[Original Paper]

Antioxidant Activity of Various Part of Koshu Grape

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The antioxidant activity of various parts of Koshu grape was examined for possible utilization. The antioxidant activity of the ethanol extracts of Koshu grape stem, leaf, shoot, and skin was determined by measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and performing the crocin bleaching assay. The ethanol extract of Koshu grape stem had stronger antioxidant activity than the ethanol extracts of Koshu grape leaf, shoot, and skin, and the 50% ethanol extracts of Koshu grape stem and seed had similar DPPH radical scavenging activities. On the basis of ultra-performance liquid chromatography-diode array detection and time-of-flight mass spectrometry (UPLC-DAD-TOFMS) and other NMR spectroscopic techniques, the major constituents of Koshu grape stem were identified as flavan-3-ols, cinnamoyl tartrates, dihydroflavonol glycosides, flavonol glycosides, and *trans/cis*-resveratrols and their oligomers. These compounds were the major contributors to the antioxidant activity of Koshu grape stem. The possibility of using Koshu grape stem as an antioxidant food product was shown.

Key words: Koshu, Stem, Leaf, Shoot, Skin, Antioxidant activity

Introduction

Grape is one of the world's largest fruit crops and its annual production amounts to approximately 68 million metric tons (Food and Agriculture Organization (FAO), 2008). Approximately 71% of the world's grapes are used for winemaking; 27%, as fresh fruit; and 2%, as dried fruit. It has been reported that grape, wine, and grape seed extract exert favorable effects on human health due to their high phenol contents (Corder et al. 2006, Lea et al. 1979). Phenolics in grape and wine reduce platelet aggregation, possess anti-inflammatory properties (Demrow et al. 1995), modulate nitric oxide production, which promotes vascular relaxation (Fitzpatrick et al. 1993), and show cancer cell growth inhibitory effects (Schneider et al. 2000).

Wine industry waste consists mainly of solid by-products, such as pomace and stem may account on average for almost 20-30% (w/w) of the total weight of grapes used for winemaking (Spigno et al. 2007). Grape seed oil contains more than 80% unsaturated fatty acids with linoleic acid being the most predominant. Linoleic acid is produced by pressing the seeds of various grape varieties and manufactured commercially as vegetable oil. In addition, grape seed is a rich source of phenols, including catechins and proanthocyanidins (Waterhouse et al. 2000). Most studies have focused on seed derived from wine production and other by-products have received scant attention. In Japan, total grape production in 2009 amounted to 202,200 metric tonnes and vineyard area was 18,300 hectares. Koshu grape is indigenously grown in Japan and has been traditionally cultured for white wine production in Yamanashi Prefecture. Goto-Yamamoto conducted a genetic analysis of its origin and found that Koshu grape is an oriental variety of *Vitis vinifera* (Goto-Yamamoto et al. 2006). Koshu grape seed extract shows neuroprotective activity (Narita et al. 2011). In order to recycle the by-products of winemaking and minimize their environmental impact, one alternative would be to use them as organic fertilizers by directly mixing with soil or after composting. It is possible that Koshu grape stem, leaf, shoot, and skin contain potentially bioactive phenolic compounds because Koshu wine has relatively high phenol content

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(Okamura et al. 1981).

The crocin bleaching assay is a suitable means for screening radical scavenging activity. This assay determines the ability of antioxidants to inhibit bleaching of crocin, a naturally occurring carotenoid derivative, by the free radical generator 2,2-azobis(2-amidinopropane)dihydrochloride (AAPH). At that time, the chemistry behind the assay was considered to be the same as that of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay, which was regarded as a hydrogen atom abstraction method. In the crocin bleaching assay, the abstraction of hydrogen atoms and/or the addition of a radical to the polyene structure of crocin results in the disruption of the conjugated system responsible for crocin bleaching (Huang et al. 2005). The latter is recorded as a reduction of absorbance in the presence or absence of radical scavengers.

In this work, the antioxidant activity of the by-products of Koshu winemaking was examined for possible is utilization. The antioxidant activities of the ethanol extracts of various parts of Koshu grapevine (stem, leaf, shoot, and skin) were determined by measuring DPPH radical scavenging activity and performing the crocin bleaching assay. In addition, the isolation and structure elucidation of the constituents of Koshu grape stem was conducted.

Materials and Methods

General Procedure. ^{1}H (500 MHz), ^{13}C (125 MHz), and two-dimensional (2D) nuclear magnetic resonance (NMR) spectra were recorded with a Varian Unity 500 instrument (Varian Inc., Palo Alto, CA). LC-MS analysis was performed by ultra-performance liquid chromatography-diode array detection and time-of-flight mass spectrometry (UPLC-DAD-TOFMS). UPLC-DAD was performed on a Waters Acquity UPLC system (Waters, Milford, MA) with a PDA 2996 photo diode array (Waters) using an Acquity UPLC HSS T3 column, 1.8 μm, 2.1×100 mm (Waters). TOFMS was performed on a Waters LCT premier XE mass spectrometer (Waters). DPPH radical scavenging activity was measured with a microplate reader (MTP-450Lab, Corona Electric Co., Japan). Crocin bleaching assay was carried out with a UV-visible spectrophotometer (U-3010 Spectrophotometer, Hitachi, Japan). Column chromatography was performed using Toyopearl TSK HW-40 (F) (Tosoh Corp., Japan) and Chromatorex ODS DM1020T (100-200 mesh, Fuji Silysia Chemical Ltd., Japan).

Chemicals. All of the solvents used were of HPLC grade and water was supplied by a Milli-Q water purification system from Millipore (Bedford, MA). 2,2-Azobis(2-amidinopropane)dihydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent, (+)-catechin, and (-)-epicatechin were purchased from Wako Pure Chemical Industries, Ltd., Japan. *trans*-Resveratrol was from Tokyo Chemical Industry Co., Ltd. *trans*-Caftaric acid, (-)-epicatechin gallate, *trans*-piceid, and procyanidins B1 and B2 were obtained from ChromaDex, Inc. (Irvine, CA). Dried saffron was purchased from Sigma-Aldrich, Ltd. (St. Louis, MO). All other chemicals and reagents were of analytical grade.

Grapes. Koshu grapes were grown at the experimental vineyard of University of Yamanashi and harvested in early October 2007.

Extraction of Koshu Grape Stem, Leaf, Shoot, and Skin. Koshu grape stem, leaf, shoot, and skin were lyophilized and pulverized. Ten grams of each part was extracted with 30%, 50%, 70%, and 100% ethanol solution. The extraction at each ethanol concentration was repeated three times. The extracts were concentrated in vacuo to remove the solvent and obtain soluble fractions.

Extraction, Isolation, and Identification of Constituents of Koshu Grape Stems. Freeze-dried Koshu grape stems (500 g) were extracted with 2 L of ethanol/water (1:1). The organic solvent in the aqueous ethanol extract was evaporated in vacuo to obtain the aqueous portion and this, in turn, was partitioned into hexane-soluble, ethyl acetate-soluble, and water-soluble portions. Each soluble portion was concentrated in vacuo to obtain the hexane-soluble (0.8 g), ethyl acetate-soluble (21.1 g), and water-soluble (129.2 g) fractions. An aliquot of the ethyl acetate-soluble fraction (1 g) was further fractionated by Toyopearl TSK HW-40 (F) column chromatography into the monomer fraction (551 mg) using ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v) and the polymer fraction (428 mg) using acetone/water (60:40, v/v).

The phenolic monomer fraction was further chromatographed over ODS with water/acetonitrile (2:1) to obtain three compounds.

Evaluation of DPPH Radical Scavenging Activity. DPPH radical scavenging activity was measured as previously described (Hisamoto et al. 2011). Two hundred micromolar DPPH solution in ethanol (100 μL) was added to an ethanol sample solution (100 μL) in a 96-well flat-bottomed microtiter plate. Absorbance at 520 nm was determined after incubating for 30 min at room temperature in a multilabel counter. Scavenging activity was calculated by comparing the absorbance with that of a blank containing only DPPH and solvent. The activities of each extract and each soluble fraction from different parts of Koshu grapevine were evaluated until the reaction reached a steady state at room temperature. Measurements were performed in triplicate.

Crocin Bleaching Assay. Crocin bleaching assay was carried out in accordance with the reported method (Bors et al. 1984, Friend and Mayer 1960, Ordoudi et al. 2006). Saffron (0.5 g) was washed five times with diethyl ether (5 mL × 5 min) and t residual ether was evaporated under a nitrogen stream. Purified saffron was suspended in 25 mL of methanol, stirred manually for 5 min, and filtered (0.50 μm, PTFE, Advantec, Japan). Estimation of crocin concentration to 10 μM was based on an extinction coefficient reported in the literature, $e^{MeOH}_{433} = 1.33 \times 10^5\ M^{-1}\ cm^{-1}$. Each extract was dissolved in 50% (v/v) ethanol to make a concentration of 1-5000 mg/L depending on the activity of the extract. To a test tube were added 1 mL of crocin working solution and 500 μL of sample solution. After mixing, 500 μL of fresh 0.5 M AAPH solution in 0.1 M phosphate buffer solution was added. The bleaching reaction rate (V) of the reaction mixture containing the antioxidant (AH) and the blank (V_0) was determined at the same time by monitoring the absorbance decrease at 443 nm at 40°C for 20 min using a UV-visible spectrophotometer equipped with a thermostable multicell block. V_0/V ratios were plotted as a function of concentration ratio, [antioxidant]/[crocin], and slopes were calculated by linear regression analysis. All the tests were carried out in triplicate and the results were averaged.

Total Phenol Content. Total phenol content was measured by the Folin-Ciocalteu method as described by Slinkard and Singleton (Slinkard and Singleton 1997) and is expressed as mg/L, (+)-catechin equivalent. All assays were conducted in triplicate.

UPLC-DAD-TOFMS. The 50% ethanol extract of Koshu grape stem was dissolved in 50% ethanol (1 mg/mL). All solutions were filtered through a PTFE membrane filter (0.50 μm) (Advantec, Japan) and an aliquot of the filtrate (2 μL) was injected into a UPLC-DAD-TOFMS system. The conditions for UPLC-DAD-TOFMS were set according to a previous report (Narita et al. 2011).

Results and Discussion

The recovery rates of 30%, 50%, 70%, and 100% ethanol extracts of Koshu grape leaf, shoot, skin, and stem are shown in Table 1. The 50% and 70% ethanol extracts of Koshu grape stem and skin showed very high recovery rates. The antioxidant activity of each ethanol extract was evaluated by measuring DPPH radical scavenging activity and performing the crocin bleaching assay. The measurement of DPPH radical scavenging activity is one of the simplest methods to evaluate the hydrogen-donating capacity of antioxidants that are responsible for the chain-breaking activity of oxidation. Figure 1 shows the DPPH radical scavenging activities of the ethanol extracts (100 μg/mL) after a 60-min reaction. All the extracts showed

Table 1 Recovery rates of ethanol extracts of various parts of Koshu grapevine and proportion of various parts of grape cluster.

	Recovery rate (%, dry weight)[z]				Proportion of various parts of grape cluster (%, w/w, fresh weight)
	30% ethanol	50% ethanol	70% ethanol	100% ethanol	
Juice	-	-		-	76.2
Leaf	11.9	11.2	15.0	6.6	-
Seed	-	9.7[y]	-	-	2.5
Shoot	7.6	7.9[y]	10.7	3.0	-
Skin	32.0	49.1	49.2	43.3	18.1
Stem	32.0	45.0	42.1	35.3	3.2

[z] Ten grams of each part was extracted with 30%, 50%, 70%, and, 100% ethanol solutions.

[y] Data were previously reported by Hisamoto et al. 2011.

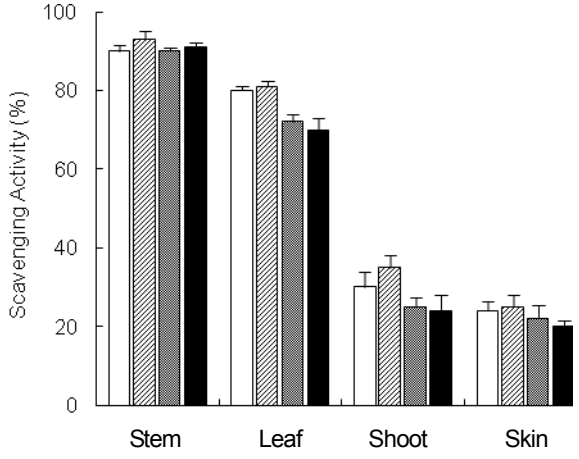

Fig. 1 DPPH radical scavenging activities of ethanol extracts of Koshu grape stem, leaf, shoot, and skin. The reaction mixture consisted of DPPH radicals (100 μM) and the following samples (100 μg/mL): □ , 30% ethanol extract; ▨ , 50% ethanol extract; ▩ , 70% ethanol extract; and ■ , 100% ethanol extract. Data are shown as means ± standard deviation of triplicate determinations.

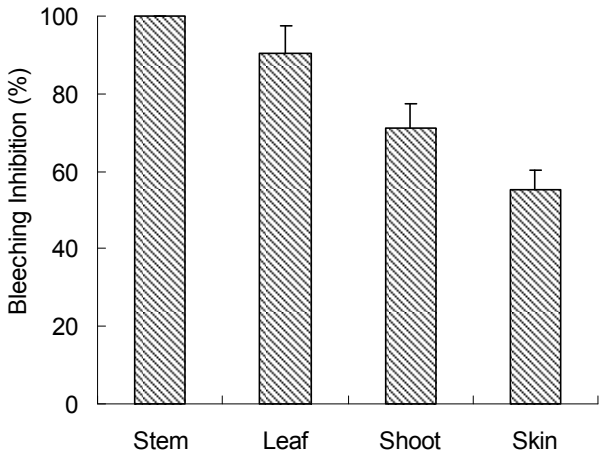

Fig. 2 Crocin bleaching assay of 50% ethanol extracts of Koshu grape stem, leaf, shoot, and skin. The reaction mixture consisted of crocin (5 μM) and sample (1000 μg/mL). Data are shown as means ± standard deviation of triplicate determinations.

significantly different DPPH radical scavenging activities. The activity decreased in the order of stem > leaf > shoot > skin. The 50% ethanol extract of Koshu grape stem showed the strongest activity.

In the crocin bleaching assay, the ability of a sample to react with peroxyl radicals is measured in terms of competition kinetics, i.e., the antioxidant competes with crocin for peroxyl radicals produced by the thermal decomposition of a diazo compound, AAPH (Bortolomeazzi et al. 2007). The reaction mixture consisted of crocin (5 μM) and the test extract (1000 μg/mL). As shown in Fig. 2, the extract of Koshu grape stem was the most effective after incubation for 20 min, followed by those of leaf, shoot, and skin.

Total phenol content and flavonoid and nonflavonoid contents in the 50% ethanol extract were determined by the Folin-Ciocalteu method. It was revealed that Koshu stem has the highest total phenol content at 275.7 ± 21.3 mg/g ((+)-catechin equivalent), followed by leaf at 190.2 ± 12.4 mg/g (Fig. 3). The flavonoid content of the 50% ethanol extract of Koshu grape stem was 94% of the total phenol content. Very strong antioxidant activity and high phenol contents were observed for the 50% ethanol extract of Koshu grape stem.

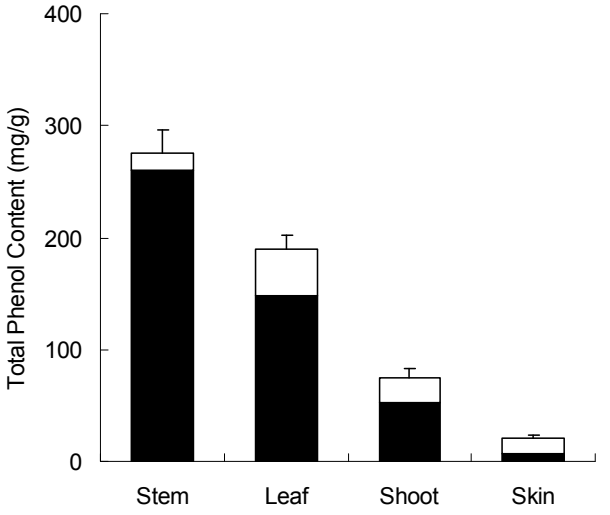

Fig. 3 Total phenol content in 50% ethanol extracts expressed as mg (+)-catechin/g dry weight determined by the Folin-Ciocalteu method: ■, flavonoids and □, nonflavonoid phenols. Data are shown as means ± standard deviation of triplicate determinations.

Then, the 50% ethanol extract of Koshu grape stem was separated into three fractions: hexane-soluble, ethyl acetate-soluble, and water-soluble fractions. Table 2 shows the DPPH radical scavenging activities and the recovery rates of the hexane-soluble, ethyl acetate-soluble, and water-soluble fractions from Koshu stem and seed. The radical scavenging activity was expressed in terms of IC_{50} (50% inhibitory concentration). The ethyl acetate-soluble fraction from Koshu grape stem showed the highest activity,

and the activity was almost the same as that of the ethyl acetate-soluble fraction from Koshu grape seed. In the crocin bleaching assay, the ethyl acetate-soluble fraction from Koshu grape stem also showed the highest inhibitory activity against peroxyl radicals (Fig. 4). When the total phenol content of each soluble fraction was measured by the Folin-Ciocalteu method, the ethyl acetate-soluble fraction exhibited the highest total phenol content (Fig. 5).

Table 2 DPPH radical scavenging activity and recovery rate of hexane, ethyl acetate and aqueous fractions from Koshu stem and seed.

	DPPH radical scavenging activity IC_{50} (mg/L)[z]	Recovery rate (%, dry weight)
Stem		
50% ethanol extraction	22.7 ± 0.3	-
Hexane -sol. fraction	22.1 ± 0.1	0.16
Ethyl acetate-sol. fraction	9.8 ± 0.0	3.3
Water-sol. fraction	29.8 ± 0.2	12.8
Seed[y]		
50% ethanol extraction	20.8 ± 1.2	-
Hexane -sol. fraction	-	10.5
Ethyl acetate-sol. fraction	7.2 ± 0.2	1.2
Water-sol. fraction	16.7 ± 0.1	8.5

[z] Data are shown as means ± standard deviation of triplicate determinations.
[y] Data were previously reported by Hisamoto et al. 2011.

The chromatograms of the 50% ethanol extract of Koshu grape stem obtained by UPLC-DAD-TOFMS with the absorbance at 280 nm and the negative mode of total ion current (TIC) are presented in Fig. 6. The peaks represent the main phenolic compounds (**1-20**) and are numbered according to the order of elution. Table 3 lists the theoretical and experimental masses of the deprotonated molecular ions of the 20 constituents. All the compounds were identified by analyzing their retention times, UV absorption spectra, and TOFMS spectra, and by comparison with some reference compounds. The constituents were identified as flavan-3-ol (**2**, **4-9,** and **11**), cinnamoyl tartrates (**1** and **3**) (Shibasaki et al. 1988), and flavonol glycosides (**12** and **13**). In addition, stilbenoid *trans/cis*-resveratrols and their oligomers were also detected, which were represented by **10**, **15**, and **16-20**.

The active ethyl acetate-soluble fraction from Koshu grape stem was further separated into ten fractions (fractions 1-10) by Toyopearl HW-40F column chromatography using

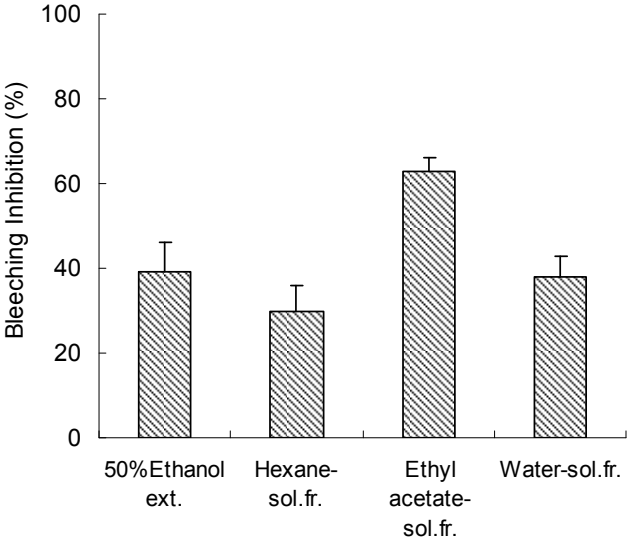

Fig. 4 Crocin bleaching assay of each extract and soluble fraction from Koshu grape stem. The reaction mixture consisted of crocin (5 μM) and samples (250 μg/mL). Data were reported as mean ± standard deviation of triplicate determinations.

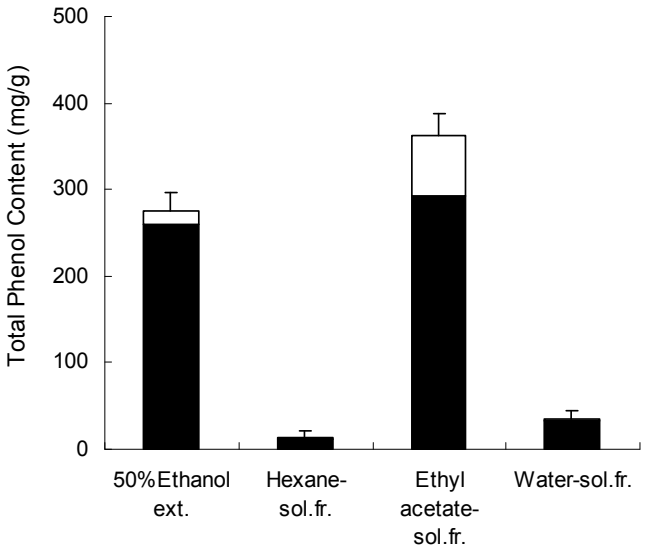

Fig. 5 Total phenols in 50% ethanol extract, hexane-soluble fraction, ethyl acetate-soluble fraction, and water-soluble fraction from Koshu grape stem expressed as mg (+)-catechin/g dry weight determined by the Folin-Ciocalteu method: ■, flavonoids and □, nonflavonoids. Data are shown as means ± standard deviation of triplicate determinations.

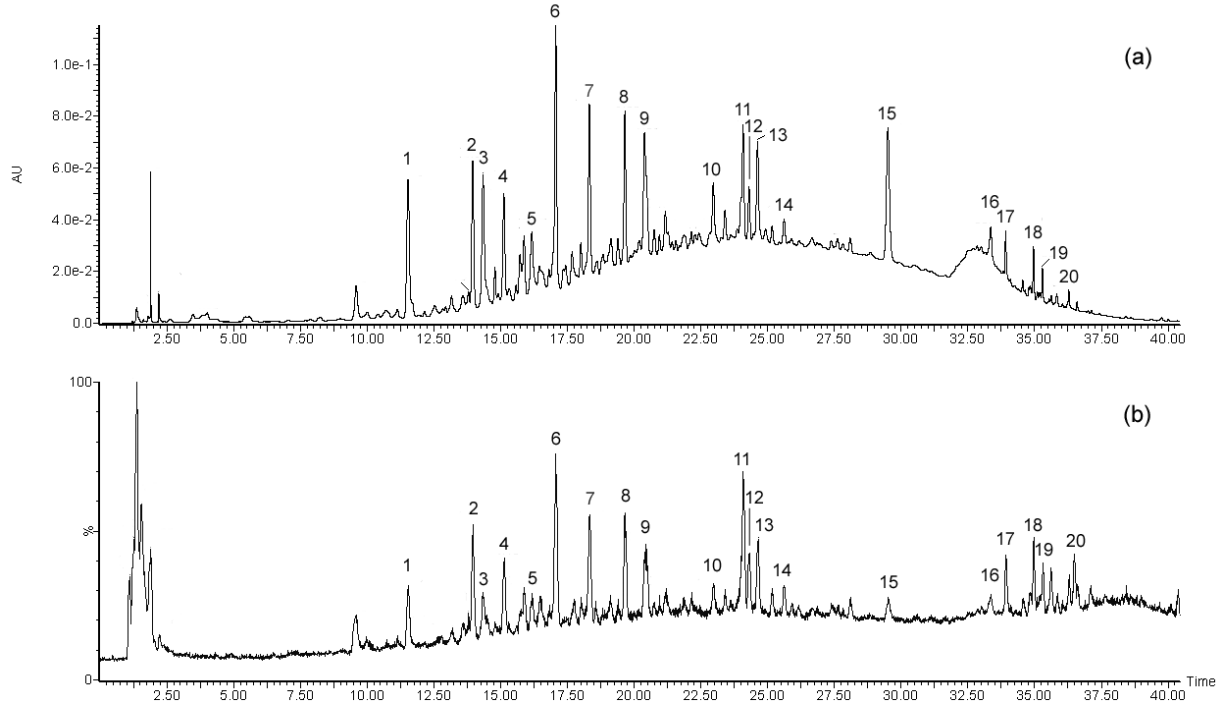

Fig. 6 Chromatograms of 50% ethanol extract of Koshu grape stem obtained by UPLC-DAD-TOFMS with absorbance at 280 nm (a) and negative mode of total ion current (TIC) (b).

Table 3 Mass measurements of deprotonated molecules of major constituents and their amounts from 50% ethanol extract of Koshu grape stem by UPLC-DAD-TOFMS.

No.	t_R [min]	Experimental *m/z* $[M-H]^-$	Theoretical *m/z* $[M-H]^-$	Formula	Compound	Amount* (mg/g ± SD)
1	11.53	311.0450	311.0403	$C_{13}H_{12}O_9$	*trans*-Caftaric acid	1.89 ± 0.17
2	13.95	577.1317	577.1346	$C_{30}H_{26}O_{12}$	Procyanidin B1	1.71 ± 0.04
3	14.34	295.0465	295.0454	$C_{13}H_{12}O_8$	*trans*-Coutaric acid	1.75 ± 0.11
4	15.12	289.0753	289.0712	$C_{15}H_{14}O_6$	(+)-Catechin	1.02 ± 0.05
5	16.17	865.1957	865.1980	$C_{45}H_{38}O_{18}$	Procyanidin trimer	-
6	17.06	577.1359	577.1346	$C_{30}H_{26}O_{12}$	Procyanidin B2	2.88 ± 0.18
7	18.32	289.0743	289.0712	$C_{15}H_{14}O_6$	(-)-Epicatechin	1.61 ± 0.13
8	19.65	865.2001	865.1980	$C_{45}H_{38}O_{18}$	Procyanidin trimer	-
9	20.39	1153.2661	1153.2614	$C_{60}H_{50}O_{24}$	Procyanidin tetramer	-
10	22.96	389.1258	389.1236	$C_{20}H_{22}O_8$	*trans*-Piceid	0.67 ± 0.07
11	23.04	441.0817	441.0822	$C_{22}H_{18}O_{10}$	Epicatechin-3-*O*-gallate	0.42 ± 0.03
12	24.08	477.0708	477.0669	$C_{21}H_{18}O_{13}$	Quercetin-3-*O*-glucuronide	0.54 ± 0.03
13	24.30	463.0912	463.0877	$C_{21}H_{20}O_{12}$	Quercetin-3-*O*-glucoside	0.20 ± 0.02
14	24.62	449.1094	449.1084	$C_{21}H_{22}O_{11}$	Astilbin	0.36 ± 0.04
15	29.50	227.0716	227.0708	$C_{14}H_{12}O_3$	*trans*-Resveratrol	1.82 ± 0.15
16	33.37	227.0701	227.0708	$C_{14}H_{12}O_3$	*cis*-Resveratrol	0.21 ± 0.01
17	33.91	905.2684	905.2611	$C_{56}H_{42}O_{12}$	Resveratrol tetramer	-
18	34.96	453.1357	453.1338	$C_{28}H_{22}O_6$	ε-viniferin	0.29 ± 0.01
19	35.29	679.1977	679.1968	$C_{42}H_{32}O_9$	Resveratrol trimer	-
20	36.28	679.1987	679.1968	$C_{42}H_{32}O_9$	Resveratrol trimer	-

* Dry weight of Koshu grape stem. Values were determined by the integration of UPLC-DAD signals and the response factors calculated from standards. Each value is the mean of three separate experiments.

ethanol/water/trifluoroacetate (55:45:0.05, v/v/v) in order to determine the chemical structure of **14**. Fraction 5 was further chromatographed over ODS with water/methanol (2:1) to give **14** (9 mg), **4** (36 mg), and **7** (38 mg). Compounds **4, 7,** and **14** were determined as (+)-catechin, (-)-epicatechin (Martin et al. 2000), and (2*R*,3*R*)-5,7,3',4'-tetrahydroxydihydroflavonol 3-*O*-α-L-rhamnopyranoside (astilbin), a dihydroflavonol glycoside (Britto et al. 1995) on the basis of ^{1}H NMR, ^{13}C NMR, and heteronuclear multiple bond coherence (HMBC) spectra, and TOFMS data.

The 20 compounds from the 50% ethanol extract of Koshu grape stem were quantified by UPLC-DAD-TOFMS. The amounts of compounds **1-4, 6, 7,** and **10-15** were calculated on the basis of the linear relationship between the peak areas and the injected concentrations (Table 3). The 50% ethanol extract of Koshu grape stem had the highest procyanidin B2 (**6**) (2.88 ± 0.18 mg/g) content and high contents of flavan-3-ols. These compounds have been reported to possess strong radical scavenging and antioxidant activities (Ricardo Da Silva et al. 1991). Astilbin showed strong antioxidant activity (Haraguchi et al. 1996) and is the main dihydroflavonol present in the stem of white and red varieties (Souquet et al. 2000). The antioxidant activities of flavan-3-ols and the dihydroflavonol, astilbin, were dependent on the number of hydroxyl groups on the benzene ring and the *ortho* substituents. Moreover, *trans/cis*-resveratrols and their oligomers were detected in Koshu grape stem. Recent reports have indicated the presence of several stilbenes in the stem of *Vitis* spp. (Amico et al. 2009, Anastasiadi et al. 2009, Püssa et al. 2006, Vivas et al. 2004). Considerable attention has been given to naturally occurring stilbene oligomers because they have been found to have multiple bioactivities (Kim et al. 2010, Yim et al. 2010).

It has been reported that Toyopearl TSK HW-40 (F) column chromatography is effective for the separation of monomeric and polymeric phenolic compounds (Souquet et al. 2000). Phenolic acids, dihydroflavonol (specifically astilbin), flavonols, and flavanol monomers were eluted with ethanol/water/trifluoroacetic acid (55:45:0.05, v/v/v), and the polymer fraction was eluted with acetone/water (60:40, v/v). In this study, the recovery rate of the polymer fraction from the 50% ethanol extract of Koshu grape stem was as high as 42.8%. Stem-condensed tannins are qualitatively intermediate between seed and skin but could not be differentiated between red and white varieties (Souquet et al. 2000).

In conclusion, the present study showed that the ethanol extract of Koshu grape stem exhibited stronger antioxidant activities than those of Koshu grape leaf, shoot, and skin, based on measurements of DPPH radical scavenging activity and the crocin bleaching assay. Although the proportions of Koshu grape seed and skin were similar and the DPPH radical scavenging activities of the 50% ethanol extracts of Koshu grape stem and seed were also similar, Koshu grape stem showed higher recovery than the Koshu grape seed in 50% ethanol extract. In this regard, Koshu grape stem would have an advantage over other parts of grapevine as it affords higher yields in the extraction of phenolics. Moreover, it is relatively easy to collect grape stem during the destemming process of winemaking. The phenolic content of Koshu grape stem was considerably higher than that of Chardonnay, Niagara, and Riesling stems (Shibasaki et al. 1988).

We conclude that the by-products of winemaking, particularly Koshu grape stem, show promise for use as an antioxidant food product. The exceptional results obtained for stem, which were assessed on the basis of phenolic content and antioxidant activity, were obtained after extraction with 50% ethanol. The antioxidant activity of Koshu grape stem is due to the presence of monomeric phenolic compounds and procyanidin B2, together with oligomeric and polymeric phenolic compounds as well as other yet unknown compounds. Therefore, phenolic compounds in grape stem may have benefits to health.

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［研　究　報　文］

甲州種ブドウの各部位の抗酸化活性

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要　約

ブドウには多岐にわたるフェノール性化合物が含まれており、ガン、脳疾患、糖尿病などの生活習慣病の予防に有効であることが多く報告されている。一方，白ワイン醸造で発生する副産物は、飼料や肥料に利用されているが、他の有効な利用方法はほとんどない。白ワイン醸造で発生する副産物を有効利用するため、甲州種ワイン醸造において利用されない部位の抗酸化活性を指標に、各部位の活性の比較、ならびに成分分析を行い、その有用性を評価することを目的とした。

甲州種ブドウの各部位（果梗、葉、梢、果皮）を乾燥・粉砕し、エタノール濃度を変えて抽出を行った。得られた抽出物について 1,1-diphenyl-2-picrylhydrazyl（DPPH）ラジカル捕捉活性及びクロシン退色法で測定した。両方の結果より、果梗の 50%エタノール水溶液抽出画分は葉、梢、果皮よりに強い抗酸化活性が認められ、種子と同様の活性であった。総フェノール量及びフラボノイド量についても、果梗の抽出物は、他の抽出物より多く含まれていた。強い活性が認められた果梗に含まれる成分を解明するために、Ultra Performance Liquid Chromatography-diode array Time-of-flight Mass Spectrometry（UPLC-DAD-TOFMS）分析ならびにカラムクロマトグラフィーによる分離・単離を行った。その結果、甲州種ブドウの果梗には、主にカテキンやプロアントシアニジン、レスベラトロールやその重合体、シンナム酸酒石酸エステル、ジヒドロフラボノール配糖体、及びフラボノール配糖体が含まれていた。これらの化合物の中には、強い抗酸化活性を有しているものがあり、果梗抽出物の強い抗酸化活性に寄与しているものと考えられた。以上より、甲州種ブドウの果梗を食品や資材などに有効利用できる可能性が示唆された。