PAPER

IDENTIFICATION OF NEOCHLOROGENIC ACID AS THE PREDOMINANT ANTIOXIDANT IN *POLYGONUM CUSPIDATUM* LEAVES

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ABSTRACT

To identify the predominant antioxidant compound in *Polygonum cuspidatum* leaves, the methanol extract of fresh samples were separated by liquid–liquid partitioning, octadecylsilyl Sep-pak® cartridge and high-performance liquid chromatography. The main active compound was identified as (1*R*,3*R*,4*S*,5*R*)-3-{[(2*E*)-3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid (neochlorogenic acid) by nuclear magnetic resonance and liquid chromatography-mass spectroscopic analysis. Its content was found to be 2.31 mg/g of fresh leaves. As shown by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and superoxide anion scavenging assays, the contributions of neochlorogenic acid as an antioxidant were 16.5% and 36.5%, respectively, suggesting that neochlorogenic acid is the predominant antioxidant in *P. cuspidatum* leaves.

- Keywords: *Polygonum cuspidatum*, antioxidant, polyphenol, neochlorogenic acid -

INTRODUCTION

Polygonum cuspidatum, commonly known as Japanese knotweed, originated in East Asia and has spread widely to European and American countries where it has been listed as one of the most invasive plants. In some invaded areas it has become a severe environmental problem and governmental actions have been taken to thwart its spread (GREVSTAD *et al.*, 2013). However, the chemical and mechanical methods that have been used have not been successful in eliminating this plant, owing to its viability. Contrastingly, in other areas, *P. cuspidatum* has been used as medicine and consumed as a food. For example, in China its dried rhizomes are used in traditional Chinese medicine to treat inflammatory diseases, hepatitis, tumors, and diarrhea (CHEN *et al.*, 2013). It is also reported that the young stems of *P. cuspidatum* were consumed by native people of North America (CHEN *et al.*, 2013). In some areas of Japan, such as Kochi prefecture, the edible portions of young stems are pickled and cooked to be served as traditional dishes even today. The young leaves have also been recognized as edible (HASHIMOTO, 2003).

Over the past few decades the health-promoting effects of *P. cuspidatum* have attracted the attention of researchers and several bioactive compounds, particularly those with antioxidant activity, have been identified. Resveratrol, or *trans*-3,5,4′-trihydroxystilbene, also found in grape skins and wine, is abundant in the rhizomes of *P. cuspidatum*. Numerous health-promoting effects of resveratrol, including anticancer, anti-inflammatory, antiviral, and antifungal activities have been described (PENG *et al.*, 2013). Polydatin, a glycoside precursor to resveratrol, is also found in abundance in the rhizomes of *P. cuspidatum*. Polydatin has been linked with beneficial lipid-regulating, melanogenesis-inhibitory and hepatoprotective effects (PENG *et al.*, 2013; CHU *et al.*, 2005). Besides stilbene compounds, other antioxidants including anthraquinones, such as emodin and physcion, and flavonoids, such as catechin and quercetin, that possess health-promoting properties have also been found in the rhizomes of *P. cuspidatum* (CHEN *et al.* 2013; PENG *et al.*, 2003; CHU *et al.*, 2005).

Less research has been performed on the different parts of the plant. The rhizomes have been the most studied however the health-promoting effects of other parts of *P. cuspidatum* have not been studied. Although the stems and leaves are not as commonly used as the rhizomes, in a previous study we observed the antioxidant effect of the leaves was comparable to that of the rhizomes (KURITA *et al.*, 2014). Despite their high antioxidant capacity, only a few studies have been performed to identify antioxidant compounds in the leaves. In this study, we isolated and identified the predominant antioxidant compounds in the leaves of *P. cuspidatum*.

MATERIALS AND METHODS

Instruments

To determine 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, a TECAN CTS-R R-10 microplate reader (TECAN, Mannedorf, Switzerland) was used. High-performance liquid chromatography (HPLC) was performed with an LC-7100 pump, L-2300 column oven, and L-2420 UV VIS detector (Hitachi, Tokyo, Japan). Liquid chromatography-mass spectroscopy (LC-MS) was performed with a Waters ACQUITY UPLC system (Waters, Milford, USA) with a Cosmosil® $5C_{18}$ AR-II Column (150 × 4.6 mm i.d., particle size 5 μm, pore size 12 nm), (Nacalai Tesque Inc., Kyoto, Japan). The mobile phase of LC-MS included 20% MeOH, 1% acetic acid and 79% H_2O at a flow rate of 0.5 mL. Positive ion ESI with the capillary voltage at 3 kV was used. The source and desolvation temperatures were 150°C and 400°C, respectively, and the eluted compounds were detected at 254 nm. ^{1}H- and ^{13}C-NMR data for compound 1 were measured using a JEOL JNM-ECX500 (JEOL Resonance Inc., Tokyo, Japan) at 500 MHz. The letters (br.) s, d, t, q and m represent (broad)singlet, doublet, triplet, quartet, and multiplet, respectively, and coupling constants are expressed in Hz. Specific rotation was determined by Horiba SEPA-500 (HORIBA Ltd., Kyoto, Japan), and the UV spectrum was measured with a Pharmacia Biotech Ultraspec 3000 UV/Visible Spectrophotometer (GE Healthcare UK Ltd., Buckinghamshire, UK). For the Folin–Ciocalteu method, UVmini-1240 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) was used for measurement.

Chemicals and reagents

All reagents used were of analytical grade or better. DPPH and HPLC-grade methanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Neochlorogenic acid was obtained from Sigma Chemical Co. (St. Louis, USA), and chlorogenic acid was from MP Biomedicals, LCC (Santa Ana, USA). Phenol reagent solution for Folin–Ciocalteu assay was purchased from Nacalai Tesque Inc. (Kyoto, Japan). Superoxide dismutase (SOD) Assay Kit-WST was purchased from Dojindo Laboratories (Kumamoto, Japan).

Isolation of antioxidants from *P. cuspidatum*

Sample materials were collected in Murotoshi, Kochi prefecture, Japan, in May 2013. The roots, stems, and leaves of *P. cuspidatum* were separated and extracted in an aqueous solution containing 80% methanol (MeOH) for 24 h, and the extraction was repeated twice. The extract was filtered using Minisart® RC 15 Syringe Filters made from regenerated cellulose

with a pore size of 0.45 μm (Sartorius Stedium, Göttingen, Germany). Twenty grams equivalents of fresh leaf weight (f.w.) were evaporated until dry under reduced pressure (1110 mg) and subjected to liquid–liquid partitioning. The residue of the MeOH extract was dissolved in 27.7 mL of water, and the solution was partitioned between hexane (19.6 mL × 3) and water and then between ethyl acetate (19.6 mL × 3) and water. The hexane (53.3 mg), ethyl acetate (93.2 mg), and water (960 mg) layers were collected. The water layer (1 g f.w. equivalent) was applied to a Sep-Pak® Plus C18 cartridge (Waters, Milford, USA), containing 360 mg of octadecylsilyl (ODS), and eluted with increasing concentrations of MeOH to obtain four fractions: 0% MeOH (25.6 mg), 20% MeOH (8 mg), 40% MeOH (3.6 mg), and 100% MeOH (trace amount) fractions. The ODS 20% MeOH fraction was further separated into six fractions by reverse-phase semipreparative HPLC (Cosmosil® $5C_{18}$ AR-II column, 250 × 10 mm i.d., particle size 5 μm, pore size 12 nm, Nacalai Tesque Inc.) and eluting with 20% MeOH containing 1% acetic acid at a flow rate of 3 mL/min and detected at 254 nm.

Compound 1 was isolated from fraction 2, and its structure is *(1*R*,3*R*,4*S*,5*R*)-3-{[(2*E*)-3-(3,4-dihydroxyphenyl)-2-propenoyl]oxy}-1,4,5-trihydroxycyclohexanecarboxylic acid, neochlorogenic acid.* $[\alpha]_D^{20}$ + 12.00° (*c* = 0.02, MeOH). UV λ_{max} (MeOH) nm (ε): 238.5 (5383), 324.2 (8729). Positive-ion ESI-MS: *m/z* 355 $[M+H]^+$, 163 [M-quinic acid]$^+$. NMR spectral data were as follows. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.44 (d, 1H, *J* = 16.0 Hz, H_C-3), 7.00 (d, 1H, *J* = 2.5 Hz, H_C-2′), 6.93 (dd, 1H, *J* = 8.0, 2.5, Hz, H_C-6′), 6.75 (d, 1H, *J* = 8.0 Hz, H_C-5′), 6.21 (d, 1H, *J* = 16.0 Hz, H_C-2), 5.16 (dt, 1H, *J* = 3.5, 8.5 Hz, H_Q-3), 3.84 (dt, 1H, *J* = 7.5, 4.0 Hz, H_Q-5), 3.15 (m, 1H, H_Q-4), 2.00 (dd, 1H, *J* = 15.0, 4.0 Hz, H_Q-2′), 1.89 (dd, 1H, *J* = 15.0, 7.5Hz, H_Q-2), 1.83 (m, 2H, H_Q-6). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 176.1 (C_Q-7, s), 166.2 (C_C-1, s), 148.2 (C_C-4', s), 145.6 (C_C-3', s), 144.5 (C_C-3, d), 125.9 (C_C-1', s), 121.2 (C_C-6', d), 115.9 (C_C-5', d), 115.2 (C_C-2, d), 114.6 (C_C-2', d), 73.1 (C_Q-1, s), 71.6 (C_Q-5, d), 71.1 (C_Q-3, d), 67.2 (C_Q-4, d), 39.5 (C_Q-2, t), δ 35.2 (C_Q-6, t).

Structural determination of compound 1

The structure of compound 1 was established by independent injection and co-injection of fraction 2 with an authentic preparation in HPLC to confirm the retention times. The following conditions were used to identify the compound found in fraction 2: a Cosmosil® $5C_{18}$ AR-II column (150 × 4.6 mm i.d., particle size 5 μm, pore size 12 nm, Nacalai Tesque Inc.) was used with a mobile phase of 20% MeOH containing 1% acetic acid at a flow rate of 0.5 mL/min, and UV detection was set at 254 nm.

Determination of total phenolic content

The polyphenol content of *P. cuspidatum* leaves was determined by the Folin-Ciocalteu method as described by Singleton *et al.* with some modifications (SINGLETON *et al.*, 1999). In a test tube, 0.25 mL of sample solution, 0.1 mL of phenol reagent (1.8 N), and 0.25 mL of saturated sodium carbonate were added within 15 s and mixed. Then, 2.15 mL of water was added and mixed, followed by 1 h of incubation at room temperature. After incubation, the sample was measured at 725 nm. The measured value for the crude extract was expressed as gallic acid equivalent (GAE) per gram of the sample material.

DPPH radical scavenging activity assay

Antioxidant activity was measured using the DPPH method as described in our previous study (KURITA *et al.*, 2014). In a 96-well plate, 20 μL of sample solution, 80 μL of 0.1 M Tris-HCl buffer (pH 7.4), and 0.2 mM DPPH in ethanol solution were added and mixed. The mixture was incubated in the dark at room temperature for exactly 30 min. The radical scavenging rates of each sample and a control solution were measured at 517 nm. All experiments were performed in triplicate. The radical scavenging rate was calculated using following equation:

$$\text{Scavenging rate (\%)} = \\ = (A_{Control} - A_{Sample}) / A_{Control} \times 100$$

where $A_{Control}$ is the absorbance of the control and A_{Sample} is that of the sample. SC_{50}, which is the sample concentration at 50% of the scavenging ratio, was used to express the antioxidant capacity of each sample. To determine the contribution rate, SC_{50} was then converted to 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) equivalent (TE) antioxidant capacity, TEAC, using the following equation (SHIMAMURA *et al.*, 2014):

$$\text{TEAC (mg TE/mg)} = \text{Trolox } SC_{50} \text{ (mg/mL)} / \\ / \text{Sample } SC_{50} \text{ (mg /mL)}$$

The contribution rate of the active compound was calculated using the following equation:

Contribution rate (%) = (TEAC of active compound × concentration of active compound in *P. cuspidatum*) / (TEAC of crude extract) × 100

Superoxide anion scavenging assay

A SOD Assay Kit-WST was used to determine the superoxide scavenging activity (SOSA) of each sample. The assay was performed according to the manufacturer's procedure. The resulting 50% inhibitory concentration (IC_{50}) was used to determine the SOSA, which was further used to evaluate the contribution of compound 1 to

the total antioxidative capacity. SOSA was defined using following equation:

$$\text{SOSA (unit/g)} = [1/IC_{50}\ (\text{mg/mL})] \times 0.02\ \text{mL} \times \times 1000\ \text{mg/g}$$

The contribution rate from SOSA was calculated using the following equation:

Contribution rate (%) = (SOSA of active compound × concentration of active compound in *P. cuspidatum*) / (SOSA of crude extract) × 100

RESULTS

Antioxidant capacities of different parts of *P. cuspidatum*

All results of DPPH radical scavenging activity assays had relative standard deviations (RSD) of < 5%. Among the MeOH extracts of the different parts of *P. cuspidatum*, the strongest activity was observed in the leaves (SC_{50}: 1.24 mg f.w./mL), followed by the rhizomes (SC_{50}: 1.63 mg f.w./mL) and stems (SC_{50}: 14.1 mg f.w./mL). This is consistent with our previous study which also found that the leaves and rhizomes showed almost equivalent antioxidant capacities (KURITA *et al.* 2014).

Fractionation and antioxidant activity of the leaf extract

The fractionated leaf extracts and antioxidant activities are shown in Fig. 1. Among all the layers, the water layer showed the highest activity (SC_{50}: 1.90 mg f.w./mL), followed by the ethyl acetate layer (SC_{50}: 13.2 mg f.w./mL). The separated hexane, ethyl acetate, and water layers were further combined for measurement. The combined sample yielded an SC_{50} of 1.8 mg f.w./mL, although some activity had been lost in the separation process. The combinations of the hexane and water layers (SC_{50}: 1.93 mg f.w./mL), and the ethyl acetate and water layers (SC_{50}: 1.69 mg f.w./mL) were measured and compared with the water layer only. The results suggest that the antioxidants were present mainly in the water layer because the activities of these combinations were close to that of the water layer only.

Antioxidant activities were observed in the ODS water and in the 20% and 40% MeOH fractions (Fig. 1). The ODS 20% MeOH fraction showed the highest activity, yielding an SC_{50} of 4.3 mg f.w./mL. All the fractions combined had an SC_{50} of 1.55 mg f.w./mL. When the ODS 20% MeOH fraction was combined with the second highest fraction, the ODS water fraction (SC_{50}: 5.56 mg f.w./mL), the SC_{50} of the combined sample was 1.68 mg f.w./mL. This suggests that the ODS water and the 20% MeOH fractions account for the majority of the antioxidant capacity of the water layer.

The ODS 20% MeOH fraction was further fractionated by reversed phase semipreparative HPLC, and the chromatogram is shown in Fig. 2. The highest antioxidant capacity was seen in fraction 6 (SC_{50}: 22.4 mg f.w./mL), followed by fraction 1 (SC_{50}: 32.4 mg f.w./mL) and fraction 2 (SC_{50}: 36.1 mg f.w./mL). A further HPLC analysis with multiple-wavelength detection using a SPD-M10A photodiode array detector (Shimadzu, Kyoto, Japan) detected no other distinct peaks in fraction 1 or 6. Fractions 1 and 6 were further separated to isolate and identify the compound; however, the antioxidant activity was dispersed during the process. In contrast fraction 2, which exhibited relatively high antioxidant activity, contained a major single peak at the retention time of 10.07 min. This major peak was assigned as compound 1, which was further purified. Compound

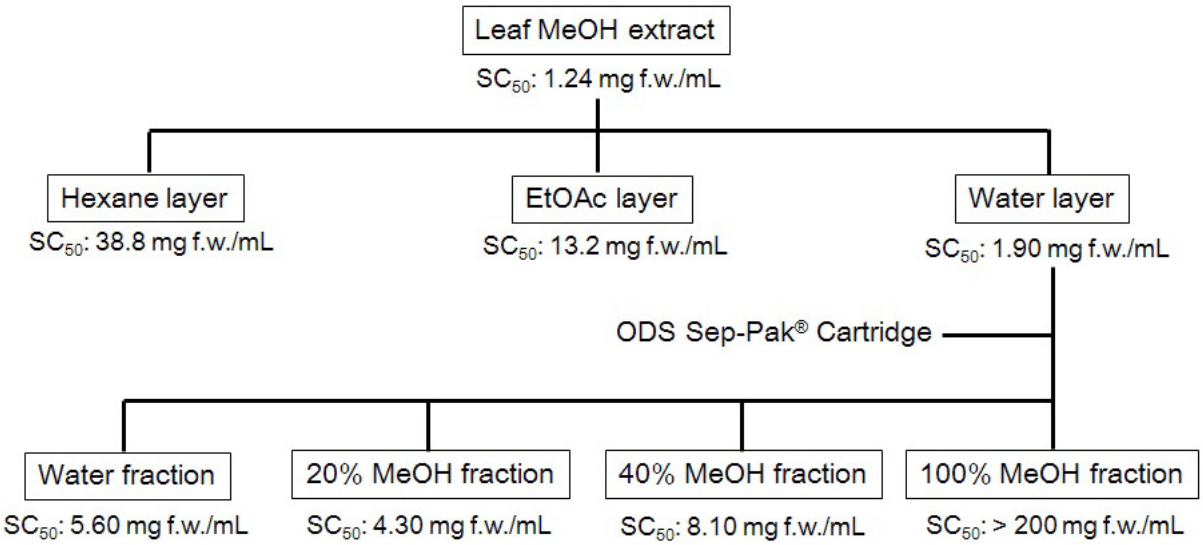

Fig. 1 - The separation process of the leaf extract and the antioxidant capacity of each fraction.

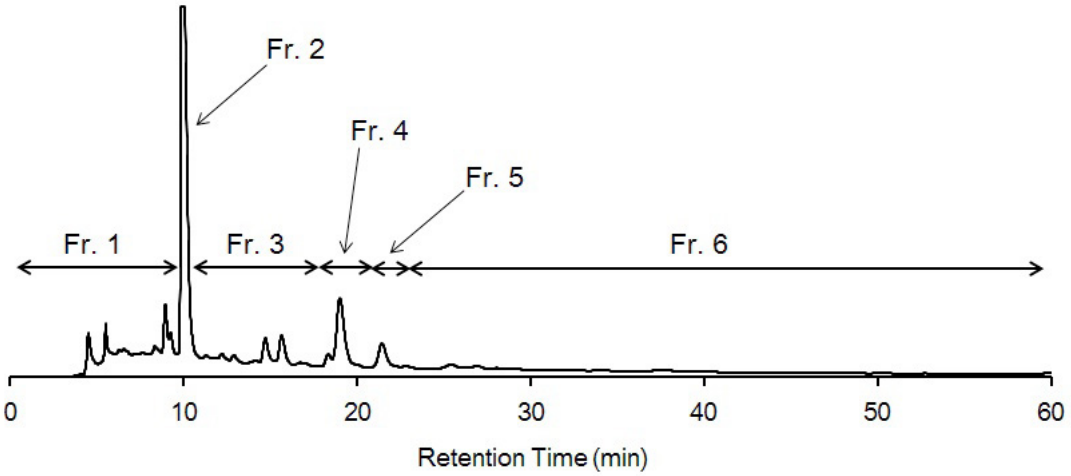

Fig. 2 - The chromatogram of ODS 20% MeOH fraction of the leaf extract. The SC_{50} of Fr. 1, 2, 3, and 6 were 32.4, 36.1, 62.4 and 22.4 mg f.w./mL, respectively. Fr. 4 and 5 was not determined since their SC_{50} were over 200 mg f.w./mL.

1 was present not only in the ODS 20% MeOH fraction but also in the ODS water fraction, which showed the second highest antioxidative activity among the ODS fractions. In the ODS water fraction, compound 1 was also found abundantly and accordingly was inferred to be the major compound in the water layer of the leaf extract.

Identification of compound 1

Compound 1 was found to have sixteen carbon atoms consisting of two methylene, eight methine, and six quaternary carbon atoms including two carbonyl groups (C_Q-7, δ 176.1 and C_C-1, δ 166.2) as a result of ^{13}C-NMR. This result was consistent with ^{1}H-NMR, which showed the presence of twelve hydrogen atoms in the spectrum. This compound contains a *trans*-form double bond (C_C-2, δ 115.2 and C_C-3, δ 144.5) signified by two hydrogen signals (δ 6.21 and δ 7.44) corresponding to a double bond where doublet with 16 Hz coupling constant. This double bond and a carbonyl group (C_C-1, δ176.1) were observed to be conjugated, consistent with these chemical shifts and the result from Heteronuclear Multiple Bond Correlation (HMBC). Because the observed six aromatic carbons in the ^{13}C-NMR spectrum corresponded to an ABX system at δ 6.75 (Hc-5′, d, J = 8 Hz), δ 6.93 (Hc-6′, dd, J = 2, 8 Hz), and δ 7.00 (Hc-2′, d, J = 2 Hz) in ^{1}H-NMR, compound 1 was found to contain a 1,2,4-trisubstituted benzene ring. For the abovementioned reasons, compound 1 was inferred to contain a caffeic acid moiety.

In the rest of the structure, three methine carbon atoms with oxygen atoms, one quaternary carbon, two methylene carbon atoms, and one carbonyl carbon were found. Two-dimensional NMR spectral data imply a six-membered ring substituted with four oxygen atoms. The methylene proton at δ 1.85 and the carbonyl carbon (C_Q-7, δ 176.1) were interrelated in HMBC spectroscopy. The other moiety was thus determined to be a quinic acid derivative.

The proton corresponding to the carbon of quinic acid (C_Q-3, δ 71.1) showed a downfield shift at 5.16 ppm, suggesting that this compound formed a caffeate ester. The molecular formula of a caffeoylquinic acid is $C_{16}H_{18}O_9$ and its molecular weight is calculated to be 354. Based on the ESI mass data (m/z 355 $[M+H]^+$), the molecular weight of compound 1 was found to be 354; therefore, compound 1 was assigned the molecular formula $C_{16}H_{18}O_9$. The data in the literature from ^{13}C-NMR and ^{1}H-NMR studies on chlorogenic acid (5-caffeoylquinic acid), cryptochlorogenic acid (4-caffeoylquinic acid) and neochlorogenic acid (3-caffeoylquinic acid) were compared with our observed data and most of the values for compound 1 matched with those of neochlorogenic acid (Fig. 3) (QIN *et al.*, 2006; HYUN *et al.*, 2010). The specific rotation value of compound 1 was also consistent with that of a neochlorogenic acid standard.

To determine the structure, fraction 2 was further analyzed using HPLC under the conditions described in *Determination of structure of compound 1*, and the result is shown in Fig. 4. The peak of compound 1 was observed at 8.1 min (Fig. 4a). The retention time of neochlorogenic acid was clearly different from that of chlorogenic acid; the neochlorogenic acid peak appeared at 8.04 min, whereas the peak of chlorogenic acid appeared at 16.67 min (Fig. 4b and 4c). Co-injection analysis showed that the peak of compound 1 was identical to that of neochlorogenic acid. Accordingly, compound 1 was assigned as neochlorogenic acid.

Quantification of neochlorogenic acid and its contribution to the whole leaf extract

The leaves of *P. cuspidatum* were freshly collected in Otoyo-cho in May 2014 to determine neochlorogenic acid content. One gram of fresh *P. cus-*

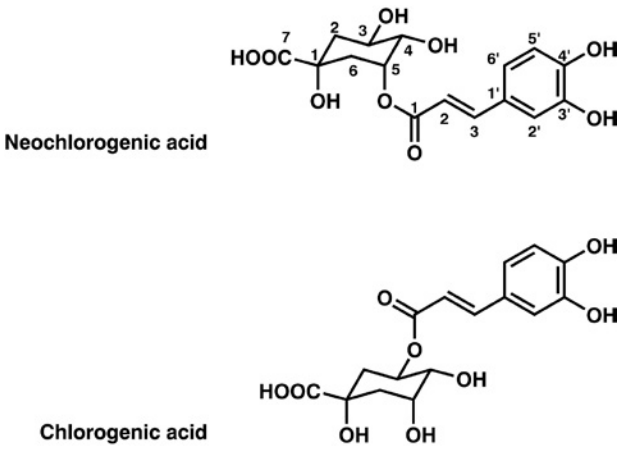

Fig. 3 - The structures of neochlorogenic acid and chlorogenic acid.

pidatum leaves contained 2.31 mg of neochlorogenic acid. By the Folin–Ciocalteu method, 17.9 mg GAE of phenolic compounds were found to be present in the fresh leaves; thus, neochlorogenic acid comprises 12.8% of the total polyphenol content. To evaluate the antioxidant capacity of neochlorogenic acid in *P. cuspidatum* two different assays, each measuring the sample's ability to quench reactive oxygen species in a different way, were performed. Antioxidant capacity cannot be evaluated by a single method because reactive oxygen species in the body do not always operate through the same mechanisms. The assays we used in this study were the DPPH radical scavenging (TEAC) and superoxide anion scavenging assays (SOSA). The TEAC values of the crude extract and neochlorogenic acid were 59.7 mg TE/g f.w. and 4.25 mg TE/mg, respectively, indicating the neochlorogenic acid contribution is 16.5%.

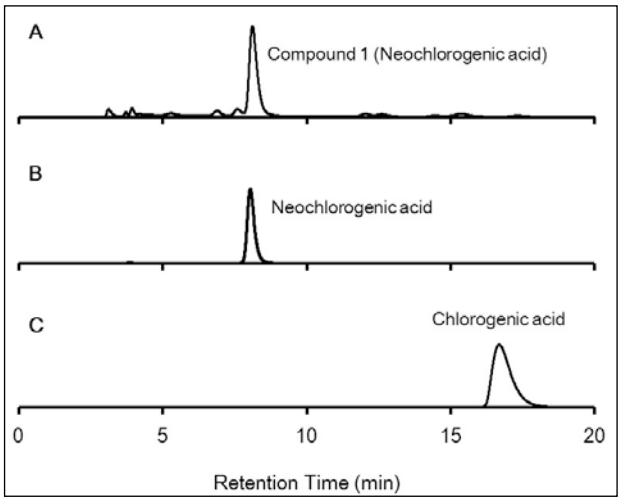

Fig. 4 - The chromatogram of the water layer of the leaf extract, neochlorogenic acid and chlorogenic acid.
In the water layer of leaf extract (A), compound 1 was observed at 8.11 min. Neochlorogenic acid (B) was found at 8.04 min whereas chlorogenic acid (C) was at 16.67 min.

However, by the superoxide anion scavenging assay, the SOSA values of the crude extract and neochlorogenic acid were 22.7 unit/g f.w. and 3.57 unit/mg, respectively, suggesting 36.5% of the antioxidant activity is by neochlorogenic acid. The disparate results may be explained by the different mechanisms of the two antioxidant activities (SHIMAMURA *et al.*, 2007). In the DPPH method, free radical scavenging activity is achieved by single electron transfer, and the assay simply measures the rate of free radical quenching. The superoxide anion scavenging assay, however, measures the sample's ability to scavenge superoxide anions produced by xanthine oxidase, thus evaluating the SOD-like activity of the sample. The superoxide anion further reduces 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium (WST-1) to produce formazan, which is detectable at a wavelength of 450 nm. Thus, the superoxide anion scavenging assay involves competition by the sample antioxidants with WST-1 in addition to the enzymatic reactions of xanthine oxidase. Taken together our results indicate that neochlorogenic acid in *P. cuspidatum* contributes a large part of its antioxidant activity, particularly as a superoxide anion scavenger.

In a study by Kirino *et al.* (2012) chlorogenic acid was reported as one of the major polyphenols in the leaves of *P. cuspidatum* (KIRINO *et al.*, 2012). The amount of chlorogenic acid was reported to be 0.36 mg/g of fresh leaves, which is only 1/6th of the neochlorogenic acid content observed in this study. In the chromatogram in Fig. 4, chlorogenic acid appeared to be a small peak in the water layer of the leaf extract. However, according to the data from Kirino *et al.*, the peak of chlorogenic acid was much more distinct than in our study. The contents of such antioxidants in *P. cuspidatum* may differ depending on its origin and harvest season, as we mentioned in a previous report (KURITA *et al.*, 2014). Stress factors such as sunlight and insects can influence antioxidant production levels as well.

Comparison of neochlorogenic acid contents in other food sources and its possible effects on human health

To the best of our knowledge, this is the first study to report the presence of neochlorogenic acid in *P. cuspidatum* leaves. Neochlorogenic acid is also found in Rosaceae fruits such as plums, cherries, and apples and Brassica vegetables such as broccoli and kale (BALLISTRERI *et al.*, 2013; KIM *et al.*, 2003; KAULMANN *et al.*, 2014). Among different kinds of sweet cherries, its content varied between 6.27–71.5 mg/100 g f.w. (BALLISTRERI *et al.*, 2013). Plums contain even higher amounts of up to 179 mg/100 g f.w., unsurprisingly neochlorogenic acid has been recognized as the predominant polyphenol in plums (KIM *et al.*, 2003). Brassica vegetables are also rich in the compound. Green vegetables such

as kale, broccoli, and Brussels sprouts contain 7.06, 5.61, and 4.59 mg/100 g f.w., respectively, of neochlorogenic acid (KAULMANN *et al.*, 2014). In comparison with these neochlorogenic-rich fruits and vegetables, the content was much higher in the leaves of *P. cuspidatum*, which yielded 231 mg of neochlorogenic acid per 100 g of fresh material. Our study suggests that the leaves of *P. cuspidatum* are a rich source of neochlorogenic acid.

Besides its antioxidant activity, neochlorogenic acid has been shown to exert health-promoting effects. As an antitumor agent, neochlorogenic acid has been found to suppress the growth of estrogen-independent MDA-MB-435 breast cancer cells (NORATTO *et al.*, 2009). This suppressive effect is selective for cancer cells and is more pronounced than that of chlorogenic acid. The compound has also been investigated in a weight-control study (SHIMODA *et al.*, 2006). In the study performed by Shimoda *et al.* (2006), experimental mice were fed a diet containing neochlorogenic acid (0.028% and 0.055%, respectively) extracted from green coffee beans for 6 days. The hepatic carnitine palmitoyltransferase activity of the experimental mice increased, indicating they had improved fat metabolism. These studies suggest that neochlorogenic acid could play a role in preventing chronic diseases and preserving healthy body weight when consumed in the diet. As a natural source of neochlorogenic acid, the leaves of *P. cuspidatum* may be used to improve human health in modern society.

CONCLUSIONS

For their medicinal effects the antioxidants in *P. cuspidatum* have been of interest to researchers, but other than the rhizomes the plant has not been extensively studied. The leaves possess high antioxidant activity and can be consumed in the diet as they currently are in Japan. Given the reports of health-promoting effects of neochlorogenic acid, our result that neochlorogenic acid is a main antioxidant in the leaves of *P. cuspidatum* may increase the utility of this hardy and prolific plant.

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