may be the above-mentioned diseases and accelerated aging. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage [4,12,13].

Bay leaves (*Laurus nobilis* L., Family: Lauraceae) are traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence [14]. The aqueous extract is used in Turkish folk medicine as an anti-hemorrhoidal, anti-rheumatic, diuretic, as an antidote in snakebites and for the treatment of stomachache [14,15]. Other sources cite that bay leaves have been used for skin diseases, rheumatism, urinary problems and stones [16].

It has been reported that bay leaves repel, are toxic to and inhibit the reproduction of insects [17], in addition to having cytotoxic properties [18], therapeutic effects for ethanol intoxication [19] and alkyl peroxy radical scavenging activity [20]. Also, there are some purification studies investigating the composition of bay leaves. Essential oils, non-polar flavonoids, sesquiterpenoid lactones, isoquinoline alkaloids, and vitamin E have been isolated from bay leaves [18,21].

There is no information about the *in vitro* antioxidant activity of water or ethanol extracts of BL. However, from a toxicological point of view, ethanol and water, as solvents, are safer than other organic solvents, and therefore more suitable for the food and pharmaceutical industries. Thus water and ethanol extracts are used in the present study. The purpose of the present study is to evaluate the antioxidant activity of the water and ethanol extracts of BL to elucidate their antioxidative capacity.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's phenol reagent, linoleic acid, α-tocopherol, nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck. All other chemicals used were of analytical grade and obtained from either Sigma-Aldrich or Merck.

Plant Material and Extraction Procedures

Dried BL was harvested from Black Sea region of Turkey. In the preparation for water extraction, 20 g bay leaves were ground into a fine powder in a mill and mixed with 400 ml boiling water by magnetic stirrer for fifteen minutes. Then, the extract was filtered over a Whatman No. 1 paper. The filtrates were frozen and lyophilized in a lyophilizer (Labconco, Freezone 1) at 5 μm Hg pressure at -50 ^{o}C. For ethanol extraction, 25 g bay leaves were ground into a fine powder in a mill and mixed with 500 ml ethanol. The residue was re-extracted under the same conditions until the extraction solvents became colorless. The obtained extracts were filtered over a Whatman No. 1 paper and the filtrate was collected. The ethanol was then removed under reduced pressure at 50 ^{o}C to obtain the dry extract. Both extracts were placed in plastic

bottles and stored at -20 °C until used.

Antioxidant Activity Determination in Linoleic Acid Emulsion Using Ferric Thiocyanate Method

The antioxidant activity of BL was determined according to the ferric thiocyanate method in linoleic acid emulsion [22]. Lyophilized water BL extract (10 mg) was dissolved in 10 ml water. Ethanol BL extract (10 mg) was dissolved in 10 ml ethanol. BL extracts (20, 40, and 60 μg ml^{-1}) or standard samples in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.0) were added to a linoleic acid emulsion (2.5 ml). The linoleic acid emulsion (50 ml) consisted of 175 μg Tween-20, 155 μl linoleic acid, and 0.04 M potassium phosphate buffer (pH 7.0). The control (50 ml) consisted of 25 ml linoleic acid emulsion and 25 ml potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37 °C in a glass flask, protected from light. After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm in a spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan), after reacting with $FeCl_2$ and thiocyanate at intervals during the incubation. During the linoleic acid oxidation, peroxides formed, which oxidized Fe^{2+} to Fe^{3+}. The Fe^{3+} ions form a complex with SCN^- and this complex has maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions, without added extracts, were used as blank samples. All antioxidant activity data were the average of duplicate analyses. The inhibition of lipid peroxidation was calculated by following equation:

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

where A_o was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of BL extract [23,24].

Total Reduction Capability by Fe^{3+}-Fe^{2+} Transformation

The total reducing power of the BL extracts was determined according to the method of Oyaizu [25]. Briefly, different concentrations of BL extracts (20, 40, and 60 μg ml^{-1}) in 1 ml of distilled 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. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min at 1000 × g (MSE Mistral 2000, UK). The upper layer of 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 using a UV-Vis spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan). Higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide Anion Radical Scavenging Activity in PMS-NADH Systems

Measurement of superoxide anion scavenging activity of BL extracts was based on the method described by Liu [26] with slight modification [27]. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). Tris-HCl buffer (3 ml, 16 mM, pH 8.0) containing 1 ml NBT (50 μM) solution, 1 ml NADH (78 μM) solution and a sample solution of BL extract (from 20 to 60 μg ml^{-1}) in water were mixed. The superoxide radical-generating reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance was read at 560 nm using a UV-Vis spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan) and measured against blank samples. L-ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percent inhibition of superoxide anion generation was calculated using the following formula

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

where A_o was the absorbance of the control and A_1 was the absorbance of BL extracts and standards [28].

Free Radical Scavenging Activity Measured by 1,1-Diphenyl-2-picryl-hydrazil

The free radical scavenging activity of BL extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH·) using the method of Blois [29]. Briefly, a 0.1 mM solution of DPPH· in ethanol was prepared and 1 ml of this solution was added to 3 ml of BL extract solution in water at different concentrations (20-60 μg ml^{-1}). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-Vis

spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan). Lower absorbance values of the reaction mixture indicated higher free radical scavenging activity. The DPPH· concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression ($R^2 = 0.999$):

$$\text{Absorbance} = 0.00107 \times [\text{DPPH}\cdot] - 0.0184$$

The capability to scavenge the DPPH radical was calculated using the following equation:

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

where A_o was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of BL extracts [30].

Ferrous Ion Chelating Activity

The chelation of ferrous ions by the BL extracts and standards was estimated by the method of Dinis [31]. Briefly, extracts (20-60 μg ml^{-1}) 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 standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a UV-Vis spectrophotometer (Jasco V-530 Japan Servo Co. Ltd., Japan). All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated as follows:

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

where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the samples of BL extracts and standards. The control contained $FeCl_2$ and ferrozine complex molecules [32].

Scavenging of Hydrogen Peroxide

The ability of the BL extracts to scavenge hydrogen peroxide was determined according to the method of Ruch [33]. 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 (Jasco V-530, Japan Servo Co. Ltd., Japan). Extracts (20-60 μg ml^{-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 BL extracts and standard compounds was calculated as follows:

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

where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample of BL extract and standard [34].

Determination of Total Phenolic Compounds Using Folin-Ciocalteu Phenolic Reagent

The total phenolic compounds in the BL extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [35] using gallic acid as a standard phenolic compound. Briefly, 1 ml of extract solution (1000 μg of extract) in a volumetric flask diluted with distilled water (46 ml). Folin-Ciocalteu reagent (1 ml) was added and the contents of the flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (Jasco V-530, Japan Servo Co. Ltd., Japan). The amount of total phenolic compounds in the BL extracts was determined in micrograms of gallic acid equivalent, using the equation obtained from the standard gallic acid graph:

$$\text{Absorbance} = 0.0053 \times \text{Total phenols [Gallic acid equivalents (μg)]} - 0.0059$$

RESULTS AND DISCUSSION

Antioxidant methods and modifications have been proposed to evaluate antioxidant characteristics and to explain how antioxidants function. Of these, antioxidant activity, reducing power, metal chelation, free radical scavenging, superoxide anion radical scavenging, and hydrogen peroxide scavenging

Table 1. Yield and Inhibition of Lipid Peroxidation in Linoleic Acid Emulsion of Bay Leaf Extracts

Solvent	Yield (g)	Inhibition of lipid peroxidation (%)
Water	1.614	97[a]
Ethanol	1.600	99[a]

[a]The antioxidant activity of extracts (60 μg ml^{-1}) was determined by the thiocyanate method.

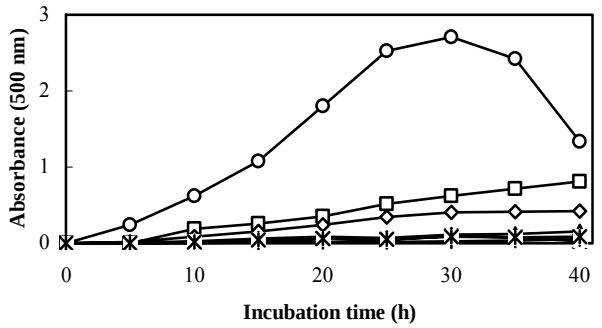

Fig. 1. Antioxidant activity of different concentrations of water BL extract and α-tocopherol in linoleic acid emulsion by the thiocyanate method: (○) Control, (×) BHA-60 μg ml^{-1}, (+) BHT-60 μg ml^{-1}, (□) α-Tocopherol-60 μg ml^{-1}, (◇) Bay-Water-20 μg ml^{-1}, (Δ) Bay-Water-40 μg ml^{-1}, (∗) Bay-Water-60 μg ml^{-1}.

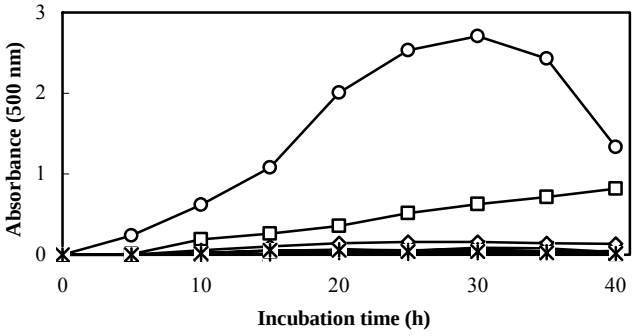

Fig. 2. Antioxidant activity of different concentrations of ethanol BL extract and α-tocopherol in linoleic acid emulsion by the thiocyanate method: (○) Control, (×) BHA-60 μg ml^{-1}, (+) BHT-60 μg ml^{-1}, (□) α-Tocopherol-60 μg ml^{-1}, (◇) Bay-Ethanol-20 μg ml^{-1}, (Δ) Bay-Ethanol-40 μg ml^{-1}, (∗) Bay-Ethanol-60 μg ml^{-1}.

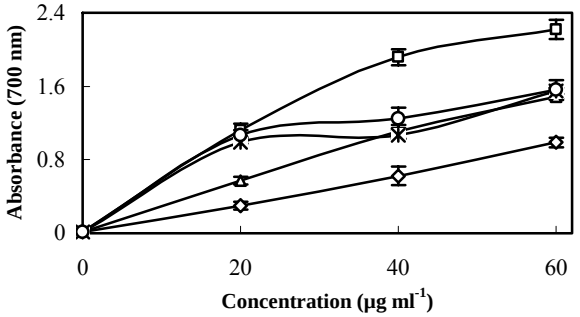

Fig. 3. Reducing power of BL extracts, BHA, BHT, and α-tocopherol by spectrophotometric detection of the Fe^{3+}-Fe^{2+} transformation: (◇) α-Tocopherol, (□) BHA, (Δ) BHT, (∗) Bay-Water, (○) Bay-Ethanol.

activities are most commonly used for the evaluation of the total antioxidant behavior of extracts [36,37].

Table 1 shows the yields and antioxidant activity of water and ethanol extracts of BL. The antioxidant activity of BL extracts was determined by the thiocyanate method. Both BL extracts exhibited effective antioxidant activity at all concentrations. The effects of various concentrations of water and ethanol extracts of BL (from 20 μg ml^{-1} to 60 μg ml^{-1}) on peroxidation in linoleic acid emulsion are shown in Figs. 1 and 2. The antioxidant activity of both BL extracts increased with increasing concentration. Different concentrations (20, 40, and 60 μg ml^{-1}) of water and ethanol extracts of BL showed higher antioxidant activities than that of 60 μg ml^{-1} concentration of α-tocopherol. The inhibition of peroxidation in the linoleic acid system was 85, 96, 97, 94, 98, and 99%, respectively, greater than that 60 μg ml^{-1} of α-tocopherol (77%). On the other hand, inhibition of BHA and BHT (60 μg ml^{-1}) was 97 and 99%, respectively.

Figure 3 shows the reductive capabilities of the BL extracts compared to BHA, BHT, and α-tocopherol. For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of BL extract using the method of Oyaizu [25]. The reducing capacity of acompound may serve as a significant indicator of its potential antioxidant activity [38]. The antioxidant activity of putative antioxidants has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity, and

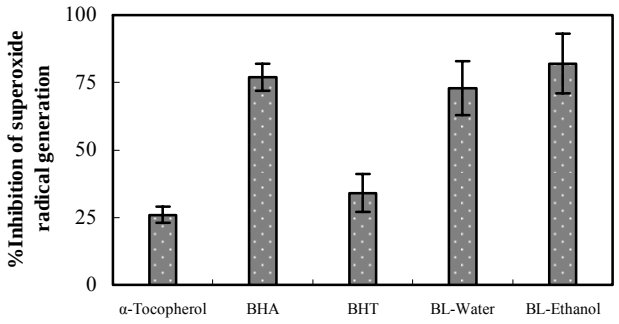

Fig. 4. Inhibition of superoxide anion radical generation activity of 60 μg μl^{-1} BL extracts, BHA, BHT, and α-tocopherol by the PMS-NADH-NBT method.

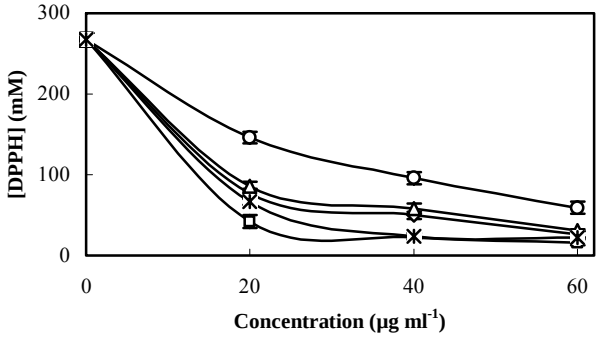

Fig. 5. Free radical scavenging activity of BL extracts, BHA, BHT and α-tocopherol by 1,1-diphenyl-2-picrylhydrazyl radicals: (◇) BHA, (□) BHT, (Δ) α-Tocopherol, (○) Bay-Water (∗) Bay-Ethanol.

radical scavenging [39]. Like the antioxidant activity, the reducing power of both BL extracts increased with increasing concentration. All BL extract concentrations tested showed higher activities than the BHT and α-tocopherol controls and these differences were statistically significant ($p < 0.01$). The ethanol extract exhibited a stronger reducing power than the water extract, but the difference between the two BL extracts was not significant ($p > 0.05$). The reducing power of water and ethanol extracts of BL and standard compounds followed the order: BHA > ethanol extract > water extract > BHT > α-tocopherol.

Superoxide anion radicals ($O_2^{\cdot -}$) are formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils, and the production of $O_2^{\cdot -}$ is an important factor in the killing of bacteria by phagocytes. In the PMS-NADH-NBT system, superoxide anion, derived from dissolved oxygen from the coupling reaction of PMS-NADH, reduces NBT. The decrease in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Figure 4 shows the percent inhibition of superoxide radical generation by 20, 40, and 60 μg ml^{-1} of BL extracts compared to the same doses of BHA, BHT, and α-tocopherol. Both extracts of BL have strong superoxide radical scavenging activity (73, and 82%), higher than that of BHT (34%) and α-tocopherol (26%) ($p < 0.05$), and comparable to that of BHA (77%), at the same dose The superoxide radical scavenging activity of the tested compounds followed the order: BL (EtOH) > BHA > BL (H_2O) > BHT > α-tocopherol.

The stable DPPH radical model is a widely-used, relatively quick method for the evaluation of free radical scavenging activity. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability [40].

DPPH· is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [41]. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH· is usually used as a substrate to evaluate the antioxidative activity of antioxidants [37]. Figure 5 illustrates a significant ($p < 0.01$) decrease in the concentration of DPPH radicals due to the scavenging ability of the both BL extracts and standards. The ethanol extract of BL showed a significantly stronger DPPH scavenging activity than the water BL extract ($p < 0.01$). We used BHA, BHT, and α-tocopherol as standards. The strength of the scavenging activity of water and ethanol extracts of BL and standards on the DPPH radical followed the order of BHT > ethanol BL extract > BHA > α-tocopherol > water BL extract, with inhibitions of 94, 92, 90, 88, and 78% (at 60 μg ml^{-1}), respectively. These results indicated that both BL extracts have a noticeable effect on scavenging free radicals. Free radical scavenging activity also increased with increasing concentration.

It has been reported that oxidative stress, which occurs

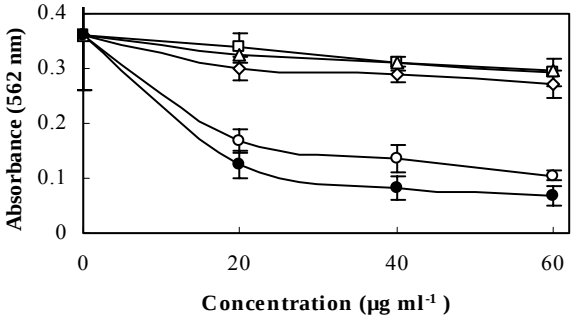

Fig. 6. Metal chelating effect of different concentrations of BL extracts, BHA, BHT and α-tocopherol on ferrous ions: (◇) α-Tocopherol, (□) BHA, (Δ) BHT, (○) BL-Water, (●) BL-Ethanol.

when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as arteriosclerosis [42]. Based on the data obtained from this study, both BL extracts are free radical inhibitors or scavengers, as well as primary antioxidants that react with free radicals, which may limit free radical damage occurring in the human body.

The chelation of ferrous ions by BL extracts was estimated by the method of Dinis [31], in which ferrozine quantitatively forms complexes with Fe^{2+}. In the presence of chelating agents, the formation of this complex is disrupted, thereby impeding the formation of the red color imparted by the complex as well. Measurement of this color change therefore allows for the estimation of the chelating activity of the coexisting chelator [43]. In this assay, both the BL extracts and the standard antioxidant compounds interfered with the formation of ferrous-ferrozine complex, suggesting that they have chelating activity, capturing the ferrous ion before it can form a complex with ferrozine.

Iron can stimulate lipid peroxidation by the Fenton reaction ($H_2O_2 + Fe^{2+} = Fe^{3+} + OH^- + OH\cdot$), and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [37,44].

As shown in Fig. 6, the formation of the Fe^{2+}-ferrozine complex is not complete in the presence of the water and ethanol BL extracts, indicating that both extracts chelate the iron. The absorbance of Fe^{2+}-ferrozine complex linearly decreased in a dose-dependent manner (from 20 to 60 μg ml^{-1}). The difference between both extracts of BL and the control was statistically significant ($p < 0.01$). The metal chelating capacities of water and ethanol extracts of BL, α-tocopherol, BHA, and BHT (all at 60 μg ml^{-1}) were 71, 82, 25, 19, and 18%, respectively, which proved to be a significant difference between the extracts and the controls ($p < 0.05$). The strength of the metal scavenging effect of the BL extracts and the standards followed this order: ethanol BL extract > water BL extract > α-tocopherol > BHA > BHT.

The metal chelating capacity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [23]. It has been reported that chelating agents, which form σ-bonds with metals, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [45]. The data shown in Fig. 6 reveal that both BL extracts demonstrate a marked capacity for iron binding, suggesting that their protective action against peroxidation may be related to its iron binding capacity.

Scavenging of H_2O_2 by both BL extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water [7]. The differences in H_2O_2 scavenging capacities between the two extracts may be attributed to the structural features of their active components, which determine their electron donating abilities [8].

The ability of the both extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch [33], is displayed in Fig. 7, where it is compared with that of BHA, BHT and α-tocopherol as standards. The BL extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. Water and ethanol BL extracts (60 μg ml^{-1}) exhibited 42 and 22% hydrogen peroxide scavenging activity, respectively, while, at the same concentration, BHA, BHT, and α-tocopherol showed 19, 25, and 23% activity (ethanol BL extract > water BL extract > BHT > α-tocopherol > BHA). The correlation between the BL extract values and those of the controls was statistically significant ($p < 0.05$). 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 [44].

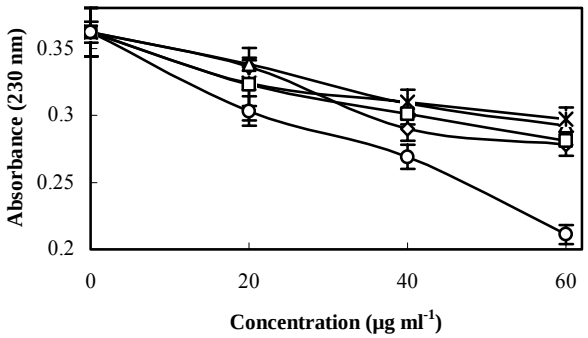

Fig. 7. Hydrogen peroxide scavenging activities of BL extracts, BHA, BHT, and α-tocopherol: (◇) α-Tocopherol, (Δ) BHA, (∗) BHT, (□) BL-Water, (○) BL- Ethanol.

Phenolic constituents are very important in plants because of their scavenging ability due to their hydroxyl groups [46]. One milligram each of water and ethanol BL extracts contained 81.7 and 84.5 µg gallic acid equivalents of phenols, respectively. These phenolic compounds may contribute directly to the antioxidative action. It has been suggested that up to 1.0 g polyphenolic compounds (from a diet rich in fruits and vegetables) ingested daily have inhibitory effects on mutagenesis and carcinogenesis in humans [47]. In addition, it has been reported that phenolic compounds are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [48].

CONCLUSIONS

On the basis of the results of this study, both BL extracts have significant antioxidant activity compared to other, well characterized, standard antioxidant systems *in vitro*. Moreover, BL can be used as an easily accessible and rich source of natural antioxidants, as a food supplement or in the pharmaceutical industry. The various antioxidant mechanisms of BL extract may be attributed to its strong abilities as a hydrogen donor, metal chelator, and scavenger of hydrogen peroxide, superoxide, and free radicals. In addition, the antioxidant activity may be due to phenolic compounds in BL extracts. However, the components responsible for the antioxidative activity of BL extracts are currently unclear. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidant components of BL.

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