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α-Glucosidase Inhibitory and Antihyperglycemic Effects of Polyphenols in the Fruit of *Viburnum dilatatum* Thunb.

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Small crimson fruit of *Viburnum dilatatum* Thunb. (gamazumi), a wild deciduous low tree belonging to a family of Caprifoliaceae, has strong antioxidant activity, and cyanidin 3-sambubioside (C3S) and 5-caffeoyl quinic acid (5-CQA) are identified as active compounds. The freeze-dried powder of *V. dilatatum* fruit juice (CEV) was orally administered to streptozotocin-induced diabetic rats for 4 weeks repeatedly. Consequently, the elevation of plasma glucose level after oral administration of 2 g/kg glucose was suppressed by the repeated administration of CEV. The action was dependent on the dose of CEV, and plasma glucose level in rats administered 500 mg/kg of CEV was decreased significantly from that in rats without CEV. Increase of insulin secretion was not found in rats with or without administration of CEV. It was expected that CEV had some effects on glucose uptake. In five compounds identified from *V. dilatatum* fruit, C3S and 5-CQA showed inhibitory activity on sucrase and maltase. Inhibitory activity of cyanidin 3-glucoside and cyanidin aglycon (Cy) was not found markedly, and so it was thought that the activity was a characteristic property in Cy diglycosides. Moreover, 5-CQA and C3S were main polyphenol in the fruit of *V. dilatatum*. These results suggest that *V. dilatatum* fruit has the α-glucosidase inhibitory activities and the antihyperglycemic action.

KEYWORDS: ***Viburnum dilatatum*; α-glucosidase; cyanidin 3-sambubioside; 5-caffeoyl qunic acid; diabetes**

INTRODUCTION

Viburnum dilatatum Thunb. (gamazumi), a wild deciduous low tree, is found widely in the hills of northern Japan. Its small crimson fruit ripens in autumn (*1*). We found that a squeezed extract of *V. dilatatum* fruit had strong antioxidant and radical scavenging activity as measured by the XYZ-dish method and electron spin resonance (*2*).

The physiological actions of *V. dilatatum* fruit were studied in rats, which were given a crude extract of *V. dilatatum* fruit (CEV) and subjected to water immersion restraint stress. In these studies, CEV significantly depressed the gastric ulcer formation, elevation of tissue lipid peroxidation, and organ functional damage in rats subjected to stress (*2*). The effect of CEV was similar or stronger than that of (−)-epigallocatechin gallate, which is the main antioxidative component in teas. Furthermore, although hepatic antioxidant capacity increased in normal rats given CEV, induction of antioxidative enzymes was not observed. These observations suggested that antioxidant components in CEV were absorbed into the body and prevented oxidation induced by stress (*3*).

The previous study was performed to investigate the antioxidative effect of CEV on oxidative damages in rats with experimental diabetes induced by administration of streptozotocin (STZ). Generation of reactive oxygen species and free radicals is accelerated in diabetes (*4*), which increases oxidative stress and decreases the activities of the radical scavenging system (*5*, *6*). Furthermore, it is well known that specific complications, such as disorders of microcirculation and cellular function, arise in diabetes (*7*, *8*). In our previous study (*9*), we reported that the administration of CEV depressed the oxidation in STZ-induced diabetic rats. Consequently, ingestion of CEV inhibited the elevation of lipid peroxidation in plasma and erythrocytes, which were shown to increase in the rats that ingested water with the experimental periods for 10 weeks. Moreover, inhibition of increase of plasma glucose and total cholesterol levels was found in the rats that ingested CEV (*9*).

In this paper, an oral glucose tolerance test (OGTT) was performed on the diabetic rats, which were administrated CEV for 4 weeks, to clarify the detail of inhibitory action of CEV on plasma glucose elevation in diabetes. Moreover, the inhibitory effects of five compounds (*10*, *11*) from CEV on α-glucosidase were investigated.

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MATERIALS AND METHODS

1. Reagents. STZ for biochemical grade, quercetin (Qrt), rat small intestinal acetone powder, and α-amylase from porcine pancreas were purchased from Sigma Chemical Co. (St. Louis, MO). Cyanidin 3-glucoside (C3G; Funakoshi Co., Tokyo, Japan), cyanidin (Cy; Funakoshi Co.), 5-caffeoyl quinic acid (5-CQA; Wako Pure Chemical Industries Ltd., Osaka, Japan), α-glucosidase from *Sacchromyces* species (140 U/mg of specific activity, Wako Pure Chemical Industries Ltd.), and 1-deoxynojirimycin (DNJ; Toronto Research Chemicals Inc., Toronto, Canada) were used. The highest available grade of all other reagents was used.

2. Preparation of CEV and Phenolic Compounds. CEV was prepared by condensing and freeze-drying an extract of *V. dilatatum* fruit, which was harvested in November, obtained by squeezing fruit with an extractor followed by filtration through filter cloths without addition of any solvent (*2*). Yield of CEV was 50.4 g from 1 kg of *V. dilatatum* fruits. Total polyphenol content in CEV was 53.0 mg/g of gallic acid equivalent, which was measured by the Folin-Denis method (*12*).

CEV was applied onto Sephadex LH-20 (Amersham Biosciences Co., Piscataway, NJ) column chromatography, 0.1 N HCl, 20% MeOH/0.1 N HCl, 40% MeOH/0.1 N HCl, 60% MeOH/0.1 N HCl, and 100% MeOH were eluted, and Fr.1−Fr.5 were collected. Furthermore, five major phenolic compounds, cyanidin 3-sambubioside (C3S), C3G, 5-CQA, 5-caffeoyl-4-methoxy quinic acid (MeO-CQA), and Qrt, were isolated from Fr.1−Fr.3 by preparative high performance liquid chromatography (*10*, *11*).

3. Animal Experiment. Male Sprague−Dawley rats were purchased from Clea Japan Inc. (Tokyo, Japan), and they were treated under the control of the guidelines for animal experiments of Notification 6 of the Cabinet Office of the Japanese government. They were divided into normal (N), control (C), CEV-Low (VL), and CEV-High (VH) groups of five rats each, and then they were kept individually in stainless wire netting cages in a room maintained at 22 ± 1 °C with 12 h light/dark cycles. All rats, except for the N group, were administered 50 mg/kg of STZ intraperitoneally (*13*). STZ solution was prepared at a concentration of 25 mg/mL by dissolution in 50 mM citrate buffer (pH 4.5) and dilution with 0.15 M NaCl solution. The N group was injected intraperitoneally with 2 mL/kg of 0.15 M NaCl solution containing 50 mM citrate buffer (pH 4.5).

Blood was collected with heparinization from the tail artery of rats before and 2 days after administration of STZ. Plasma and red blood cells (RBC) were immediately separated from blood by centrifugation at 15 000 rpm for 1 min. Diabetic status was identified by measurement of plasma glucose level by the Fuji Dri-Chem 3000 (Fuji Medical System Co., Tokyo, Japan).

In the experiment, all rats were given free access to a commercial diet (type MF; Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water. Rats in the VL and VH groups were orally administered 100 and 500 mg/kg/d of CEV for 4 weeks, respectively. CEV solutions were prepared at concentrations of 50 and 250 mg/mL by dissolution with distilled water.

After all experiments, all rats were fasted and received tap water for 20 h, and then blood was collected from the abdominal artery under ether anesthesia. After blood collection, the liver, kidney, and pancreas were removed and weighed.

The concentration of glycated hemoglobin A_1 in the blood of fasted rats after experimental period for 4 weeks was measured with the glycated hemoglobin kit (Sigma Chemical Co.).

4. Oral Glucose Tolerance Test. OGTT was carried out before and after repeated oral administration of CEV for 4 weeks. All rats were fasted for 20 h before OGTT. Blood was collected with heparinization from the tail artery before and at 0.5, 1, and 2 h after oral administration of 2 g/kg of glucose. Plasma and RBC were immediately separated from blood by centrifugation at 10 000 rpm for 1 min. Plasma was diluted with 0.15 M NaCl solution, and then the plasma glucose level was measured via the Dri-Chem 3000. Moreover, the insulin concentration in plasma was measured with the kit of Rat-Insulin ELISA (Mercodia AB, Uppsala, Sweden).

5. Measurement of α-Glucosidase. Inhibitory activities of C3S, C3G, 5-CQA, MeO-CQA, and Qrt, which were isolated from CEV, on α-glucosidases were measured using DNJ and Cy as controls. They were prepared at 0.2, 0.5, 2, 5, and 20 mg/mL by dissolution and dilution with optimal buffer for assay. Inihibitory activity of α-glucosidases was measured by following a procedure according to the method of Suzuki et al. (*14*).

Ten U/mL of α-glucosidase from *Sacchromyces* species solution was prepared with 50 mM phosphate buffer (pH 7.4). Samples and 20 mg/mL of maltose solution as a substrate were dissolved and prepared in 50 mM phosphate buffer (pH 7.4).

Rat small intestinal acetone powder was homogenized in a 9-fold volume of cold 56 mM maleate buffer (pH 6.0). The homogenate was centrifuged at 3000 rpm for 10 min, and then the supernatant was used as a crude enzyme solution for an assay of maltase, sucrase, isomaltase, and glucoamylase. Maltose, sucrose, palatinose, and soluble starch were used as substrates of maltase, sucrase, isomaltase, and glucoamylase, respectively. Crude enzyme solution was diluted with 56 mM maleate buffer (pH 6.0) to 1/20, 1/2, 1/4, and 1/2, respectively. All 20 mg/mL of substrates and samples were dissolved in 56 mM maleate buffer (pH 6.0).

Briefly, 20 μL of sample was added to 150 μL of substrate solution and mixed. After incubation at 37 °C for 5 min, 20 μL of enzyme solution was added into the mixture, and then they were mixed and incubated at 37 °C. At 0, 5, 15, 30, 60, 90, and 120 min of incubation, 15 μL of incubated mixture was placed into other tubes, and the enzymatic reaction was immediately stopped by boiling for 10 min. After the reaction mixture was cooled, glucose concentration in the mixture was measured by using the Glucose C-II Test Wako kit (Wako Pure Chemical Industries Ltd.).

All experiments were carried out in duplicate, and the kinetics were calculated from the average of glucose production. Inhibitory rate of sample (%) was standardized 100% of kinetics without sample. The 50% inhibition concentration (IC_{50}) of the sample was obtained from the linear regression curve of the sample concentrations and inhibitory rates.

6. Measurement of α-Amylase. Measurement of α-amylase was performed according to the method of Amylase Test-Wako (*15*). α-Amylase (Type VI-B from porcine pancreas, Sigma Chemical Co.) was prepared to 0.5 U/mL solution by dissolution in 100 mM phosphase buffer (pH 7.0). Briefly, 60 μL of sample solution was added into 540 μL of 0.4 mg/mL starch solution, and then they were incubated at 37 °C for 5 min. Next, 60 mL of α-amylase solution was added, and they were incubated at 37 °C. After 0, 5, 20, 40, 60, 90, and 120 min of incubation, 60 μL of reaction mixture was collected, and the enzyme was altered by boiling immediately. Next, 40 μL of cooled mixture was put into the well of a micro plate, and 40 μL of 0.01 N iodine solution and 100 μL of water were added into the cooled mixture. After the plate was shaken for 10 s, their absorbance at 620 nm was measured by a micro plate reader iEMS (Dainippon Pharmaceutical Co., Tokyo, Japan). The inhibition rate of the sample was calculated from the kinetics of enzyme that was calculated from the residual starch concentration. Finally, the IC_{50} value was calculated from the linear regression curve of the sample concentrations and inhibition rates.

7. Statistical Analysis. Data were exhibited as the mean and standard deviation (SD) and analyzed by the Scheffe test after one-way analysis of variance (ANOVA) using the Stat View System (SAS Institute Inc., Cary, NC). Significant differences in the mean values were tested at $P < 0.05$.

RESULTS

Effect on Growth of Rats. The body weight of the N group was smoothly increased, but that of the STZ-diabetic groups was inhibited significantly from that of the N group. There was no significant difference among the three groups, which were administered STZ (**Figure 1**).

Table 1 shows the final body weight, body weight gain, tissue weight, and the tissue-to-body weight ratio of liver, kidney, and pancreas in rats fasted for 20 h after the experiment for 4 weeks. The fasted body weights, body weight gains, and liver and pancreas weights of the three diabetic groups were significantly lower than those of the N group, and there were no significant differences in these weights among the diabetic groups.

Table 1. Fasted Body Weight, Body Weight Gain, Tissue Weight, Ratio of Tissue-to-Body Weight, and Hemoglobin A_1 of Rats after Experiment for 4 weeks[a]

	N	C	VL	VH
final body wt (g)	337.8 ± 17.7	238.2 ± 22.6 c	239.5 ± 19.4 c	244.7 ± 12.6 c
body wt gain (g)	142.4 ± 14.5	55.2 ± 16.2 c	56.4 ± 14.2 c	60.1 ± 7.7 c
liver wt (g)	10.65 ± 0.73	8.96 ± 0.72 c	8.30 ± 0.64 c	8.02 ± 0.83 c
kidney wt (g)	2.462 ± 0.189	2.208 ± 0.263	1.895 ± 0.310 c	1.846 ± 0.359 c
pancreas wt (g)	0.946 ± 0.180	0.597 ± 0.076 c	0.632 ± 0.151 c	0.664 ± 0.081 c
tissue-to-body wt ratio[b]				
liver (g/100 g)	3.162 ± 0.294	3.771 ± 0.216	3.485 ± 0.378	3.288 ± 0.434
kidney (g/100 g)	0.730 ± 0.071	0.930 ± 0.111 c	0.789 ± 0.093	0.751 ± 0.115
pancreas (g/100 g)	0.279 ± 0.042	0.252 ± 0.032	0.266 ± 0.067	0.271 ± 0.032
hemoglobin A1 (%)	2.16 ± 0.18	4.27 ± 0.60 c	3.29 ± 0.84 c	2.65 ± 0.36 d

[a] Rats were fasted for 20 h after the experimental period of 4 weeks. Data represent the mean ± SD of five rats. [b] Tissue weight per 100 g of body weight. Significant difference is indicated from the N (c) and C (d) groups at $P < 0.05$.

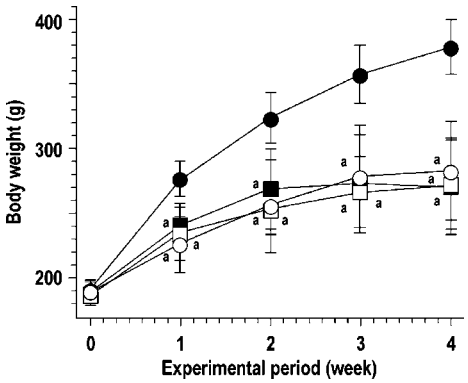

Figure 1. Body weight change of rats: ●, N (normal); ■, C (diabetic control); □, VL (diabetic with CEV 100 mg/kg); ○, VH (diabetic with CEV 500 mg/kg) groups. Data represent the mean ± SD of five rats. Significant difference is indicated from the N (**a**) and C (**b**) groups at $P < 0.05$.

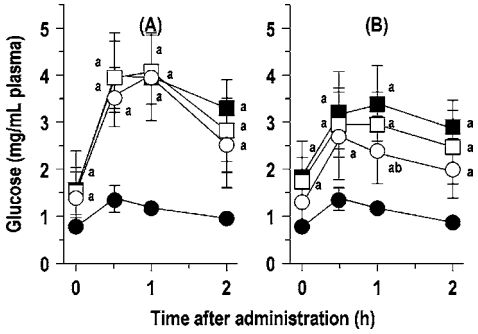

Figure 2. Change of plasma glucose levels in rats after oral administration of glucose at 0 (**A**) and 4 (**B**) weeks of experiment: ●, N (normal); ■, C (diabetic control); □, VL (diabetic with CEV 100 mg/kg); ○, VH (diabetic with CEV 500 mg/kg) groups. All rats were fasted for 20 h before oral administration of 2 g/kg glucose. Data represent the mean ± SD of five rats. Significant difference is indicated from the N (**a**) and C (**b**) groups at $P < 0.05$.

Although the kidney weights of the VL and VH groups were also decreased significantly as compared to the N group, that of the C group was similar to that of N group, and the ratio of kidney to body weight in the C group was significantly higher than that in the N group. There were no significant differences in other ratios among all groups.

The hemoglobin A_1 level increased in the diabetic groups, and the C and VL groups showed a significantly higher level than the N group. However, the VH group showed a significantly lower level than the C group (**Table 1**).

Changes of Plasma Glucose Levels in OGTT. Figure 2 shows the changes of plasma glucose levels in rats after oral administration of glucose at 2 g/kg. Before repeated administration of CEV, the plasma glucose level at each point of three groups, which were administered STZ, was significantly higher than that of healthy N group (**Figure 2A**). There was no significant difference among the three diabetic groups. After 4 weeks, N and C groups showed the same change of glucose level similar to that at 0 week. However, VL and VH groups showed a change of plasma glucose level lower than that of the C group. Especially, it was found that the plasma glucose level of the VH group had a tendency to be lower than that of the VL group (**Figure 2B**).

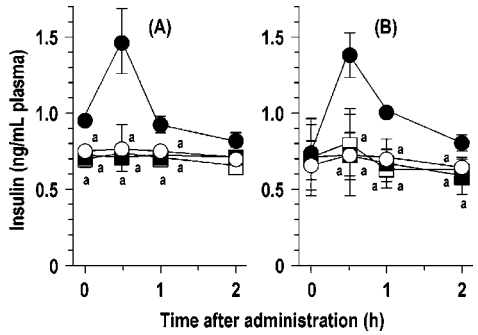

Figure 3. Change of plasma insulin levels in rats after oral administration of glucose at 0 (**A**) and 4 (**B**) weeks of experiment: ●, N (normal); ■, C (diabetic control); □, VL (diabetic with CEV 100 mg/kg); ○, VH (diabetic with CEV 500 mg/kg) groups. All rats were fasted for 20 h before oral administration of 2 g/kg glucose. Data represent the mean ± SD of five rats. Significant difference is indicated from the N (**a**) and C (**b**) groups at $P < 0.05$.

Changes of Plasma Insulin Levels in OGTT. The changes of plasma insulin concentrations were shown in **Figure 3**. The N group showed the maximum level of plasma insulin at 0.5 h after administration of glucose and decrease after that. However, in the three diabetic groups, the plasma insulin level did not increase after oral administration of glucose at each test.

Inhibitory Activities of Phenolic Compounds from *V. dilatatum* on α-Glucosidase. Table 2 shows α-glucosidase inhibitory activities of five compounds, which were identified from the fruit of *V. dilatatum*. C3S was found to have the strongest inhibitory activities on α-glucosidase from *Sacchromyces* species, maltase from rat small intestine, and α-amylase from porcine pancreas in the five compounds. The inhibitory activity on sucrase from rat small intestine was found in 5-CQA, and MeO-CQA showed the strongest inhibitory activity on isomaltase and glucoamylase. On the other hand, DNJ, as a positive control compound, showed stronger inhibitory activity than the five compounds. It was found that Cy aglycon had the tendency to be weaker on all enzymes than C3S.

Table 2. Inhibitory Activities of Phenolic Compounds from *V. dilatatum* Fruit on α-Glucosidases and α-Amylase

	inhibitory activity (IC_{50}, mM)					
	α-glucosidase[a]	sucrase[b]	maltase[b]	isomaltase[b]	glucoamylase[b]	α-amylase[c]
C3S	3.19	3.26	3.85	12.86	5.61	0.96
C3G	25.55	6.50	26.00	102.07	77.01	42.48
MeO-CQA	11.12	31.60	114.91	4.08	3.76	106.38
Qrt	29.41	141.51	12.67	7.89	237.26	162.20
5-CQA	82.18	1.40	24.87	23.46	5.07	37.16
DNJ	0.77	0.11	1.83	2.24	8.23	83.44
Cy	63.29	59.82	18.15	42.69	68.77	113.84

[a] From *Sacchromyces* species. [b] From rat small intestine. [c] From porcine pancreas.

DISCUSSION

The natural habitat of *V. dilatatum* fruit is limited in Japan, Aomori, and it is a wild species, not a common fruit. In our previous studies, 40 mg/mL of CEV solution was ingested in healthy rats for 2 weeks (*2*), and 16.8 mg/mL of CEV solution was given to STZ-diabetic rats for 10 weeks (*9*). In both studies, alterations of body weight and tissue weights were not found by ingestion of CEV, and it was concluded that the fruit of *V. dilatatum* had no bad influence on growth. In this study, 100 and 500 mg/kg of CEV administration did not show a marked effect on body weight change, body weight gain, and liver, kidney, and pancreas weights of STZ-diabetic rats. Similar to previous results, it is suggested that the high dose of CEV or *V. dilatatum* fruit for 4 weeks has no semi acute influence.

Diabetes increases oxidative stress (*5*−*7*), and increased glycosylated hemoglobin, which is caused by glycation of proteins as a result of hyperglycemia, is a feature of diabetes (*16*). In this study, increased hemoglobin A_1 was found in diabetic rats, but the administration of CEV suppressed that level. This result is similar to our previous result (*9*), and it suggests that CEV can depress glycosylation of hemoglobin in diabetic rats via its antioxidative actions on oxidative stress.

Insulin has an important role for translocation of glucose transporter 4 (GLUT4) in blood glucose homeostasis. In the OGTT after repeated oral administration of CEV for 4 weeks, the suppression of plasma glucose level was found, and the marked increase of plasma insulin level was not observed. Plasma glucose level in diabetic rats, which were administered CEV, was higher than that in normal rats. However, it was already found that the elevation of steady plasma glucose level was inhibited by free ingestion of CEV in our previous study (*9*). So, the phenomenon in the OGTT was dependent on the CEV dose, and it was considered that its reaction was neither stimulation nor recovery of insulin secretion. Accordingly, we expected that its action was induced by inhibition of intestinal glucose uptake, enhancement of hepatic glucose uptake via GLUT2, or increase of glucose uptake in adipocytes and muscles via GLUT4. Dietary fiber is well known to have inhibition and delay of glucose absorption from a digestive tract. Tabuchi et al. (*17*) have found that ukogi (*Acanthopanax sieboldianus*) leaves improved the blood glucose level and glucose tolerance. They have reported that the participation of ukogi polyphenol could not be denied, although there was high possibility that its effect was based on fibers. Moreover, there were reports that inhibition of intestinal glucose uptake was induced by an interaction of catechins and flavonoids with sodium-dependent glucose transporter 1 (SGLT1) (*18*, *19*). CEV was crude extract that was obtained by squeezing the fruits of *V. dilatatum*. Therefore, it is necessary to investigate the possibility that the compositions including polyphenols, which were identified in our previous study, have insulin-like activity, which contributes to translocation of GLUT4, and interaction with SGLT1.

On the other hand, to clarify the inhibitory effect of CEV on elevation of steady glucose level in plasma of STZ-induced diabetic rats in a previous study, α-glucosidase inhibitory activities of isolated compounds from CEV were measured. Although some α-glucosidase inhibitory activity of C3G was stronger than that of Cy aglycon, the activity of C3S was almost stronger than that of C3G and Cy aglycon. We did not investigate the inhibitory activity of diglycosides, except for C3S, but these results were very interesting and indicated the structure-related activities of aglycon, monoglycoside, and diglycosides. Moreover, as C3S has a xylose, there is undeniably a difference in sugar species.

C3G is a typical anthocyanin glycoside, which is contained in many fruits such as grapes and blueberry, while the range of C3S distribution is comparatively limited in the plant family of Caprifoliaceae including *V. dilatatum*. From the above, although α-glucosidase inhibitory activity of C3S is weaker than that of DNJ, the major anti-diabetic compound of mulberry, it is very interesting in that C3S has α-glucosidase inhibitory activities.

5-CQA and MeO-CQA, derivatives of chlorogenic acid, which were classed in phenylpropanoides, also had inhibitory activity on some α-glucosidase. There are reports that chlorogenic acids have hypoglycemic effects (*20*, *21*). Moreover, the experiment of synthesized chlorogenic acid derivatives suggested that the hypoglycemic action was caused by the inhibition of chlorogenic acids on glucose-6-phosphate translocase in hepatic glucose-6-phosphatase systems (*22*). Because the elevation of plasma insulin level was not observed in diabetic rats administered CEV repeatedly, it was suggested that the suppressing action on hyperglycemia was induced by inhibitory effects of phenolic compounds in CEV on glucose transportation such as insulin-like action, glucose uptake inhibition, or α-glucosidases.

Recently, various physiological actions of anthocyanins have attracted a great deal of attention. The radical scavenging activity and antioxidant effect of Cy on low-density lipoprotein oxidation were reported (*23*−*25*), and also C3G was found to have antioxidant activity (*26*), decrease consumption of tocopherols and improvement of lipid metabolisms (*27*), stimulatory effect on regeneration of rhodopsin (*28*), and gastropreventing effect (*29*). It was reported that some acylated anthocyanin glycosides had α-glucosidase inhibitory activity (*30*, *31*); we had also discussed the same matter in our previous study (*9*). After that, five phenolic compounds were identified from the fruit of *V. dilatatum*, and it was expressed that the sum of these compounds accounted for one-half of the total polyphenol contents (*11*). In these compounds, 5-CQA has the largest quantity, followed by C3S, which contained 10 times as much as C3G in this fruit. The radical scavenging activities of these compounds have already been reported (*10*); in this study, it is suggested that antihyperglycemic actions of CEV were induced by the α-glucosidase inhibitory activities of C3S and 5-CQA, which were the main polyphenols in *V. dilatatum* fruit. Moreover, the

possibility that other compounds in the fruit have an insulin-like action in the GLUT4 translocation system or contribution to glucose uptake inhibition via SGLT1 is speculated.

These results demonstrate that the fruit of *V. dilatatum* may be a functional fruit for prevention of life style-related diseases through the inhibitory actions of polyphenol on oxidation and hyperglycemia. For manufactural use, future subjects of investigation on the possibility of glucose uptake inhibition and insulin-like action of constituents including these phenolic compounds, their absorption and metabolism of these phenolic compounds in animal and human, their stability during processing, and efficient extract procedure are necessary.

ABBREVIATIONS USED

CEV, crude extract of *V. dilatatum* fruit; C3S, cyanidin 3-sambubioside; C3G, cyanidin 3-glucoside; 5-CQA, 5-caffeoyl quinic acid; MeO-CQA, 5-caffeoyl-4-methoxy quinic acid; Qrt, quercetin; STZ, streptozotocin; Cy, cyanidin; OGTT, oral glucose tolerance test; DNJ, 1-deoxynojirimycin.

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