

## Effects of blueberry leaf and stem extracts on hepatic lipid levels in rats consuming a high-sucrose diet

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### ABSTRACT

**Background:** Blueberry stems, a by-product of blueberry leaf tea production, are typically discarded. We evaluated the effects of hot-water extracts of rabbiteye blueberry (*Vaccinium virgatum* Aiton; RB species) leaves and stems on hepatic lipid levels in rats consuming a high-sucrose diet.

**Aim:** This study focused on the stems of rabbiteye blueberry (*Vaccinium virgatum* Aiton; RB species), a major blueberry species, and evaluated the effects of daily consumption of blueberry stems on hepatic lipid levels and hepatic lipogenic enzyme activity in rats consuming a high-sucrose diet.

**Methods:** Male Sprague-Dawley rats were divided into groups that received a control high-sucrose diet alone or supplementation with 2.0% blueberry leaf extract or 0.5% or 2.0% blueberry stem extract. Blood and hepatic lipid levels, hepatic lipogenic enzyme activity, and hepatic quercetin metabolites were evaluated after 28 days of *ad libitum* consumption.

**Results:** Supplementation with the extracts did not affect body weight gain, food intake, liver and white adipose tissue weights, or serum lipid levels. Hepatic triglyceride and total cholesterol levels were reduced in the groups that received 2.0% supplementation of either extract. Hepatic malic enzyme activity was also reduced in those groups. Quercetin and its glycosides, the major polyphenols identified in the extracts, accumulated in the liver as quercetin aglycone and quercetin metabolites.

**Conclusion:** We demonstrated how daily consumption of blueberry leaf and stem extracts can decrease hepatic lipid levels, potentially downregulating malic enzyme activity. These effects were intensive in leaf extracts. The active compounds existed in both extracts may be quercetin and its glycosides. Therefore, blueberry stems and leaves may be an attractive candidate novel functional food.

**Keywords:** Blueberry leaf; blueberry stem; quercetin; hepatic lipid; rat; functional food

## BACKGROUND

Evidence indicates that multiple beneficial ingredients exist in daily food items. Foods rich in beneficial ingredients are termed “functional foods.” Their daily consumption is believed to offer health-promotion and disease-prevention benefits. For example, the consumption of berry fruits may prevent carcinogenesis, liver damage, obesity, inflammation, and glaucoma, and improve eyesight [1-4]. Berries are rich in flavonoids, mainly anthocyanins and phenolic acids/esters such as chlorogenic acid [3, 5]. These ingredients are known to exert beneficial effects such as antioxidant activity [6].

However, the leaves of berry-bearing plants have typically been ignored and discarded, although they contain high quantities of bioactive ingredients such as phenolic acids/esters, flavonoids, and procyanidins [7] which exert anti-diabetic and anti-glycation effects [8,9]. Hot-water leaf extracts of blueberry have been reported to have hepatic lipid-lowering, anti-hepatitis C virus, and hypolipidemic effects to suppress the proliferation of adult T-cell leukemia cells and ameliorate insulin resistance [10-14]. These findings indicate that blueberry leaves may be a potential functional food. Moreover, blueberry leaf teas, are commercially available worldwide as hot-water extracts.

During the production of blueberry leaf tea, the plant stems are discarded as waste. If blueberry stems contain compounds with beneficial effects, the stems may offer a novel source of functional food. However, information on blueberry stems is limited.

## METHODS

### Chemicals

Cellulose,  $\beta$ -cornstarch, sucrose, vitamin mixture (AIN-76), and mineral mixture (AIN-76) were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). Casein, corn oil, DL-methionine, choline bitartrate, trans-cinnamic acid, 3,4-dihydroxycinnamic acid, ferulic acid, rutin (quercetin-3-*O*-rutinoside), cyanidin chloride, luteolin, apigenin, and kaempferol were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). P-coumaric acid was from MP Biomedicals, LLC (Santa Ana, CA, USA), chlorogenic acid was from Cayman Chemical (Ann Arbor, MI, USA), and quercetin was from ChromaDex (Irvine, CA, USA).  $\beta$ -glucuronidase/sulfatase from *Helix pomatia* (type H-5) and  $3\alpha$ -hydrosteroid dehydrogenase were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of the highest grade available.

### Plant materials

Rabbiteye blueberry cultivar Kunisato No. 35 was cultivated in the Miyazaki area of Japan and taxonomically identified on the basis of morphological characteristics by author H.K. The fresh leaves and stems were separated in December 2012 (Figure 1). Then both leaves and stems were blanched, air-dried for 5 h (leaves) and 8 h (stems) using a hot-air dryer (FV-1000, ADVANTEC Ltd., Tokyo, Japan), and then powdered using an R-10 food processor (Robot Coupe Ltd., Vincennes, France). The blueberry leaf and stem powders were stored away from light at  $-20^{\circ}\text{C}$  until extraction.

**Figure 1.** Rabbiteye blueberry (*Vaccinium virgatum* Aiton; RB species) used in the study. (A) General view of typical blueberry field. (B) Leaves. (C) Stems. Photographs by author Y.M.



***Hot-water extraction***

Blueberry leaf powder (0.5 kg) and stem powder (2.5 kg) were mixed with 50 L and 62.5 L of water respectively at 95°C. After agitation for 30 min, the filtrates were obtained via filtration through 300-mesh nylon, and concentrated into approximately 1 L under reduced pressure using a rotary evaporator (CEP-lab, Okawara Mfg. Co. Ltd., Shizuoka, Japan). The concentrates were then lyophilized using a freeze dryer (Dura Dry MP, FTS Systems Inc., Stone Ridge, NY, USA). The leaf and stem yields were 49.4% and 12.0% respectively.

***Institutional approval of the study protocol***

All procedures for animal experiments were conducted according to the guidelines for the care and use of laboratory animals of the University of Miyazaki (Miyazaki, Japan). The experimental protocol was registered under the number 2010-013-3.

***Animal experiments***

Male Sprague-Dawley rats (4 weeks old) were obtained from Kyudo Co. Ltd. (Saga, Japan) and housed in an air-conditioned room (temperature:  $23 \pm 1^\circ\text{C}$ ; humidity:  $55 \pm 5\%$ ) under a 12-h dark/light cycle (light on between 9:00 and 21:00) with free access to deionized water and laboratory chow (Type CE-2, CLEA Japan, Tokyo, Japan). After a 1-week acclimatization, the animals were submitted to the study according to the following protocol.

Twenty-eight rats were randomly divided into four groups. The first group consumed AIN-76 control diets that contained 72.2% carbohydrate, 5.5% fat, and 22.2% protein as a percentage of the total energy (Table 1). The second group consumed AIN-76 diets containing 2.0% blueberry leaf extracts (BLEx). The third and fourth groups consumed 0.5% and 2.0% blueberry stem extract (BSEx). After 28 days, blood was drawn from the abdominal vein under anesthesia with sodium pentobarbital (30 mg/kg body weight) without fasting. After 30 min at room temperature, serum was separated by centrifugation at  $1,000 \times g$  for 20 min. Livers and white adipose tissue were removed and weighed. Liver and serum samples were stored at  $-80^\circ\text{C}$  for subsequent analysis, with the exception of liver samples analyzed for enzymatic activities.

***Blood chemistry***

Serum lipid parameters (triglycerides, total cholesterol, high-density lipoprotein [HDL] cholesterol, phospholipids) were analyzed using test kits from Wako Pure Chemical Industries, Ltd. All standards were used in accordance with the manufacturer's instructions.

**Table 1.** Composition of experimental high-sucrose diets (%).

Constituent	Control diet	BLEx-containing diet	BSEx-containing diet	
		2.0%	0.5%	2.0%
Sucrose	50.0	48.0	49.5	48.0
Casein	20.0	20.0	20.0	20.0
$\beta$ -Cornstarch	15.0	15.0	15.0	15.0
Cellulose	5.0	5.0	5.0	5.0
Corn oil	5.0	5.0	5.0	5.0
Mineral mixture	3.5	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0	1.0
DL-methionine	0.3	0.3	0.3	0.3
Choline bitartrate	0.2	0.2	0.2	0.2
Blueberry extract	—	2.0	0.5	2.0

BLEx, hot-water extract of blueberry leaves; BSEx, hot-water extract of blueberry stems.

### *Hepatic lipid analysis*

Lipids from liver samples were extracted as described by Folch and colleagues [15]. The extracts were saponified to measure total cholesterol as described by Sperry and Webb [16]. Triglycerides and phospholipid quantities were analyzed using classical methods [17, 18].

### *Enzymatic activities*

Cytosolic fractions were prepared from fresh liver samples using the modified method described by Graham [19]. Briefly, the liver samples were immediately placed in 10× ice-cold buffer (pH 7.4) containing 0.25 mol/L sucrose, 0.01 mol/L tris(hydroxymethyl) aminomethane-HCl, and 1  $\mu$ mol/L ethylenediaminetetraacetic acid disodium salt and then homogenized on ice. The homogenates were centrifuged at 700 ×g for 10 min at 4°C. The supernatants were further centrifuged at 10,000 ×g for 10 min at 4°C. Finally, the cytosolic fractions were obtained by centrifugation at 126,000 ×g for 60 min at 4°C. Enzymatic activities of fatty acid synthase (FAS), malic enzyme, glucose-6-phosphate dehydrogenase (G6PDH), and carnitine palmitoyltransferase II (CPT2) were evaluated according to previous methods [20-23]. Protein contents in the cytosolic fractions were quantified using a Lowry method [24] with slight modifications.

### *Polyphenol analysis in blueberry leaf and stem extracts*

Polyphenols in blueberry leaf and stem extracts were analyzed using our previous method [5]. Briefly, the stored powder (50 mg) was placed in a test tube and mixed with 4 mL of 62.5% aqueous methanol containing 0.5 mg/mL of *tert*-butylhydroquinone and 1 mL of 2N HCl. The test tube was heated at 90°C for 2 h and then the sample was extracted with two volumes of ethyl acetate. The extract was dried under nitrogen gas. The extract and another aliquot of the stored powder (50 mg) were dissolved in 500  $\mu$ L of methanol containing 0.5% trifluoroacetic

acid and filtered through a 0.2- $\mu$ m membrane filter (SLLGH04NK, Millex-LG, Merck Millipore, Burlington, MA, USA) prior to analysis by high-performance liquid chromatography (HPLC).

#### ***Extraction of quercetin metabolites from liver***

One-gram aliquots of liver were mixed with 0.5 mL of ice-cold acetate buffer (pH 5.0) and homogenized at 3,800 rpm for 2 min (Micro Smash MS-100, TOMY Seiko Co., Tokyo, Japan). The homogenate was transferred to another test tube and 4.5 mL of ice-cold acetate buffer (pH 5.9) were added. After mixing well, the supernatant was collected by centrifugation (3,000 rpm, 10 min, 4°C) and then incubated with and without  $\beta$ -glucuronidase/sulfatase (100 units) for 45 min at 37°C. Next, 5 mL of ethyl acetate were added and mixed well. After centrifugation at 1,500  $\times$ g for 10 min at 4°C, a 2-mL aliquot of the supernatant was dried with a centrifugal concentrator (CC-105; TOMY Seiko Co.). The residues were dissolved in 100  $\mu$ L of methanol containing 0.5% trifluoroacetic acid and filtered through a 0.2- $\mu$ m membrane filter before HPLC analysis.

#### ***HPLC diode-array detection (DAD)***

Polyphenols were analyzed by HPLC in combination with DAD as described in our previous report [5] with small modifications. The HPLC system used was a JASCO LC-NetII/ADC system control program (Tokyo, Japan) equipped with a ChromNAV chromatography data station, PU-2089 Plus pump, AS-2057 Plus autosampler, CO-2060 Plus column oven, and MD-2018 Plus DAD system to monitor all 200–600 nm wavelengths. The column, Capcell Pak UG120 (4.6 mm i.d.  $\times$  250 mm, 5  $\mu$ m, Shiseido Co. Ltd., Tokyo Japan), was used at 35°C under the following conditions.

Condition I for analysis of polyphenols in leaf and stem extracts: Gradient elution was performed with solution A, composed of 50 mM of sodium phosphate buffer (pH 3.3) and 10% methanol, and solution B, containing 70% methanol, delivered at a flow rate of 1.0 mL/min as follows: Initially 100% of solution A; for the next 15 min, 70% A; for another 30 min, 65% A; for the next 20 min, 60% A; then 5 min of 50% A, and then finally 0% A for 25 min.

Condition II for analysis of quercetin metabolites in liver samples: Isocratic elution was performed with 60% methanol containing 50 mM of sodium phosphate buffer (pH 3.3).

#### ***Statistical analysis***

Data were analyzed using StatView for Windows (version 5.0, SAS Institute, Cary, NC, USA) and one-way analysis of variance. The Dunnett method was applied for multiple comparisons

between control and treatment groups. Results were considered significant if the probability of error was <5%.

## RESULTS

### Food intake and body and organ weights

After 28 days, body weight, average weight gain, food intake, and liver and white adipose tissue weights did not differ between the control diet and treatment groups (Table 2).

**Table 2.** Effects of dietary blueberry leaf and stem extracts on biological parameters.

Parameter	Control diet	BLEx-containing diet 2.0%	BSEx-containing diet	
			0.5%	2.0%
Body weight (g)				
initial (day 0)	145 ± 3	145 ± 3	145 ± 3	145 ± 3
final (day 28)	372 ± 8	358 ± 11	364 ± 11	367 ± 10
average gain (g/day)	8.1 ± 0.3	7.6 ± 0.3	7.8 ± 0.3	7.9 ± 0.3
Food intake (g/rat/day)	20.2 ± 0.5	19.5 ± 0.7	20.2 ± 0.7	20.4 ± 0.4
Total quercetin consumption (µmol/rat/day) <sup>a</sup>	–	34.5 ± 1.2	1.4 ± 0.1	5.6 ± 0.1
Organ weight (g/100 g body weight)				
Liver	4.98 ± 0.12	4.74 ± 0.09	4.84 ± 0.07	4.86 ± 0.13
White adipose tissue				
epididymal	2.08 ± 0.16	2.09 ± 0.20	2.11 ± 0.17	2.20 ± 0.21
perirenal	1.68 ± 0.10	1.62 ± 0.12	2.00 ± 0.23	1.70 ± 0.12
mesentery	1.72 ± 0.13	1.54 ± 0.10	1.83 ± 0.16	1.66 ± 0.11

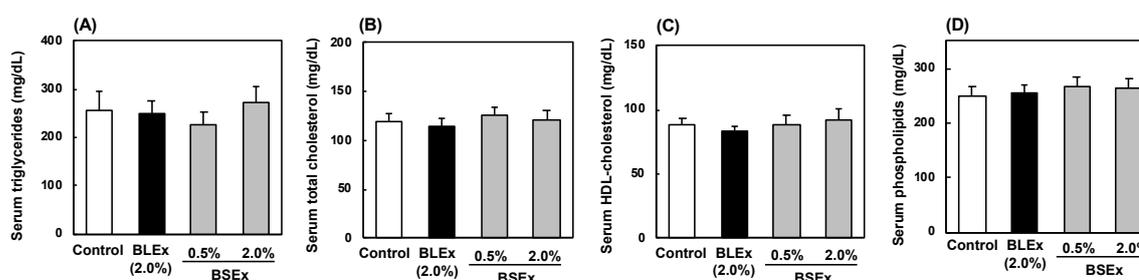
BLEx, hot-water extract of blueberry leaves; BSEx, hot-water extract of blueberry stems. Values indicate mean ± standard error (n = 7).

<sup>a</sup>Total amounts of quercetin glycosides + rutin + quercetin.

### Serum lipid parameters

After 28 days, serum levels of triglycerides, total cholesterol, HDL cholesterol, and phospholipids did not differ between the control and treatment groups (Figure 2).

**Figure 2.** Effects of dietary blueberry leaf and stem extracts on serum lipid levels.

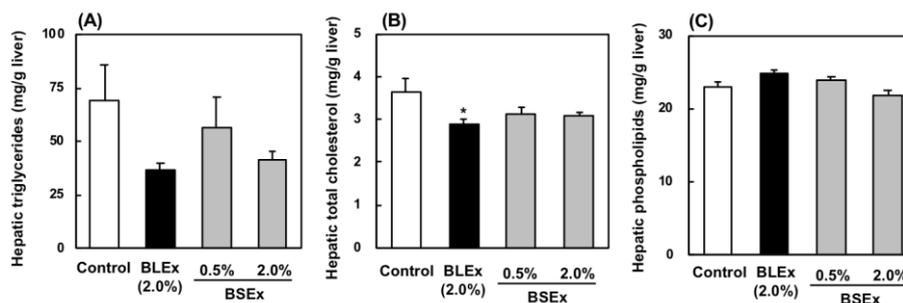


(A) Serum triglycerides. (B) Serum total cholesterol. (C) Serum high-density lipoprotein (HDL) cholesterol. (D) Serum phospholipids. Values indicate mean ± standard error (n = 7). BLEx, blueberry leaf extract; BSEx, blueberry stem extract.

### Hepatic lipid levels

We quantified major hepatic lipids such as triglycerides, total cholesterol, and phospholipids. Hepatic triglyceride levels decreased notably in rats fed 2.0% BLEx or 2.0% BSEx, but these decreases did not reach statistical significance (Figure 3A). Levels of total cholesterol decreased in all treatment groups and rats fed 2.0% BLEx had significantly lower levels of total cholesterol than the controls (Figure 3B). Hepatic phospholipids did not differ between the control and treatment groups (Figure 3C).

**Figure 3.** Effects of dietary blueberry leaf and stem extracts on hepatic lipid levels.

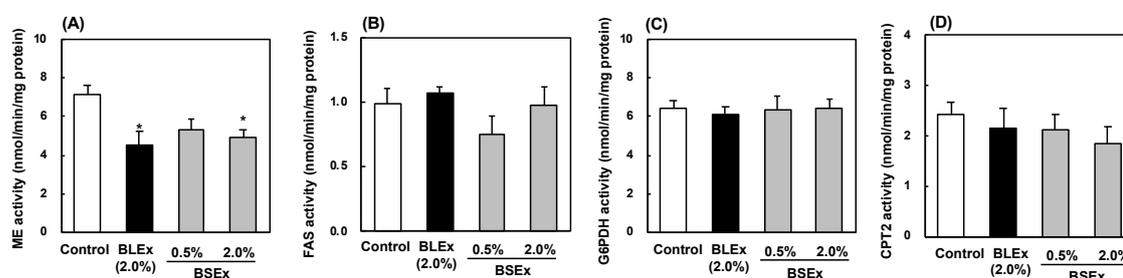


(A) Hepatic triglycerides. (B) Hepatic total cholesterol. (C) Hepatic phospholipids. Values indicate mean  $\pm$  standard error (n = 7). \* $p < 0.05$  vs. the control group (Dunnett’s test). BLEx, blueberry leaf extract; BSEx, blueberry stem extract.

### Enzymatic activities associated with hepatic lipid metabolism

Daily consumption of 2.0% BLEx or BSEx significantly inhibited hepatic malic enzyme activity (Figure 4A). The enzymatic activities of FAS, G6PDH, and CPT2 were not affected by supplementation with 2.0% BLEx or 0.5% or 2.0% BSEx (Figure 4B–D).

**Figure 4.** Effects of blueberry leaf and stem extracts on enzymatic activity related to hepatic lipid metabolism.



(A) malic enzyme (ME). (B) Fatty acid synthase (FAS). (C) Glucose-6-phosphate dehydrogenase (G6PDH). (D) Carnitine palmitoyltransferase II (CPT2). Values indicate mean  $\pm$  standard error (n = 7). \* $p < 0.05$  vs. the control group (Dunnett’s test). BLEx, blueberry leaf extract; BSEx, blueberry stem extract.

***Polyphenols in blueberry leaves and stems***

Analysis without hydrolysis identified more than 10 peaks at 370 nm. Two of these peaks were consistent with rutin (quercetin-3-*O*-rutinoside) and quercetin standards (Table 3). The other peaks did not correspond to any standard used in this study, but displayed a spectrum typical of cinnamic acid or quercetin. Therefore, we hydrolyzed and analyzed it again. Hydrolysis yielded a single peak, which corresponded to the quercetin standard in retention time and spectrum. This meant that the peaks indicated a spectrum similar to that of quercetin aglycone. Then we examined them as quercetin glycosides with the calibration curve of rutin. Additionally, the peaks that indicated a spectrum similar to that of cinnamic acid were calculated as cinnamic acids using the calibration curve of chlorogenic acid. Table 3 summarizes the quantities of phenolic compounds in hot-water BLEx and BSEx; quantities were higher in BLEx.

**Table 3.** Quantities of phenolic compounds in hot-water extracts from blueberry leaves and stems.

Compounds	BLEx	BSEx
	$\mu\text{mol/g extract}$	
Total cinnamic acids <sup>a</sup>	345.7	20.9
Total quercetin glycosides <sup>b</sup>	85.9	11.6
Rutin	11.1	2.1
Quercetin	1.5	0.1

<sup>a</sup>Amounts of total cinnamic acids were calculated using chlorogenic acid.

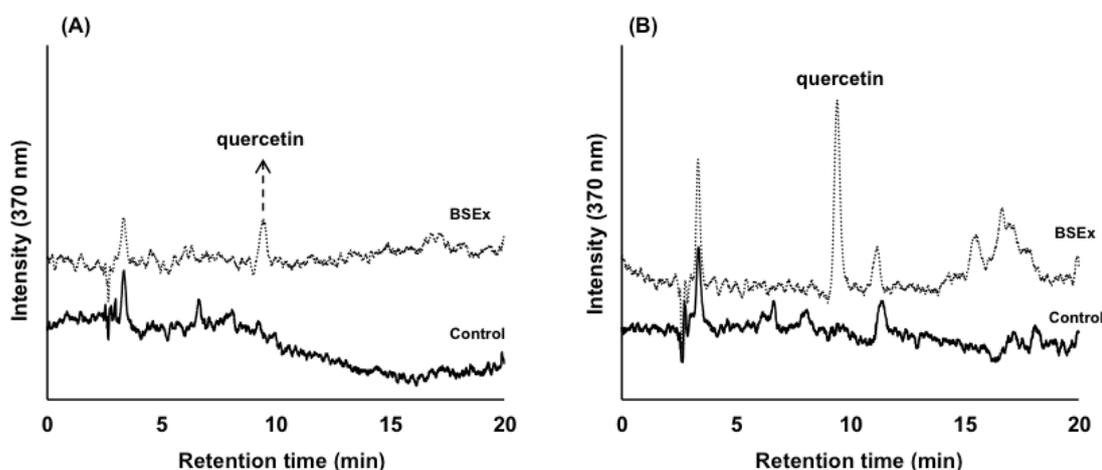
<sup>b</sup>Amounts of total quercetin glycosides (with the exception of rutin) were calculated using rutin.

BLEx, blueberry leaf extract; BSEx, blueberry stem extract.

***Quercetin accumulation in liver***

In rats that consumed diets containing 2.0% BLEx or 0.5% or 2.0% BSEx, the mean daily intake of total quercetin (quercetin + rutin + quercetin glycosides) was 34.5, 1.4, and 5.6  $\mu\text{mol/rat/day}$  respectively (Table 2). Quercetin was detected in liver samples from rats that consumed BSEx (Figure 5A). After hydrolysis, the peak identified as increased quercetin (Figure 5B). Table 4 lists the quercetin amounts measured in liver samples. No quercetin was detected in liver samples from rats that consumed the control diet. Total quercetin and quercetin glycoside amounts in the livers of rats that consumed BLEx were considerably higher than the amounts measured in livers from rats that consumed BSEx.

**Figure 5.** Typical high-performance liquid chromatography readouts at 370 nm of quercetin accumulation.



Quercetin accumulation in livers of rats consuming a control diet (solid line) and blueberry stem extract (BSEx, dotted line), and the accumulated quercetin levels before (A) and after (B) hydrolysis.

**Table 4.** Quercetin amounts in livers of rats fed blueberry leaf and stem extracts.

	Control diet	BLEx-containing diet 2.0%	BSEx-containing diet	
			0.5%	2.0%
			pmol/g liver	
Aglycone	u.d.	46.1 (u.d.–155.6)	u.d.	17.9 (u.d.–46.3)
Metabolites	u.d.	146.2 (41.7–190.8)	18.0 (u.d.–48.2)	20.6 (6.4–29.7)
<b>Total</b>	<b>u.d.</b>	<b>199.3 (150.6–251.7)</b>	<b>12.9 (u.d.–48.2)</b>	<b>38.5 (24.9–58.9)</b>

Data indicate mean (minimum–maximum). BLEx, blueberry leaf extract; BSEx, blueberry stem extract; u.d., under the detection limit.

## DISCUSSION

Blueberry leaves and fruit are potential functional food candidates. However, blueberry stems have been overlooked. In this study, we evaluated the effects of *ad libitum* consumption of hot-water extracts of blueberry stem and leaves on serum and hepatic lipid levels in rats. Rodent studies commonly use a purified diet, the American Institute of Nutrition AIN-93 formula [25] that substitutes cornstarch for the sucrose in the older AIN-76 diet. High dietary concentrations of sucrose have been associated with several metabolic complications, including dyslipidemia and fatty liver [26-28]. We used the sucrose-based AIN-76 diet so that our control rats would present mild dyslipidemia.

The addition of BLEx or BSEx to the AIN-76 diet in treatment groups did not affect body weight, weight gain, food intake, or the weights of the liver and white adipose tissue.

Triglyceride and total cholesterol levels in serum collected from control rats were much higher than those reported in fasted rats administered a normal diet (AIN-93) [29], confirming that the high-sucrose diet successfully induced mild dyslipidemia. Serum lipid levels in rats consuming BLEx did not differ from levels in the controls, although hepatic total cholesterol levels decreased significantly, in accordance with the findings of Yuji and colleagues for a different blueberry species [13]. In rats consuming 2.0% BSEx, serum lipid levels did not differ from levels in the controls, and while some hepatic lipid levels were reduced, the reduction did not reach statistical significance. We also evaluated hepatic lipogenic enzyme activity [30] and discovered that malic enzyme activity decreased in the rats that consumed a diet containing 2.0% BLEx or BSEx. These data indicate that consumption of 2.0% BSEx or BLEx may decrease hepatic lipid levels, perhaps by downregulating malic acid activity.

We screened the extracts to identify the ingredients responsible for the hepatic lipid-lowering effects. Vegetables, fruits, and teas rich in polyphenols have been recognized as providing beneficial effects [5]. Therefore, we quantified the polyphenolic compounds in BLEx and BSEx, and identified them as cinnamic acid, quercetin, and quercetin glycosides. The quantities of cinnamic acid and quercetin were much higher in BLEx than in BSEx. Quercetin has been reported to exhibit anti-obesity activity and to regulate lipid metabolism in diabetes mellitus [31-33]. We found quercetin aglycone in the liver of rats that consumed diets supplemented with 2.0% BLEx or BSEx, as well as quercetin metabolites. This indicates that ingested quercetin and its metabolites accumulate in the liver and may be a factor in reducing hepatic lipid levels. Metabolites such as quercetin 3,4'-di-O- $\beta$ -glucoside, quercetin 3-O- $\beta$ -glucoside, and quercetin 4'-O- $\beta$ -glucoside are known to circulate in the bloodstream [34]. Ingested quercetin is known to be present as metabolites in the blood and organs in rats consuming a normal diet, although not in its aglycone form [35]. We demonstrated how quercetin and its glycosides in blueberry plant extracts could be absorbed and accumulated as the aglycone form and as hepatic metabolites. This apparent contrast can be explained: A high-carbohydrate diet stimulates an inflammatory response in rats [36] and  $\beta$ -glucuronidase released from the liver may hydrolyze quercetin conjugates to free quercetin aglycone at the site of inflammation [37]. Another possible candidate for reducing hepatic lipid levels might be metabolites involvement of glycoside parts and cleavage metabolites after absorption. These are our future study topics.

## CONCLUSION

The present study demonstrated that daily consumption of hot-water extracts from rabbiteye blueberry stems exerts decreasing tendency in hepatic lipid levels, perhaps by downregulating

malic enzyme activity. These effects were intensive in daily consumption of leaf extracts. Additionally, the active candidates existed in both extracts may be quercetin and its glycosides, although their biological evidences remain unclear. Thus, blueberry stems and leaves may be an attractive candidate functional food for reducing hepatic lipid levels and ameliorating dyslipidemia. More detailed studies of the mechanism of action should be undertaken.

**List of Abbreviations:** BLEx, hot-water extracts of blueberry leaves; BSEx, hot-water extracts of blueberry stems; CPT, carnitine palmitoyltransferase; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; HDL, high-density lipoprotein; HPLC, high-performance liquid chromatography.

**Competing Interests:** There are no conflicts of interest to declare.

**Authors' contributions:** M.S. and Y.M. designed the research. H.K. cultivated the blueberry leaves and stems. C.Y. and Y.M. prepared the blueberry leaf and stem extracts. Y.M., M.K., E.M., and H.S. conducted the research. D.Y. performed statistical analyses. Y.M., H.S., and M.S. wrote the manuscript. M.S. had primary responsibility for the final content. All authors have read and approved the final version of the manuscript.

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