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Functional properties of water extracts from fully ripened silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries

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Abstract
The content of vitamin C was measured in fully ripened silver vine berries. As a result, the value (313 mg/100 g wet berries) was much higher than those of fruit species such as garden strawberry, kiwi berry, haskap, blueberry, and lemon. The antioxidative activity and scavenging activities against active oxygen species such as hydroxyl radicals, superoxide anion radicals, and DPPH radicals were investigated in water extracts prepared from fully ripened silver vine berries. The activities were extremely high similar to those of 5 mM ascorbic acid and 1 mM α-tocopherol. Moreover, these extracts possessed high ACE inhibitory activity. The present study indicates that fully ripened silver vine berries should find applications in various fields of foods and medicals and will be increasingly regarded as a health-promoting food.

Key words: Fully ripened silver vine, berry, water extract, vitamin C, antioxidative activity, scavenging activities against active oxygen species.

Introduction

Free radicals released during oxidative stress pose the major endogenous damage in the biological system [1]. This type of damage is often associated with various degenerative diseases and disorders such as cancer, cardiovascular disease, immunofunction decline, and aging [1, 2]. Free radicals are highly reactive molecules having unpaired electrons [2]. They can be produced by radiation or as by-products of the metabolic process [1, 3]. To gain stability, free radicals capture electrons quickly from other compounds. The attacked compound becomes a free radical itself, that continues to attack other compounds and leads to a chain reaction. This results in the disintegration of cell membranes and cell compounds, including protein, lipid and nucleic acids [2]. Besides damage to living cells, free radicals are the major cause of food deterioration through lipid oxidation that finally affects the organoleptic properties and edibility of foods[1]. That is, the intervention of an antioxidant may provide therapeutic functions [4]. Regular consumption of fruit and vegetables containing natural antioxidants is correlated with the decreased risk of diseases as cancer and cardiovascular diseases.

Silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] is a vined medicinal plant and is a native plant in the fields and mountains in all parts of Japan, Sakhalin, Korea, China, and the South Kurils. It belongs to Actinidiaceae and grows wild in all parts of Hokkaido, Japan. It comes into flower in August and into bearing in October (Fig. 1). So far, the unripe berries have been used for food such as pickles and fruit liquors, its buds as preservation with salt, its shoot as dressed with sauce and leaves and twigs as one of herbal medicine. On the other hand, ripe berries have been used for the processing of jam, dried fruits, and puree and so on. As consumers have become increasingly concerned about their health, their selection of products and services has been impacted. It has been assumed that increasing consumers' nutrition knowledge will lead to changes in attitudes and benefits and in turn their food selections will be improved [5]. As a result, specific health promoting

Figure 1. Silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] and berries.

marketing strategies has been developed to reach consumers[6]. It is well known that a great amount of vitamin C is contained in the berries of silver vine. Therefore, increased consumption of silver vine berries and their processed products may be beneficial in preventing the incidence of degenerative diseases.

In this investigation, we determined the chemical composition of fully ripened silver vine berries. Moreover, the paper deals with the antioxidative activity and antihypertensive activity of water extract prepared from fully ripened silver vine berries and a relationship between these functional properties and total content of vitamin C.

Materials and Methods

Materials: Fully ripened silver vine berries were harvested in Abashiri City, Hokkaido, Japan, and transported to our laboratory. The berries were stored at -20°C until used in this study. Linoleic acid, α-tocopherol, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), nitroblue tetrazolium salt (NBT), xanthine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2-deoxy-D-ribose, ACE (from bovine lung; 1U), hippuryl-L-histidyl-L-leucine as substrate peptide, and ethyl acetate for spectrochemical analysis grade were purchased from Wako Chemicals Co. Ltd. (Osaka, Japan). Xanthine oxidase from butter milk (XOD; 0.33 U/mg powder) was obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Other chemicals were of analytical grade.

Chemical analysis of fully ripened silver vine berries: AOAC methods were used[7]. Moisture content was measured using a hot oven. Protein content was determined by the Kjeldahl method using a conversion factor of 6.25. Lipid content was analyzed by ether extraction. Ash content was determined using a furnace. Carbohydrate content was estimated by difference.

The total phenolic compounds were measured spectrophotometrically at 760 nm using chlorogenic acid as standard[8]. Total vitamin C content was measured by the α,α'-dipyridyl method[9].

Fully ripened silver vine berries were weighed, thawed at half, and homogenized with 2 volumes of chilled distilled water for 5 min in ice. After centrifugation at 38,500 x g at 4°C for 30 min, the supernatants were pooled and then used in the experiment. Specific gravity, sugar content, and pH of water extract were measured. The sugar content and pH of water extract were determined using hand-held refractometer (N-50E; Atago Co., Ltd., Tokyo, Japan) and pH meter (PHL-40; DKK Co., Ltd., Tokyo, Japan), respectively.

Determination of antioxidative, radical scavenging, and antihypertensive activity of water extract from fully ripened silver vine berries: The antioxidative activity of water extract from fully ripened silver vine berries was measured in a linoleic acid oxidation system described by Nagai *et al.*[10]. A 0.083 ml sample of the solution and 0.208 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.208 ml of 2.5% (w/v) linoleic acid in ethanol. The peroxidation was initiated by the addition of 20.8 µl of 0.1 M AAPH and carried out at 37°C for 200 min in the dark. The degree of oxidation was measured according to the thiocyanate method for measuring peroxides by reading the absorbance at 500 nm after coloring with $FeCl_2$ and ammonium thiocyanate. A control was performed with linoleic acid but without sample solution. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

Hydroxyl radical scavenging activity was evaluated as the inhibition rate of 2-deoxy-D-ribose oxidation by this radical as described by Nagai *et al.*[10]. The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$-EDTA, 0.15 ml of 10 mM H_2O_2, 0.525 ml of H_2O, and 0.075 ml of sample solution in an Eppendorf tube. The reaction was started by the addition of H_2O_2. After incubation at 37°C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% trichloroacetic acid and 0.75 ml of 1.0% TBA in 50 mM NaOH. The solution was boiled for 10 min and then cooled in water. The absorbance of the solution was measured at 520 nm. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

The superoxide anion radical scavenging activity was performed as described by Nagai *et al.*[10]. The reaction mixture contained 0.48 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 ml of 3 mM xanthine, 0.02 ml of 3 mM ethylenediaminetetraacetic acid disodium salt (EDTA-2Na), 0.02 ml of 0.15% bovine serum albumin, 0.02 ml of 0.75 mM NBT and 0.02 ml of sample solution. After preincubation at 25°C for 10 min, the reaction was started by adding 6 mU XOD and carried out at 25°C for 20 min. After 20 min, the reaction was stopped by adding 0.02 ml of 6 mM CuCl. The absorbance of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of the formazan that was reduced from NBT by superoxide. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

DPPH radical scavenging activity was measured as described by Nagai *et al.*[10]. The mixture contained 0.3 ml of 1.0 mM DPPH solution, 2.4 ml of 99% ethanol and 0.3 ml of sample solution. The mixture was rapidly mixed in the dark and the scavenging activity was measured spectrophotometrically at 517 nm after 30 min. Ascorbic acid (0.1 and 1.0 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

Antihypertensive activity was measured as described by Nagai *et al.*[10]. Seventy five microlitres of 0.1 M sodium borate (pH 8.3) containing 5.83 mM hippuryl-L-histidyl-L-leucine and 1.0 M NaCl and 25µl of sample solution were preincubated at 37°C for 5 min and then incubated with 25 µl of 0.1 M sodium borate buffer (pH 8.3) containing 1 mU ACE and 1.0 M NaCl at 37°C for 60 min. The reaction was stopped by the addition of 125 µl of 1.0 M HCl. The resulting hippuric acid was extracted with 750 µl of ethyl acetate by mixing for 15 s. After centrifugation at 6,000 rpm for 3 min, 500 µl of the upper layer was transported into the tube and evaporated at 40°C for 2 h. The hippuric acid was dissolved in 500 µl of distilled water, and the absorbance was measured at 228 nm using a PerkinElmer model Lambda 11 (PerkinElmer, Tokyo, Japan) UV/VIS spectrometer. The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the ACE inhibitory activity.

Results and Discussion

Chemical properties of fully ripened silver vine berries were investigated using AOAC method. As a result, the contents of water, proteins, lipids, carbohydrates, and ash were as follows: 77.8, 2.1, 0.6, 18.6, and 0.9%, respectively (Table 1).

Table 1. Chemical analysis of fully ripened silver vine berries.

	(%)
Water	77.8
Proteins	2.1
Lipids	0.6
Carbohydrates	18.6
Ash	0.9

The content of total phenolic components of fully ripened silver vine berries was about 58.4 mg/100 g wet berries (Table 2). This was much lower than that of haskap (*Lonicera coerulea* L. var. *emphyllocalyx* Nakai) and were about one tenth in comparison with that of haskap, although silver vine was one of the functional plants in northern region as well as haskap. The content of vitamin C in fully ripened silver vine berries was about 313.4 mg/100 g wet berries (Table 2). This is remarkably higher than contents (mg/100 g wet berries) of garden strawberry (62), kiwi berry (69), haskap (44), blueberry (9), and lemon (100) [11].

Specific gravity of water extract was 1.012 and the sugar content and pH of water extract were 4.0 (Brix% at 20°C) and 4.68 (20°C), respectively (Table 3).

Table 2. The contents of total phenolic components and total vitamin C in fully ripened silver vine berries.

	mg/100 g wet berries
Total phenols	58.4
Total vitamin C	313.4

Table 3. Properties of water extracts from fully ripened silver vine berries.

Property	
Specific gravity	1.012
Brix% at 20℃	4.0
pH at 20℃	4.68

To evaluate the inhibition effects at the initiation stage of lipid peroxidation, the antioxidative activities of water extracts from fully ripened silver vine berries were investigated *in vitro*. As a result, each sample species possessed antioxidative activity and the activity increased with increasing the concentration of sample (Table 4). For 1% solution the activity was very low, but the activity for 10% solution was higher than that of 1 mM ascorbic acid and was lower than that of 5 mM ascorbic acid. On the other hand, the sample solution for 100% showed high activity, although this did not amount to that of 1 mM α-tocopherol.

Hydroxyl radical scavenging activities of water extracts from fully ripened silver vine berries were measured using the Fenton reaction system. As a result, each sample species showed the activity and the activity was increased with increasing the concentration of sample (Table 5). For 1% solution the activity was very low, but the activity for 10% solution was similar to those of 1 and 5 mM ascorbic acid. On the other hand, 100% sample solution had an activity about 56%, and this was fairly high although the activity did not amount to that of 1 mM α-tocopherol.

Superoxide anion radical scavenging activities of water extracts from fully ripened silver vine berries were investigated using xanthine/xanthine oxidase system (NBT method). The sample solution for 1% hardly showed the activity, the activity was lower than that of 1 mM ascorbic acid (Table 5). On the contrary, the solution for 10% possessed extremely high activity of about 74% and this was higher than that of 1 mM α-tocopherol. The solution for 100% sample concentration showed remarkably high scavenging activity (95.1%) and the activity was much higher than that of 5 mM ascorbic acid. These activities tended to increase with an increasing degree of the concentration of sample.

DPPH radical scavenging activities of water extracts from fully ripened silver vine berries were measured and compared with those of ascorbic acid and α-tocopherol. As a result, the activity increased with increasing the concentration of sample (Table 5). For 1% sample solution the activity was low but was higher than that of 0.1 mM ascorbic acid. With increasing the sample concentration the activity was increased and 10% sample solution scavenged this radical about 41%. On the other hand, 100% sample solution exhibited higher activity and scavenged it about 83%, although the activity did not amount to those of 1 mM ascorbic acid and α-tocopherol.

Antihypertensive activities of water extracts from fully ripened silver vine berries were measured and the IC_{50} value was calculated to 47.8%. In the present study, the content of total vitamin C was remarkably high in fully ripened silver vine berries. Next, it was investigated the correlation between the content of total vitamin C and these capacities such as antioxidative activcity, scavenging activities, and antihypertensive activity. As a result, high correlation was demonstrated between the content of total vitamin C and scavenging activities against hydroxyl radical and DPPH radical, with $R^2 = 0.976$ and $R^2 = 0.851$, respectively (Table 6). The correlation coefficient for scavenging activity against superoxide anion radical and antihypertensive activity was lower, $R^2 = 0.604$ and $R^2 = 0.634$, respectively (Table 6). The antioxidative activity correlated only weakly with the content of total vitamin C ($R^2 = 0.487$).

Antioxidant compounds play an important role as health-protective factors. Most of the antioxidant compounds in a typical diet derive from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. They can delay or inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions and are also involved in scavenging free radicals. Primary sources of naturally occurring antioxidants are fruits and vegetables. Antioxidants from plant food sources, such as vitamin C, vitamin E, phenolic acids, and flavonoids, have been recognized as

Table 4. Antioxidant activities of water extracts from fully ripened silver vine berries.

	Absorbance at 500 nm						
Time (min)	A	B	C	D	E	F	G
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50	0.082	0.064	0.038	0.022	0.016	0.006	0.379
100	0.242	0.112	0.060	0.135	0.032	0.025	0.715
200	0.920	0.296	0.131	0.469	0.090	0.028	1.406

(A) 1% solution; (B) 10% solution; (C) 100% solution; (D) 1 mM ascorbic acid; (E) 5 mM ascorbic acid; (F) 1 mM α-tocopherol; (G) control.

Table 5. Hydroxyl radical, superoxide anion radical, and DPPH radical scavenging activities of water extracts from fully ripened silver vine berries.

	Scavenging activity (%)		
Sample	Hydroxyl radical	Superoxide anion radical	DPPH radical
A	6.3	5.4	15.9
B	14.7	73.7	40.7
C	56.3	95.1	82.6
D	13.2	14.7	5.6*
E	16.1	89.9	94.7**
F	67.3	52.6	94.7
G	0.0	0.0	0.0

See sample nomenclature in Table 4. *0.1 mM ascorbic acid; **1.0 mM ascorbic acid.

Table 6. Correlation between the contents of vitamin C and the antioxidative activities and angiotensin I-converting enzyme inhibitory activities of water extracts from fully ripened silver vine berries.

	Equation	r
Antioxidant	y = 34.348 + 0.57612 x	0.487
Hydroxyl radical	y = 4.9450 + 0.49603 x	0.976
Superoxide anion radical	y = 22.133 + 0.73876 x	0.604
DPPH radical	y = 15.748 + 0.65718 x	0.851
ACE inhibition	y = 25.608 + 0.51043 x	0.634

having the potential to reduce risk for chronic diseases including cancer, cardiovascular diseases, tumor and heart disease, helping to neutralize free radicals, that are unstable molecules linked to the development of such diseases [12]. That is, regular comsumption of fruit and vegetables containing natural antioxidants is correlated with the decreased risk of these diseases[13]. Our present investigation showed that a large amount of vitamin C possessed in fully ripened silver vine berries. Moreover, water extracts prepared from these berries exhibited highly antioxidative activity and scavenging activities against hydroxyl radicals, superoxide anion radicals, and DPPH radicals: water extracts could protect oxidation of linoleic acid and capture these radicals in a concentration-dependent manner and may play an important role as an antioxidant in berries. In addition, these extracts possessed high ACE inhibitory activity.

Silver vines grow wild in all parts of Japan, Sakhalin, Korea, China, and the South Kurils, and the harvest quantities of berries are high. From these reasons, fully ripened silver vine berries may be benefit not only to the materials of health food diets, and also to patients undergoing life-style related diseases. In the future, fully ripened silver vine berries should find applications in various fields of foods and medicals and will be increasingly regarded as a health-promoting food.

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