African Journal of Food Science Vol 5(3) pp. 125 - 130, March, 2011
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2011 Academic Journals

Full Length Research Paper

The liquor made from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries possess strongly antioxidative activity and antihypertensive activity

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Accepted 10 October, 2010

Liquor was prepared from silver vine berries and its functional properties were investigated. The colour of liquor correlated with that of berries was as amber. The contents of protein, total phenolic components, and total vitamin C were about 2.3 (mg/ml), 339.5 (µg/ml), and 2.4 (mg/100 ml), respectively. The liquor possessed strongly antioxidative activity, scavenging activities against superoxide anion radical, hydroxyl radical, and DPPH radical. Moreover, the liquor showed angiotensin I-converting enzyme inhibitory activity. The present study indicates that liquor made from silver vine berries may help to prevent oxidative damage such as lipid peroxidation, associated with many diseases, including cancer, atherosclerosis, diabetes, aging, arthritis, brain dysfunction, and immune deficiency.

Key words: Antihypertensive activity, antioxidative activity, liquor, silver vine berries.

INTRODUCTION

Functional foods could potentially be used for improved health or well-being in a range of areas including cardio-vascular system, gastrointestines, growth, metabolism, defense against free radical oxidation and to enhance psychological functions. There are a wide range of products and developments that provide examples for the changing relationship between food and health because of the increasing attention to the health-diet interaction. The idea that food habits have direct influence on one's health is certainly not new, but the attention paid to this relation is increasing. Ongoing research yields new insights regarding the relation between food habits and the increase or decrease of the incidence of varies aliments, such as certain types of cancer and different forms of cardiovascular diseases. These scientific developments have not only resulted in extensive literature on the impact of food habits on health, but also in practical dietary advice.

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. 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,

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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 (Tepper et al., 1997). As a result, specific health promoting marketing strategies has been developed to reach consumers. It is well known that a great amount of vitamin C is contained in the berries of silver vine (Nagai et al., 2008). Therefore, increased consumption of silver vine berries and their processed products may be beneficial in preventing the incidence of degenerative diseases. The objectives of the present work were to prepare liquor from silver vine berries and to perform in order to find a scientific support to produce an enhanced value-added and a low cost functional food.

MATERIALS AND METHODS

Materials

Silver vine berries were harvested in Abashiri City, Hokkaido, Japan, and transported to our laboratory. The berries were stored at -85°C until used. 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 obtained from Wako Chemicals Co. Ltd. (Osaka, Japan). Xanthine oxidase from butter milk (XOD; 0.33 U/mg powder) was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). Other chemicals were of reagent grade.

Preparation of liquor from silver vine berries

After silver vine berries were thawed at half, the calyces were removed, and then were washed with water. These berries were wiped of the water with a cloth, weighed, and added an equivalent weight of crystal sugar and twice volume (w/w) of 35% ethanol. After brewing or extracting in the dark condition for 10 month, the liquor was used for the following tests.

Chemical analysis of liquor made from silver vine berries

The protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard. The total phenolic compounds were determined spectrophotometrically at 760 nm using chlorogenic acid as standard (Slinkard and Singleton, 1977). Total vitamin C content was measured by the α,α'-dipyridyl method (The Vitamin Society of Japan, 1990). The alcohol content was estimated using an alcohol checker (YSA-200; Yazaki Meter Co. Ltd., Tokyo, Japan). The sugar content and pH of liquor were determined using a hand-held refractometer (N-50E; Atago Co. Ltd., Tokyo, Japan) and a pH meter (PHL-40; DKK Co. Ltd., Tokyo, Japan), respectively. For colour analysis, a Minolta spectrophotometer M-3500d (Tokyo, Japan) with illuminant D65 was used. Colour was recorded using a CIE L^* a^* b^* colour space; L^* [lightness (0 = black, 100 = white)], a^* (-a = greenness, +a = redness), and b^* (-b = blueness, +b = yellowness).

Antioxidative assay

The antioxidative activity of liquor made from silver vine berries was determined in a linoleic acid oxidation system as described by Nagai et al. (2006). The degree of oxidation was measured according to the thiocyanate method for measuring peroxides by reading the absorbance at 500 nm using a PerkinElmer model Lambda 11 (PerkinElmer, Tokyo, Japan) UV/VIS spectrometer after coloring with $FeCl_2$ and ammonium thiocyanate. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. 18% of ethanol was used as negative control.

Superoxide anion radical scavenging assay

The effect of superoxide anion radical scavenging activity in liquor made from silver vine berries was estimated as described by Nagai et al. (2006). 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 nitroblue tetrazolium by superoxide. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. 18% of ethanol was used as negative control. The IC_{50} value was defined as the concentration of liquor required to inhibit 50% of the superoxide anion radical scavenging activity.

Hydroxyl radical scavenging assay

The effect of hydroxyl radical in liquor made from silver vine berries was assayed by using the Fenton reaction system. Hydroxyl radical scavenging activity was evaluated as the inhibition rate of 2-deoxy-D-ribose oxidation by hydroxyl radical (Nagai et al., 2006). Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. 18% of ethanol was used as negative control. The IC_{50} value was defined as the concentration of liquor to inhibit 50% of the hydroxyl radical scavenging activity.

DPPH radical scavenging assay

DPPH radical scavenging activity of liquor made from silver vine berries was measured by the method of Okada and Okada (1998) with a slight modification. The reaction mixture contained 0.3 ml of 1.0 mM DPPH in ethanol, 2.4 ml of 99% ethanol, and 0.3 ml of liquor. The solution was rapidly mixed in the dark condition and the scavenging capacity was measured at 517 nm using a spectrometer after incubation for 30 min. Ascorbic acid (0.1 and 1 mM) and α-tocopherol (1 mM) were used as positive control. 18% of ethanol was used as negative control. The IC_{50} value was defined as the concentration of liquor to inhibit 50% of the DPPH radical scavenging activity.

Angiotensin I-converting enzyme (ACE) inhibitory assay

ACE inhibitory activity of liquor made from silver vine berries was investigated as described by Nagai et al. (2007). The absorbance of the hippuric acid, the reaction product, was measured at 228 nm. The IC_{50} value was defined as the protein concentration of inhibitor required to inhibit 50% of the ACE inhibitory activity.

RESULTS AND DISCUSSION

The liquor was successfully prepared from silver vine berries. Chemical properties of liquor were investigated. As a result, specific gravity of liquor was 1.447, the alcohol sugar contents and pH of liquor were 18.0 (%),35.0 (Brix% at 20), and 5.14 (20°C), respectively (Table1). The

Table 1. Property of liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries.

Property	
Specific gravity	1.447
Alcohol%	18.0
Brix% at 20°C	35.0
pH at 20°C	5.14
Colour parameter	
*L**	93.84
*a**	0.84
*b**	23.09

Figure 1. Liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries

colour of liquor, amber correlated with that of berries (Figure 1, Table 1). The contents of protein, total phenolic components, and total vitamin C were about 2.3 (mg/ml), 339.5 (µg/ml), and 2.4 (mg/100 ml), respectively (Table 2). To evaluate the inhibition effects at the initiation stage of lipid peroxidation, the antioxidative activity of liquor made from silver vine berries was investigated. As a result, each sample species possessed antioxidative activity and the degree of the activity increased with increasing concentration of sample (Table 3). The activity for 1% solution was very low, but that for 10% solution was higher than that of 1 mM ascorbic acid. The activities for 25 and 50% solution were fairly higher than that of1 mM ascorbic acid, and were lower than that of 5 mM ascorbic acid. For 100% solution, the activity was extremely higher than that of 5 mM ascorbic acid, but did not amount to that of 1 mM α-tocopherol (Table 3).

Table 2. The contents of protein, total phenolic components, and vitamin C of liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries.

Protein	2.3 (mg/ml)
Total phenolic components	339.5 (µg/ml)
Vitamin C	2.4 (mg/100 ml)

Superoxide anion radical scavenging activity of liquor

Table 3. Antioxidant activities of liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries.

Time (min)	A	B	C	D	E	F	G	H	CN
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50	0.101	0.055	0.039	0.022	0.018	0.022	0.016	0.006	0.379
100	0.323	0.144	0.093	0.073	0.050	0.135	0.032	0.025	0.715
200	0.921	0.361	0.211	0.148	0.074	0.469	0.090	0.028	1.406

(A) 1% solution; (B) 10% solution; (C) 25% solution; (D) 50% solution; (E) 100% solution; (F) 1 mM ascorbic acid; (G) 5 mM ascorbic acid; (H) 1 mM α-tocopherol; (CN) control.

Table 4. Superoxide anion radical, hydroxyl radical, and DPPH radical scavenging activities of liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries.

Sample	Scavenging activity (%)		
	Superoxide anion radical	Hydroxyl radical	DPPH radical
A	0.0	7.0	0.0
B	19.6	26.3	0.0
C	49.6	30.0	30.8
D	79.1	41.6	31.6
E	> 100.0	53.4	64.1
F	14.7	13.2	3.1*
G	89.9	17.6	34.1**
H	52.6	67.6	87.6
CN	0.0	0.0	0.0

(a) See sample nomenclature in Table 3. *0.1 mM ascorbic acid; **1.0 mM ascorbic acid.

made from silver vine berries was measured using xanthine/xanthine oxidase system and was compared with those of ascorbic acid and α-tocopherol. As a result, the activity increased with increasing concentration of sample (Table 4). The activity for 10% solution was higher than that of 1 mM ascorbic acid, although the activity was not detected in 1% solution. For 25% solution, the activity was almost similar to that of 1 mM α-tocopherol. Moreover, the activity for 50% solution was fairly high, although this did not amount to that of 5 mM ascorbic acid. On the other hand, the solution for 100% perfectly scavenged this radical. The IC_{50} value against superoxide anion radical was calculated to 29.7%.

Hydroxyl radical scavenging activity of liquor made from silver vine berries was investigated using the Fenton reaction system *in vitro*. Each sample showed the activity and these activities tended to increase with an increasing degree of the concentration of sample (Table 4). Although the activity for 1% solution was very low, the solution more than 10% was much higher than those of 1 and 5 mM ascorbic acid. Particularly 100% sample solution showed an activity about 53%, and this was fairly high although the activity did not amount to that of 1 mM α-tocopherol (Table 4). The IC_{50} value against hydroxyl radical was 81.0%.

Table 5. ACE inhibitory activity of liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries.

Sample species	Activity (%)
A	0.0
B	0.0
C	13.7
D	53.8
E	88.6

(A) 1% solution; (B) 10% solution; (C) 25% solution; (D) 50% solution; (E) 100% solution.

DPPH radical scavenging activities of liquor made from silver vine berries was measured. As a result, the activity increased with increasing concentration of sample (Table 4). For 25 and 50% solution, the activities were similar to that of 1 mM ascorbic acid and were about 30.8 - 31.6% (Table 4). Moreover, 100% sample solution exhibited higher activity and the activity was about 64%, although the activity did not amount to that of 1 mM α-tocopherol (Table 4). On the contrary, the solution for 1 and 10%

Table 6. Correlation between the contents of total vitamin C and phenols, scavenging activities, and ACE inhibitory activity of liquor prepared from silver vine [*Actinidia polygama* (Sieb. et Zucc.) Planch. ex Maxim.] berries.

	Equation	r
Phenols	$y = 5.0653 \times 10^{-3} x - 1.2967$	0.999
Superoxide anion radical	$y = 1.1926 \times 10^{-2} x - 4.5151$	0.983
Hydroxyl radical	$y = 4.9437 \times 10^{-2} x - 0.7664$	0.794
DPPH radical	$y = 3.4218 \times 10^{-2} x - 6.8119 \times 10^{-2}$	0.896
ACE inhibition	$y = 2.4033 \times 10^{-2} x + 4.7200 \times 10^{-2}$	0.938

had no activity against this radical. The IC_{50} value against DPPH radical was calculated as 75.6%.ACE inhibitory activity was investigated in liquor prepared from silver vine berries and the results were indicated as inhibition rate. The activities for 1 and 10% solution were not detected, but 25% solution showed the activity about 13.7% (Table 5). The solution for 50% exhibited higher activity and this inhibited the activity of ACE about 54%. Moreover, the activity for 100% solution was extremely high about 89% (Table 5). On the other hand, the IC_{50} value was 51.5% (1.16 mg protein/ml).

In the present study, the contents of total vitamin C and total phenol are remarkably high in liquor made from silver vine berries. The correlation between the content of total vitamin C and scavenging activities against these radicals and ACE inhibitory activity were investigated. As a result, high correlation was demonstrated between the content of total vitamin C and scavenging activity against superoxide anion radical and ACE inhibitory activity, with R^2 = 0.983 and R^2 = 0.938, respectively (Table 6). Moreover, the correlation coefficient for scavenging activities against hydroxyl radical and DPPH radical was moderately higher, R^2 = 0.794 and R^2 = 0.896, respectively (Table 6). On the other hand, these tendencies were shown in the content of total phenol.

Sake consumption containing fermented drink (beer, wine, and Japanese sake), distilled drink (whisky, brandy, and distilled beverage), and liquor (plum wine, vermut, and campari) is moderately decreasing in Japan. The annual consumption of sake is around 9,092 million litres in 2008, a decrease of 1.9% in comparison with the previous year (Japan Wineries Association, 2010). According to the report in WHO (2008), the consumption of alcohol carries a risk of adverse health and social consequences related to its intoxicating, toxic and dependence-producing properties. Moreover, to the chronic diseases that may develop in those who drink large amounts of alcohol over a number of years, alcohol is also associated with an increased risk of acute health conditions. On the contrary, the recent paper reported that the enhanced endothelial formation of nitric oxide by red wine polyphenols involved in the protective effect of chronic intake of red wine on coronary diseases (Ndiaye et al., 2005). Lefevre et al. (2007) reported that moderate consumption of red wine (cabernet sauvignon) improves ischemia-induced neovascularization in ApoE-deficient mice. Sarr et al. (2006) reported that red wine polyphenols prevent angiotensin II-induced hypertension and endothelial dysfunction in rats.

Our present study indicates that a large amount of vitamin C and phenols possessed in liquor made from silver vine berries. Its liquor showed highly antioxidative activity and scavenging activities against superoxide anion radical, hydroxyl radical, and DPPH radical. That is, the liquor made from silver vine berries could protect oxidation of linoleic acid and capture these radicals in a concentration-dependent manner. The liquor also exhibited high antihypertensive activity. In addition, the contents of total vitamin C and total phenols correlated with these activities such as antioxidative activity, scavenging activities, and antihypertensive activity.

At the present time, the value of silver vine berries was low, but the development of an enhanced value-added and a health-promoting processed foodstuff such as the liquor may be benefit in the food industry.

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