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Antioxidant Properties and Total Phenolic Contents of Cherry Elaeagnus (*Elaeagnus multiflora* Thunb.) Leaf Extracts

Seung-Ryeul Shin[1], Ju-Yeon Hong[1], and Kyung-Young Yoon*

Department of Food and Nutrition, Yeungnam University, Gyeongsan, Gyeongbuk 712-749, Korea

[1]College of Health and Welfare, Faculty of Herbal Cuisine and Nutrition, Daegu Hanny University, Gyeongsan, Gyeongbuk 712-715, Korea

Abstract In Korea and China, cherry elaeagnus (*Elaeagnus multiflora* Thunb.) has been used traditionally to treat cough, diarrhea, itching, and foul sores. Therefore, in this study, the ethanol and water extracts of cherry elaeagnus leaves were examined for their antioxidant activities. The ethanol extract of the cherry elaeagnus leaves contained more phenolics than the water extract. All the cherry elaeagnus leaf extracts had higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability than ascorbic acid at concentrations of 250-1,000 μg/mL. The ethanol extract also showed higher superoxide dismutase (SOD)-like activity compared to the water extract. Furthermore, the SOD-like activity of the ethanol extract amounted to 89% of that of ascorbic acid at a concentration of 500 μg/mL. The nitrite scavenging ability and xanthine oxidase inhibitory (XOI) activity of the ethanol extract were higher than those of the water extract. In particular, the ethanol extract had higher XOI activity than ascorbic acid at a concentration of 1,000 μg/mL.

Key words: cherry elaeagnus, *Elaeagnus multiflora* Thunb., phenolics, antioxidant

Introduction

Oxidative modifications of DNA, proteins, lipids, and small cellular molecules by reactive oxygen species play a role in a wide range of common diseases and age-related degenerative conditions (1). To protect biological molecules from possible damage, all oxygen-consuming organisms are endowed with a well-integrated antioxidant system. The protective effects for plants are due to the presence of several components that have distinct mechanisms of action, such as natural antioxidant phytochemicals (2). Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. It has been shown that a wide variety of phenolic compounds, including flavonoids, isoflavones, flavones, anthocyanins, and catechins, act to prevent or reduce oxidative stress by scavenging free radicals (3-5). In addition, vitamins such as ascorbic acid, α-tocopherol, and β-carotene have been shown to have antioxidant properties (6). Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and to delay the oxidation process. However, commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are restricted by legislative rules because they are suspected to have certain toxic effects and are possible carcinogens (7). Recently, the food industry has been using various natural antioxidants of plant origin (8); in addition, the use of naturally occurring antioxidants extracted from plants and vegetables has been widely investigated (9,10).

Cherry elaeagnus (*Elaeagnus multiflora* Thunb.) belongs to the family Elaegnaceae (oleaster) and order Myrtales, and is called 'gumi' or cherry silverberry. The cherry elaeagnus leaves are egg-shaped, 2.5-5 cm long, dark green on top, and silver with tiny brown scales beneath. The plant is native to China and Japan, and has been cultivated for centuries as an ornamental, as well as for its tasty fruit in east Asia, including Korea. Cherry elaeagnus is drought tolerant, can grow in either salty or alkaline soils, and rarely has insect or disease problems. Cherry elaeagnus is a medicinal plant, and the fruits, leaves, and roots have been used to treat cough, diarrhea, itching, and foul sores for a long time in China (11). It contains abundant amounts of sugars, fatty acids, and phytosterols in the leaves and stems, and ascorbic acid and carotenoids in the fruit (12). Although there is published work related to the components and physiological activity of the fruits (13-15), there is still limited information on the antioxidant activity of the leaves of cherry elaeagnus.

In preliminary studies, we found that the water and ethanol extracts of cherry elaeagnus fruits have high antioxidant properties (16,17), and we anticipated that the leaves would have as much biological activity as the fruits. Therefore, the aim of present study was investigated the possibility of a potent source of antioxidant, as an alternative to synthetic compounds. Cherry elaeagnus leaves were extracted with nontoxic solvents such as water and ethanol. The antioxidant activity and xanthine oxidase inhibitory activity of cherry elaeagnus leaf extracts were examined, and the levels of phenolic compounds in these extracts were measured.

Materials and Methods

Materials The cherry elaeagnus leaves were picked at a farm in Gyeongsan, Gyeongbuk, Korea. The samples were washed, dehydrated, and stored at −75°C in preparation for antioxidant activity and phenolic compound analysis.

*Corresponding author: Tel: +82-53-810-2878; Fax: +82-810-4768
E-mail: yoonky2441@ynu.ac.kr
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Preparation of sample extract A 30 g portion of sample and 300 mL of distilled water or ethanol were combined. The water and ethanol extract solutions were collected after shaking for 3 hr at 70°C. The sample extraction was replicated 3 times. The extract was centrifuged at 16,270×g for 30 min, and then filtered. Next, it was condensed with a rotary evaporator (N-1000; Eyela, Tokyo, Japan) under reduced pressure at 60°C, and the condensed extracts were lyophilized. The lyophilized extracts were used to prepare solutions of various concentrations that were then analyzed for their antioxidant activities, as described in the following section.

Determination of L-ascorbic acid content Ascorbic acid was determined according to the method of Klein and Perry (18). Each extract (20 mg) was extracted with 10 mL of 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was mixed with 9 mL of 2,4-dichloroindophenol (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and the absorbance was measured within 15 sec at 515 nm against a blank. The content of ascorbic acid was calculated based on the calibration curve of authentic L-ascorbic acid.

Determination of total phenolic content Total phenolic content was determined according to the method of Singleton and Rossi (19). Each extract was dissolved in distilled water. One mL of each sample was mixed with 1 mL of Folin-Ciocaltue reagent and allowed to stand at room temperature for 3 min. Then, 1 mL of 2% Na_2CO_3 was added to the mixture. After standing 1 hr, the absorbance was measured with a spectrophotometer (UV-2001; Hitach, Tokyo, Japan) at 725 nm. The results are expressed as tannic acid equivalents.

Determination of antioxidant activities The free radical scavenging effects were estimated according to the method of Blois (20) with some modification. Two mL of each sample prepared at various concentrations (125, 250, 500, and 1,000 μg/mL) were added to 1 mL of 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical solution. The mixture was shaken and allowed to stand for 30 min at 37°C, and then the absorbance was measured at 517 nm with a spectrophotometer (UV-2001; Hitachi).

The superoxide dismutase (SOD)-like activity was determined by the method of Marklund and Marklund (21), where 0.5 mL of each sample, 3 mL of Tris-HCl buffer (pH 8.5), and 0.2 mL of 7.2 mM pyrogallol were mixed and kept at 25°C for 10 min. The reaction was stopped with 0.1 mL of 1 N HCl, and the absorbance was measured by spectrophotometry at 420 nm.

The nitrite scavenging ability of each sample was carried out by the method of Kato *et al.* (22). Each sample extract of 4°C different concentrations (125, 250, 500, and 1,000 μg/mL) was mixed with 1 mL of 1 mM $NaNO_2$, and adjusted to pH 1.2 using 0.1 N HCl and 0.2 N citrate buffer. The mixture was increased in volume to 5 mL, and then incubated at 37°C for 1 hr. Next, a 0.5 mL aliquot of sample was combined with 2.5 mL of 2% acetic acid and 0.2 mL of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid). After 15 min, the color intensity was measured by a spectrophotometer (UV-2001; Hitachi) at 520 nm.

Xanthine oxidase inhibitory (XOI) activity was measured by the method of Stirpe and Corte (23) with some modification. The assay mixture consisted of 0.1 mL of each extract, 0.6 mL of 2 mM xanthine solution dissolved in 0.6 mL of 0.1 M potassium phosphate buffer (pH 7.5), and 0.1 mL of enzyme solution (0.2 units/mL xanthine oxidase in phosphate buffer, pH 7.5). After incubation at 37°C for 5 min, the reaction was stopped by the addition of 1 mL of 1 N HCl, and the absorbance was measured at 292 nm using a spectrophotometer (UV-2001; Hitachi). A blank was also prepared in the same manner, but the enzyme solution was added to the assay mixture after adding 1 N HCl. One unit of xanthine oxidase (XO) is defined as the amount of enzyme required to produce 1 mmol of uric acid per min at 37°C; the XOI activity is expressed as the % inhibition of XO in the above assay system.

Ascorbic acid was prepared same concentration of cherry elaeagnus extract and measured all antioxidant activity as control by the same experiments described above.

Statistical analysis Each experiment was performed 3 times and the data are expressed as means±standard deviations. Statistical analyses were performed by Duncan's multiple range tests using SPSS 12.0 software. The level of statistical significance was set at $p<0.05$.

Results and Discussion

Contents of ascorbic acid and total phenolics There was no significant difference in ascorbic acid content between the water and ethanol extracts of the cherry elaeagnus leaves, even though the ascorbic acid content of the water extract (250.0 mg/100 g) was slightly higher than that of the ethanol extract (240.0 mg/100 g) (Table 1). The total phenolics, which are naturally occurring antioxidant components, were greater in the ethanol extract (154.3 mg/100 g) than the water extract (146.4 mg/100 g). In an earlier study, cherry elaeagnus fruit was rich in ascorbic acid and polyphenols (15), and the polyphenol content of the fruit pulp changed according to the maturity of the fruits, and was highest in the youngest fruits (12). Also, the polyphenols within the pulp and stone of ripening oleaster (Elaeagnus family) fruit were found to be mainly composed of associated tannins (proanthocyanines) (24). The plant polyphenols and ascorbic acid contained within fruits and vegetables play important roles in preventing degenerative diseases, when they are consumed as part of a daily diet (4,5). Wang and Lin (25) reported that the leaves of blackberries, raspberries, and strawberries have high amounts of phenolic compounds, and their results showed

Table 1. Total phenolic and ascorbic acid contents of cherry elaeagnus leaf extracts[1)]

	Ascorbic acid (mg/100 g)	Total phenol (mg/100 g)
Water extract	250.0±0.1[a]	146.4±3.5[b]
Ethanol extract	240.0±0.0[a]	154.3±3.5[a]

[1)]Values are the means of triplicate ± SD; means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

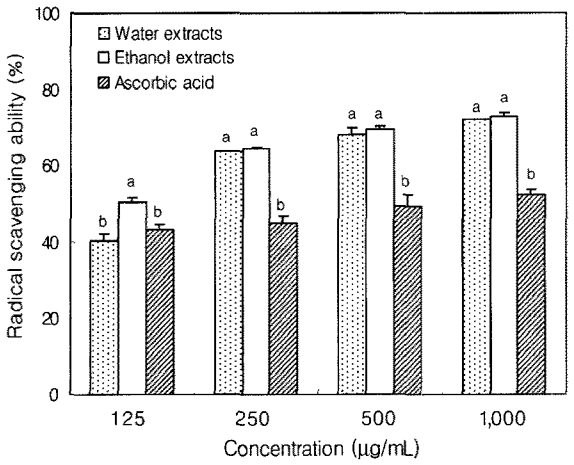

Fig. 1. DPPH radical scavenging ability of cherry elaeagnus leaf extracts. Values are the means of triplicate±SD; means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

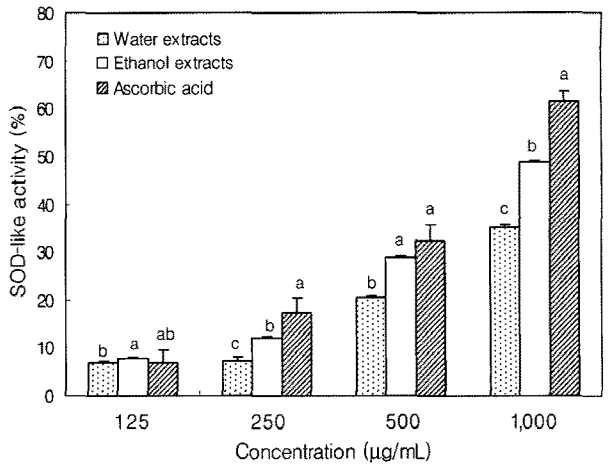

Fig. 2. Superoxide dismutase (SOD)-like activity of cherry elaeagnus leaf extracts. Values are the means of triplicate±SD; means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

a linear correlation between total phenolic content and antioxidant activity of the leaves. Yen *et al.* (26) found that the antioxidant activity of the methanolic extract of peanut hulls correlated with the content of total phenols. This suggests that the ethanol extract of cherry elaeagnus leaves, which contained a higher level of phenolics than the water extract, might have high antioxidant properties.

DPPH radical scavenging activity DPPH is a free radical compound widely used to test the free radical scavenging ability of various samples (27,28). The DPPH scavenging activities of all the cherry elaeagnus leaf extracts were increased according to increase of extract concentrations (Fig. 1). The DPPH radical scavenging ability of the ethanol extract was higher than that of the water extract at a concentration of 125 μg/mL; whereas no significant difference was shown between the ethanol and water extracts at concentrations of 250-1,000 μg/mL. All the cherry elaeagnus leaf extracts had higher DPPH radical scavenging ability than that of ascorbic acid under the same concentrations, except at 125 μg/mL. Kang *et al.* (29) reported that radical scavenging activity is an index for the antioxidant effectiveness of phenolic compounds, and it increases with increasing extract concentration. Previous results have also shown positive correlations between total phenolic content and antioxidant activity for extracts of edible mushrooms and seaweed produced in Korea (30, 31). The higher total phenol content of the ethanol extract than water extract might explain the high antioxidant properties of cherry elaeagnus leaves. The results show that cherry elaeagnus leaves are a potent source of antioxidants, as an alternative to synthetic compounds.

SOD-like activity SODs are known to catalyze the conversion of O_2^- to H_2O_2 plus O_2, and provide a defense system under oxidation conditions, in which O_2^- appears to play an important role (32). The enzyme is proven to be a useful probe for studying the participation of radicals in reactions involving oxygen such as autoxidations (21). In this study, SOD-like activity was measured by the amount of intermediate products formed from pyrogallol, which rapidly autoxidizes in aqueous solution. As shown in Fig. 2, all extracts had dosage-dependant SOD-like activity, and ascorbic acid showed the highest SOD-like activity with 7-62% at 125-1,000 μg/mL. The ethanol extract of the cherry elaeagnus leaves had significantly higher SOD-like activity compared to the water extract at all concentrations. This might be due to the ethanol extract's higher content of antioxidative materials capable of repressing the reactivity of superoxides, even though the ascorbic acid content was similar between the 2 extracts. In addition, under the same concentration, the SOD-like activity of the ethanol extract amounted to 89% of that of ascorbic acid, which has high SOD-like activity among natural antioxidants (33).

Nitrite scavenging ability Nitrosamines are known to be potent carcinogens when humans consume them in the diet, or when they are produced from endogenous biosynthesis in the body. Nitrosamines are formed by the reaction of secondary amines with nitrosating agents under acidic conditions in the stomach. Therefore, it is very important to inhibit the formation of nitrosamines in order to prevent carcinogenicity. Generally, the inhibition of nitrosamine formation is considered to occur by the degradation of nitrite itself, as well as by nitrosation inhibition of the secondary amines by chemicals having reducing ability (22).

Figure 3 shows the nitrite scavenging abilities of the extracts at pH 1.2, and that the nitrite scavenging ability was positively correlated to the extract concentration. Ascorbic acid had the highest scavenging ability of all the samples under the same concentration. The ethanol extract had higher nitrate scavenging ability than the water extract. Furthermore, the ethanol extract's activity was approximately 40-73% of that of ascorbic acid under the same concentration. A preliminary study found that the water extract of cherry elaeagnus fruit had the highest nitrite scavenging ability among tested extracts (16), which disagrees with the present results. This difference is believed to be due to the different amounts of materials related to antioxidant

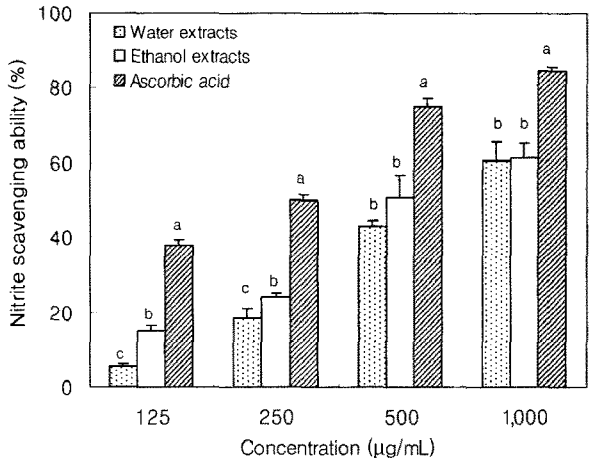

Fig. 3. Nitrite scavenging ability of cherry elaeagnus leaf extracts. Values are the means of triplicate±SD; means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

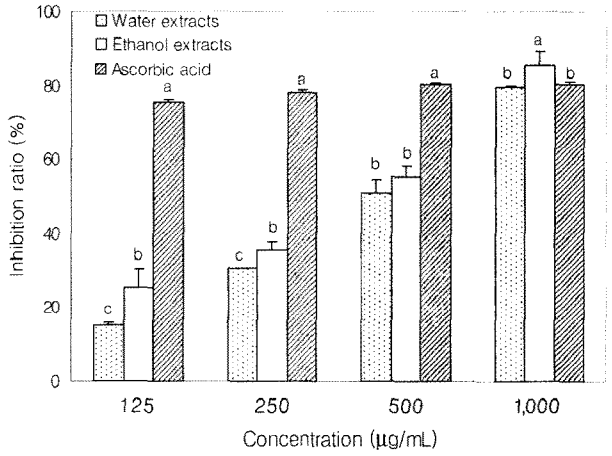

Fig. 4. Xanthine oxidase inhibitory activity of cherry elaeagnus leaf extracts. Values are the means of triplicate±SD; means with different letters are significantly different at $p<0.05$ by Duncan's multiple range test.

activity between the leaves and fruits of cherry elaeagnus. Phenolic compounds and ascorbic acid are reported to have high nitrite scavenging effects, and have even higher effects under conditions of low environmental pH (29,34). Our results show that when compared to the water extract, the higher antioxidant activity of the ethanol extract is in good accordance with its greater amount of total phenolics.

XOI activity The XOI activity of the ethanol extract of the cherry elaeagnus leaves was significantly higher than that of the water extract at all concentrations, and positive correlations were found between XOI activity and extract concentration (Fig. 4). The coefficients of correlation for concentration and XOI activity in water and ethanol extract were 0.9517 and 0.9794, respectively (data not shown). In contrast, ascorbic acid's activity did not change, regardless of increases in its concentration. Among the tested samples, ascorbic acid had the highest XOI activity at concentrations between 125-500 μg/mL; whereas the ethanol extract showed the highest activity at the 1,000 μg/mL concentration. XO is the enzyme that catalyzes the metabolism of hypoxanthine and xanthine into uric acid, and it is responsible for the medical condition known as gout, which is caused by the deposition of uric acid in the joints, leading to painful inflammation (35). The treatment of gout is to either increase the excretion of uric acid, or reduce uric acid production. XO also serves as an important biological source of oxygen-derived free radicals that contribute to oxidative damage in living tissues involved in many pathological processes (36). Therefore, XOI would be beneficial not only to treat gout, but also to combat other diseases (37). From these results, cherry elaeagnus leaves may potentially be useful for treating gout or other XO-induced diseases.

In conclusion, this study confirmed that cherry elaeagnus leaves have high DPPH radical scavenging ability, nitrite scavenging ability, SOD-like activity, and XOI activity. Therefore, cherry elaeagnus leaves show potential for use as food supplements. However, further studies are needed to analyze in detail the individual compounds related to the antioxidant activity of the extracts.

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