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Antioxidative and antimicrobial potential of residues of camu-camu juice production

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Abstract

Total phenolic contents, antioxidant- and antimicrobial-activities of residual by-products of camu-camu fruit juice production were investigated in order to clarify the potential as functional resources for food industry. The seed and peel of camu-camu juice residue contain significantly more abundant phenols, than other tropical fruits do. The phenol content was higher in the seed extracts than in those of the peel. The radical scavenging activity, reducing power and antimicrobial activity were assayed to investigate its functional properties. The fractionated seed and peel extracts, which contained high total phenolic contents, showed potent antioxidant activity. Especially, those of 25 - 75% MeOH fractions of the seed exerted stronger antioxidant activity than ascorbic acid. Moreover, the extracts of the seed and peel showed antimicrobial activity to *S. aureus*, which revealed that the lipophilic constituents were responsible for antimicrobial activities. From these results, the seed and peel of camu-camu juice residue are shown to be promising multi-functional resources.

Key words: *Myriciaria dubia* (HBK) McVaugh, polyphenol, antioxidant, antimicrobial.

Introduction

Tropical fruits such as acelora, pineapple and passion fruit have been widely attracting attention from food industry, because of their remarkable nutritional characteristics. Many kinds of tropical fruits have been applied for functional foods in industrialized countries. Recently, the products of camu-camu fruit have been commercialized in Japan and other countries.

Camu-camu (*Myriciaria dubia* (HBK) McVaugh), belonging to genus Myrtaceae, is a bush tree native to the Amazonian rainforest [1]. Its fruit, round berries averaging 2.5 cm in diameter, is known to be rich in vitamin C, and the content of vitamin C in camu-camu fruit was reported to be higher than that of acerola fruit [2]. Recently, the constituents in camu-camu fruit were extensively studied, and several reports have been made about flavonoids [3], carotenoids [4], anthocyanins [4, 5] and volatile compounds [6].

Camu-camu has a mild characteristic flavor, but its high acid content (ascorbic acid and citric acid) induces a bitter taste and, therefore, it is mainly consumed as a juice or ingredient in prepared foods [7, 8]. During the processing of juices production, the mixture of peel and seed was obtained as agricultural and industrial wastes in a large scale. Therefore, utilization of these wastes has been recommended in the commercial and ecological point of view.

Several studies on residual sources of food production as antioxidants have been reported [9-11]. Wolf *et al*. [12] reported health-promoting constituents in the apple peel obtained from apple juice production, and these constituents may provide additional values of apple peel for application as food ingredient and functional foods [12, 13]. Oliveria *et al*. [11] also reported that the extracts obtained from residual by-products of acelora, passion fruit and pineapple juice production showed anti-oxidative activities against different reactive oxygen species.

In the present study, we evaluated the total phenol contents, anti-oxidative activity and antimicrobial activities of residual by-products of camu-camu fruit production in order to evaluate the potential as functional resources for foods.

Materials and Methods

Material and chemicals: Dried powder of two by-products of camu-camu juice production, peel and seed, were obtained from Empresa Agroindustrial del Peru S.A. (Peru). These samples were used after drying at room temperature. Gallic acid, 1,1-dipheny-2-picrylhydrazy (DPPH) and other chemicals were purchased from Kanto Chemicals (Tokyo, Japan). Purified water was obtained by a Elix EV3 (Millipore, Japan).

Extraction and fractionation: Two samples (400 g each) were extracted with 50% aqueous acetone (v/v) three times at room temperature, and the combined extracts were concentrated under reduced pressure at 40°C to dryness, and crude extracts (56.8 g from peel and 66.2 g from seed) were obtained. The crude extracts (3.0 g) were dissolved with 80% methanol (MeOH, 300 ml) and applied to a column of Dioion HP-20 (5.0 × 20.0 cm, Mitsubishi

Chemical Co. Japan). The bound materials were eluted with MeOH - H_2O solvent system by stepwise gradient, successively with MeOH : H_2O (0 : 100, 55 : 75, 50 : 50, 75 : 25 and 100 : 0, each 1,500 ml) to obtain 5 fractions. The fractions of seed and peel extracts were concentrated under reduced pressure to dryness, and Water Fr. (216 mg, 150 mg), 25 % MeOH Fr. (48 mg, 45 mg), 50 % MeOH Fr. (75 mg, 18 mg), 75% MeOH Fr. (21 mg, 54 mg) and 100 % MeOH Fr. (21 mg, 48 mg) were obtained, respectively.

Determination of total phenolic content: Total phenolic contents were determined by Folin-Ciocalteau method as described by Singleton *et al*. [14] , using gallic acid as a standard. Sample solutions were prepared by dissolving the crude extracts or the fractions in water or 25 - 100 % MeOH at the concentration of 1.0 and 5.0 mg/ml. The solutions (100 µl) were mixed with Folin-Ciocalteau reagent (200 µl) and were incubated for 30 min at room temperature. After addition of 1N NaOH (500 µl), absorbance at 750 nm was measured. The total phenolic content was expressed as mg gallic acid equivalents (GAE)/mg of samples. The assays were carried out in triplicate.

DPPH radical scavenging assay: 1,1-Dipheny-2-picrylhydrazyl (DPPH) radical scavenging activity was measured by the spectrophotometric method [15] with slight modification. A freshly prepared solution of 100 µM DPPH in MeOH was used. Sample solution (100 µl) at concentrations of 5 - 400 µg/ml and 400 ml of 100 µM Tris HCl buffer (pH 7.4) were mixed with 100 µM DPPH solution (500 µl). The mixture was shaken well and was incubated in the dark for 20 min at 30°C. The absorbance was measured spectrophotometrically at 517 nm.

The anti-oxidative activity of the sample was expressed as an inhibition of the DPPH radical formation as follows:

$$\text{Inhibition (\%)} = [(A_{517(\text{control})} - A_{517(\text{sample})}) / A_{517(\text{control})}] \times 100$$

The IC_{50} value was calculated by inhibition curve. The assays were carried out in triplicate. Gallic acid and ascorbic acid were used as positive controls.

Reducing power: The reducing power was determined by the method of Oyaizu [16] . The sample (1.0 mg/ml) was mixed with 200 mM sodium phosphate buffer (pH 6.6, 500 µl) and 1% potassium ferricyanide (500 µl), and the mixture was incubated at 50°C for 20 min. After addition of 10% trichloroacetic acid (2.5 ml, w/v), the mixture was centrifuged (3,000 rpm, 10 min). The supernatant (500 µl) was mixed with water (500 µl) and 0.1 % ferric chloride (100 µl), and then the absorbance at 700 nm was measured. The assays were carried out in triplicate. Gallic acid and ascorbic acid were used as positive controls.

Antimicrobial assay: *Staphylococcus aureus* ATCC11522, *Escherichia coli* DH5α and *Saccharomyces cerevisiae* BY4741 were used for antimicrobial tests using paper disc diffusion assay. *S. aureus* and *E. coli* were grown on nutrient broth medium and *S. cerevisiae* was grown on YPD medium for 24 h to yield a final concentration of 10^6-10^7 CFU/ml. Aliquots of 0.1 ml suspension of the microorganisms were spread on nutrient agar (*E. coli* and *S. aureus*) or YPD (*S. cerevisiae*) Agar plates, respectively. Sterile filter paper discs (Advantec, Japan, 1.6 mm in diameter) were placed on the surface of the microbial agar plates. The solution of the samples (0.1 - 5.0 mg/ml) dissolved in water or 10, 30 and 40% DMSO (30 µl) were applied on the paper discs. Kanamycin (50 µg/ml, Wako Pure Chemical Industries, Japan) and/or Aureobasidin A (5.0 µg/ml, Takara bio inc., Japan) were used as positive controls against bacterial strains and yeast strain, respectively. A paper disc mounted with the solvent was used as a negative control. The plates were incubated at 30°C for 24 h. The diameter (width) of inhibitory zone was measured after 24 h incubation.

Statistical analysis: The results of total phenolics content, DPPH radical scavenging assay and reducing power assay were expressed as means ± standard error of triplicate assay. These results were analyzed using ANOVA, followed by Tukey test for statistical comparisons among groups, with a value of $p < 0.05$ or $p < 0.01$ indicating significance. A comparison of the samples between peel and seed was done by student *t*-test.

Results and Discussion

Total phenolic contents of camu-camu juice residue: The yield of 50% acetone extracts of the seed and the peel of camu-camu juice residue was 14.2% and 13.2%, respectively. Total phenolic contents of the extracts of seed and peel and their fractions were shown in Table 1. Total phenolic content of the seed extract was 369.4 ± 9.6 mg/g, which was significantly higher ($p < 0.01$) than that of peel (203.8 ± 7.7 mg/g). Comparing these results with those of other tropical fruit residue, the total phenolic contents in the camu-camu juice residue was remarkably higher than those of other fruit juice residues, such as acerola (94.6 mg/g), pineapple (9.1 mg/g) and passion fruit (41.2 mg/g) as reported by Oliveira *et al*. [11] .

Table 1. Total yield and polyphenol contents in fractions of camu-camu juice residue.

Extract and fractions	Phenol (mg/g)	
	Seed	Peel
Extract	369.4±9.6**	203.8±7.7
Water	44.5±2.0*	70.3±2.6
25% MeOH	784.1±14.1**	329.0±13.5
50% MeOH	684.8±42.2*	470.6±3.3
75% MeOH	467.6±7.9**	344.0±5.9
100% MeOH	179.1±5.5**	112.4±5.4

$n = 3$, mean ± SEM. * $p < 0.05$, ** $p < 0.01$ Seed Fr. vs Peel Fr.

The results of the factions obtained by the chromatography on HP-20 demonstrated that the total phenolic content was the highest in 25% MeOH Fr. (784.1 ± 14.1 mg/g) of the seed extract, followed by 50% MeOH Fr. (684.8 ± 42.2 mg/g) and 75% MeOH Fr. (467.6 ± 7.9 mg/g). In case of the peel extract, total phenolic contents was the highest in 50% MeOH Fr. (470.6 ± 3.3 mg/g), and followed by 75% MeOH Fr. (344.0 ± 5.9 mg/g) and 25% MeOH Fr. (329 ± 13.5 mg/g). Total phenolic contents in Water Fr. and 100 % MeOH Fr. were lower than these fractions. The fractions of seed extract were shown to contain higher phenolic contents than those of peel, except for the Water Fr. These results suggested that the composition of phenolic constituents in the seed and peel might be similar to each other.

Antioxidant activity of camu-camu juice residue: The free radical scavenging activities of the seed and peel extracts were tested using 1,1-dipheny-2-picrylhydrazyl (DPPH) assay. As shown in

Table 2, the extracts showed DPPH radical scavenging activities, indicating that the extracts contain the constituent(s) having hydrogen donating capacity providing its antioxidant-activitiy. The IC_{50} value of the seed extract was 32.2 ± 1.1 μg/ml, while that of the peel extract was 65.6 ± 4.5 μg/ml, showing the antioxidant activity of the seed extract was significantly higher ($p < 0.01$) than that of the peel extract.

The IC_{50} value of 50% MeOH Fr. (27.8 ± 1.4 μg/ml) of the peel extract was the lowest, followed by those of 25% MeOH Fr. (36.4 ± 4.0 μg /ml) and 75% MeOH Fr. (37.4 ± 0.7 μg/ml). In case of the seed extract, the IC_{50} value of 25% MeOH Fr. (12.8 ± 0.7 μg/ml) was the lowest, followed by 50% MeOH Fr. (15.6 ± 0.9 μg/ml) and 75% MeOH Fr. (20.7 ± 0.9 μg/ml). In both of the peel and seed extracts, DPPH radical scavenging activities of 25 - 75% MeOH Fr. (no statistical significance among them) were higher than those of Water Fr. and 100% MeOH Fr. ($p < 0.01$). Those values were higher than that of ascorbic acid (21.4 ± 0.1 μg/ml), and the fraction may contain compound(s) with strong antioxidant activity.

Reducing power of the extract, fractions and standards were shown in Table 2. The reducing capacity is a significant indicator of its antioxidant potential [17]. The reducing power of the seed and peel showed similar tendency to DPPH radical scavenging activities. The seed extracts showed higher activity than those of the peel ($p < 0.01$). In more details, the 25 - 75% MeOH Frs. of both the seed and peel extracts were significantly higher than those of Water Fr. and 100 % MeOH Fr. ($p < 0.01$).

The results of the reducing power and the DPPH radical scavenging potential of the fractions of the seed and peel extracts were consistent with each other, and these results were also consistent with total phenolic contents of the fractions. Remarkable correlations were observed between the total phenolic content and the DPPH radical scavenging activity (r = 0.99), or the reducing power (r= 0.97) of fractions of the extract of seed and peel. Therefore, the phenolic constituent(s) in the seed and peel of camu-camu juice residue must be responsible for their antioxidant activities.

Antimicrobial activities of camu-camu juice residue: The antimicrobial activities of the seed and peel against *Staphylococcus aureus*, *Escherichia coli* and *Saccharomyces cerevisiae* were shown in Table 3. The extracts of the seed showed antibacterial activity against *S. aureus* at 5.0 mg/ml, giving 2.7 mm of inhibition zone, but no inhibition zone was observed at 2.0 mg/ml and lower concentrations. The antimicrobial activity of the peel extracts against *S. aureus* was shown to be stronger than that of seed extract, and 3.1 mm inhibition zone was observed.

Table 2. DPPH radical scavenging activity and reducing power of camu-camu juice residue.

Extract and fractions	IC_{50} value of DPPH ragical scavenging (μg/g)		Reducing power (abs)	
	Seed	Peel	Seed	Peel
Extract	$32.3^{a} \pm 1.1$**	$65.6^{b} \pm 2.6$	$0.31^{b} \pm 0.02$**	$0.20^{b} \pm 0.01$
Water	$219.0^{c} \pm 19.5$	$232.6^{c} \pm 16.7$	$0.01^{a} \pm 0.01$	$0.01^{a} \pm 0.01$
25% MeOH	$12.8^{a} \pm 0.7$**	$36.4^{a} \pm 2.3$	$0.85^{d} \pm 0.01$**	$0.22^{b} \pm 0.01$
50% MeOH	$15.6^{a} \pm 0.9$**	$27.8^{a} \pm 1.4$	$0.87^{d} \pm 0.01$**	$0.50^{d} \pm 0.01$
75% MeOH	$20.7^{a} \pm 0.9$**	$37.4^{a} \pm 1.0$	$0.65^{c} \pm 0.01$**	$0.44^{c} \pm 0.02$
100% MeOH	$156.4^{b} \pm 9.3$*	$203.4^{c} \pm 5.3$	$0.02^{a} \pm 0.01$	$0.02^{a} \pm 0.01$
Gallic acid	8.1 ± 0.2		1.01 ± 0.01	
Ascorbic acid	21.4 ± 0.1		0.81 ± 0.01	

$n = 3$, mean ± SEM. *: $p < 0.05$, **: $p < 0.01$ Seed Fr. vs Peel Fr.
Values in the same column with different superscripts are significantly different ($p < 0.01$).

Table 3. Antimicrobial activity of camu-camu juice residue.

Sample	Conc. (mg/ml)	Inhibition zone (mm)		
		S. aureus	*E. coli*	*S. cerevisiae*
Seed	5.0	2.7	–	–
	2.0	–	–	–
	1.0	–	–	–
	0.5	–	–	–
	0.1	–	–	–
Peel	5.0	3.1	–	–
	2.0	1.0	–	–
	1.0	0.5	–	–
	0.5	–	–	–
	0.1	–	–	–
Kanamycin	0.1	3.3		

– Not detected.

The size of the inhibition zone was dose dependent, and 0.5 mm inhibition zone was observed at 1.0 mg/ml of the extracts. The activities of the peel extracts were compatible to that of kanamycin at the concentration of 0.1 mg/ml. However, these extracts showed no antimicrobial activities against *E. coli* and *S. cerevisiae*.

Among the fractions of the peel and seed extracts, the strongest antimicrobial activity against *S. aureus* was exhibited by 100% MeOH Fr. of the seed extracts, resulting the inhibition zone as large as 3.0 mm in diameter even at the concentration of 1.0 mg/ml (Table 4). While other two fractions, 75% MeOH Fr. of the seed extracts and 100 % MeOH Fr. of the peel extracts showed substantial antimicrobial activities at the concentration of 1.0 mg/ml (Table 4).

Table 4. Antimicrobial activity of fractions of camu-camu product residue against *S. aureus*.

Fraction	Conc. (mg/ml)	Inhibition zone (mm)	
		Seed	Peel
Water	5.0	–	–
	1.0	–	–
25% MeOH	5.0	–	–
	1.0	–	–
50% MeOH	5.0	–	–
	1.0	–	–
75% MeOH	5.0	4.0	–
	1.0	1.0	–
100% MeOH	5.0	4.7	3.8
	1.0	3.0	2.0

– Not detected.

Some polyphenols have been reported to show antimicrobial activities [18], and others reported that non-polar constituents of sorghum showed antimicrobial activities against *S. aureus* [19]. Our results indicated that the fractions with high total phenolic content showed no antimicrobial activity, except for 75% MeOH Fr. of the seed extract. Therefore, in the extracts of seed and peel of camu-camu juice residue, constitute with relatively high lipophilicity must be the major component responsible for the antibacterial activities. On the other hand, for the 75% MeOH Fr. of the seed extracts, it is not excluded that phenolic constituent(s) may be responsible for the antimicrobial activities.

Conclusions

The results of this study demonstrated that the seed and peel of camu-camu juice residue were rich in phenolic constituents. The total phenolic content in

the extracts of seed of camu-camu was shown to be more than three times higher than that of acelora, and it must be the highest content among the residual sources of food production. Therefore, the seed and seed extracts of camu-camu juice residue may have the higher potential as the source of effective antioxidant applicable to food industry than these tropical fruits. It would be important to characterize phenolic constituents in the seed and peel extracts. Moreover, the extracts of the seed and peel showed antimicrobial activity to *S. aureus*, although further studies are needed to clarify the antimicrobial spectra of these extracts at the molecular levels. Taken together, the seed and peel of camu-camu juice residue is proved to be a multi-functional resource for food industry. Application of the waste by-products as utilizable resources for functional foods might be a driving force for camu-camu juice industry.

References

[1]Rodrigues, R. B., Menezes, H. C., Cabral, L. M. C., Dormier, M., Rios. G. M. and Reynes, M. 2004. Evaluation of reverse osmosis and osmotic evaporation to concentrate camu-camu juice (*Myrciaria dubia*). J. Food Eng. **63**:97-102.

[2]Määttä, K. R., Kamal-Eldin, A. and Törrönen, A. R. 2003. High performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: *Ribes* species. J. Agric. Food Chem. **51**:6736-6744.

[3]Reynertson, K. A., Wallace, A. M., Adachi, S., Gil, R. R., Yang, H., Basile, M. J., D'Armiento, J., Weinstein, I. B. and Kennelly, E. J. 2006. Bioactive depsides and anthocyanins from jaboticada (*Myrciaria cauliflora*). J. Nat. Prod. **69**:1228-1230.

[4]Zanatta, C. F. and Mercadamte, A. Z. 2007. Carotenoid composition from the Brazilian tropical fruit camu-camu (*Myrciria dubia*). Food Chem. **101**:1526-1532.

[5]Zanatta, C. F., Cuevas, E., Bobbio, F. O., Winterhalter, P. and Mercadamte, A. Z. 2005. Detemination of anthocyanins from camu-camu (*Myrciria dubia*) by HPLC-PDA, HPLC-MS and NMR. J. Agric. Food Chem. **53**:9531-9535.

[6]Pino, J. A and Quijano, C. C. E. 2008. Volatile constituents of camu-camu (*Myrciria dubia* (HBK) McVaugh) leaves. J. Essent. Oil Res. **20**:205-207.

[7]Villachica, L. H. 1997. El cultivo del camu-camu (*Myrciaria dubia* (H. B. K) McVaugh) en la Amazonia Peruana. In Tratado de Cooperacion Amazonica, Lima, Peru.

[8]Franco, M. R. B. and Janzantti, N. S. 2005. Aroma of minor tropical fruits. Flavor Fragr. J. **20**:358-371.

[9]Moure, A., Cruz, J., Franco, D., Dominguez, J., Sineiro, J. and Dominguez, H. 2000. Natural antioxidants from residual sources. Food Chem. **72**:145-171.

[10]Shui, G. and Leong, L. P. 2006. Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. Food Chem. **97**: 227-284.

[11]Oliverira, A. C., Valentim, I. B., Silva, C. A., Bechara, E. J. H., Barros, M. P., Mano, C. M. and Goulart, M. O. F. 2009. Total phenolic content and free radical scavenging activities of methanolic extract powders of tropical fruit residues. Food Chem. **115**:469-475.

[12]Wolf, K. L., Wu, X. Z. and Liu, R. H. 2003. Antioxidant activity of apple peels. J. Agric. Food Chem. **51**:609-614.

[13]Wolf, K. L. and Liu, R. H. 2003. Apple peels as a value-added food ingredient. J. Agric. Food Chem. **51**:1676-1683.

[14]Shingleton, V. L., Orthofer, R. and Lamuela-Raventós, R. S. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteau reagent. Methods Enzymol. **299**:152-178.

[15]Bolis, M. S. 1958. Antioxidants determination by the use of a stable free radical. Nature **4617**:1199-1200.

[16]Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. **44**:307-315.

[17]Chung, Y. C., Chang, C. T., Chao, W. W., Lin, C. F. and Chou, S. T. 2002. Antioxidant activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK 1. J. Agric. Food Chem. **50**:2454-2458.

[18]Pietta, P. G. 2000. Flavonoids as antioxidants. J. Nat. Prod. **63**:1035-1042.

[19]Kil, H. Y., Seong, E. S., Ghimire, B. K., Chung, I. M., Kwon, S. S., Goh, E. J., Heo, K., Kim, M. J., Lim, J. D., Lee, D. and Yu, C. Y. 2009. Antioxidant and antimicrobial activities of crude sorghum extract. Food Chem. **115**:1234-1239.