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Inhibition of angiotensin I-converting enzyme (ACE-I) by aqueous extracts prepared from edible and non-edible parts of lotus root

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Abstract

Angiotensin I-converting enzyme (ACE) is known to catalyze the conversion of angiotensin-I to angiotensin-II, which is the biologically active peptide elevating blood pressure, thereby being implicated in the pathogenic process of hypertension. Therefore, ACE inhibitory substances are expected to reduce the blood pressure in hypertensive patients. Then, the aqueous extracts were prepared from the edible and non-edible joint parts of lotus root (a rhizome of *Nelumbo nucifera*), and the effects of these extracts on ACE activity were examined *in vitro* to search for potentially effective substances against hypertension. The extracts of both edible and joint parts caused their inhibitory effects on ACE activity, similar in their properties and different in their potencies. Thus, the aqueous extracts prepared from lotus root are suggested to contain novel substances inhibiting ACE activity, thereby reducing blood pressure by inhibiting the production of active hypertensive peptide.

Keywords: Angiotensin I-converting enzyme (ACE); Hypertensive, Blood pressure; Lotus root extracts; Polyphenolic compounds

Introduction

Angiotensin I-converting enzyme (ACE) is generally considered to be implicated in the pathogenic process of hypertension through the conversion of angiotensin I to angiotensin II, which is known as a biologically active peptide inducing the elevation of blood pressure. Therefore, the inhibition of this enzyme is considered to reduce blood pressure, thereby improving the physical conditions of hypertensive patients. Based on this concept, much work has previously been done to find out potentially active substances to cause the antihypertensive effect, and a variety of natural materials, such as plant foods including wheat, rice, peas,

corn, fruits and vegetables, have been evaluated by determining their inhibitory potencies on ACE activity *in vitro*. Then, many peptides derived from dietary sources have recently been reported to inhibit ACE activity, thus proposing their potential effectiveness for the treatment of hypertension (Marti'nez-Maqueda, et al., 2012). As the specific examples, the active peptides inhibiting ACE activity have been identified in the extracts prepared from cowpea and Korean rice wine (Kang, et al., 2012; Segura Campos, et al., 2010). Also, the synthetic peptides containing selenocysteine and cysteine residues have been shown to inhibit ACE activity *in vitro* (Bhuyan and Mugesh, 2012). On the other hand, many natural products derived from fruits, nuts, vegetables and other plants have been shown to contain considerable amounts of polyphenolic compounds, which may contribute to their free-radical scavenging and antioxidant activities (Johnson, et al., 2011; McDougall, et al., 2005; McDougall and Stewart, 2005; Rubilar, et al., 2011). Furthermore, polyphenolic compounds contained in plant-derived natural materials have also been suggested to be responsible for their ACE inhibitory activities (Ahmed, et al., 2010; Dong, et al., 2011; Liu, et al., 2010; Maejima, et al., 2011).

Lotus root (a rhizome of *Nelumbo nucifera*) is widely used not only as foodstuffs but also as a medicinal plant, which can be expected to have a variety of pharmacological activities, such as hypolipemic, antipyretic, antiinflammatory, antiviral and antioxidative effects, thereby being traditionally applied as a folk medicine to the treatment of diarrhea, gastritis, insomnia and nervous prostration in the countries of East Asia (Jung, et al., 2003; Kuo, et al., 2005; la Cour, et al., 1995; Liu, et al., 2007; Sinha, et al., 2000). Recently, lotus root has been analyzed, and shown to contain many phytochemicals including isoliensinine, kaempferol and procyanidins as the major active components (Kim, et al., 2007; Ling, et al., 2005). Furthermore, lotus root has also been reported to cause hypoglycemic, antidiarrheal and immunomodulatory effects (Mukherjee, et al., 2010; Mukherjee, et al., 1997; Talukder and Nessa, 1998), and suggested to have a potential ability to improve the functions of learning and memory as well (Yang, et al., 2008). Based on these previous findings, we focused on phytochemicals contained in lotus root, particularly in the joint part, which is the non-edible part connecting the edible parts each other and usually dumped as an agricultural waste, and then tried to assess the biological activities of extractable phytochemicals from lotus root using the *in vitro* experimental systems. In a series of our current studies, the aqueous extracts were prepared from the edible and joint parts of lotus root, and the biological activities of these extracts were examined to use lotus root effectively as a potential source of bioactive substances. Consequently, we have previously shown that the aqueous extracts prepared from the joint part of lotus root can inhibit both α-amylase and α-glucosidase activities *in vitro,* thus proposing the possibility that the extracts of lotus root may have potential biological activities to prevent the postprandial elevation of blood glucose levels in diabetic patients (Nishibori, et al., 2012).

Among various phytochemicals contained in lotus root, it seems possible to assume that some of them, or at least one of them, may have a potential effect to improve the conditions of patients with hypertension. Then, the aqueous extracts were prepared from the edible and joint parts of lotus root, and the effects of these extracts on ACE activity were examined to evaluate a potential ability of lotus root to ameliorate the hypertensive conditions. Consequently, the extracts of both edible and joint parts were shown to inhibit ACE activity in a similar manner, but the inhibitory potencies of these extracts were different, due to the difference in the contents of phytochemicals between these extracts. Therefore, the present study is

possibly considered to provide evidence for suggesting that the aqueous extracts prepared from the edible and joint parts of lotus root may be able to effectively reduce the blood pressure in the hypertensive patients, and moreover seems to propose polyphenolic compounds as one of the hypothetical candidates for a putative hypotensive factor contained in lotus root.

Materials and Methods

Chemicals

Rabbit lung-derived angiotensin I-converting enzyme was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hippuryl-L-histidyl-L-leucine (HHL) tetrahydrate was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals used were commercially available reagent grade.

Preparation of lotus root extracts

Lotus root (a rhizome of *Nelumbo nucifera*) was kindly donated by the Naruto Agricultural Cooperative in Tokushima (Naruto, Japan), and the aqueous extracts were prepared according to the methods reported previously (Nishibori, et al., 2012). Briefly, the edible and joint parts of lotus root were sliced in approximately 1 cm thickness, and dehydrated by exposing to wind stream for several weeks, and then ground into coarse powder. The powder was soaked in distilled water in the ratio of 4 g to 100 ml, and kept it at 4^{o}C for 1 h. Then, the suspension was filtered through a Whatman No. 1 filter paper, and the obtained filtrate was centrifuged at 6,000 x g for 20 min to remove the insoluble materials. The supernatant fraction was further centrifuged at 12,000 x g for 20 min to clarify the solution. The obtained extracts were filtered through a 0.2 μm syringe-top disk filter, and stored in small aliquots at -20^{o}C until use.

Determination of ACE activity

The enzyme activity was determined as described previously (Mallikarjun-Gouda, et al., 2006). Briefly, the reaction mixture containing 0.1 M Borate buffer (pH 8.3), 1 M NaCl, 1 mM HHL, 0.5 mU of the enzyme and 100 μl of the test samples in 150 μl of total volume was made up on ice, and then incubated at 37^{o}C for 60 min. The reaction was terminated by adding 10 μl of 5 M HCl, and the mixture was kept on ice for 30 min or longer, then filtered through a syringe-top filter (0.45 μm-pore size). The amount of hippuric acid formed enzymatically during the reaction period was determined using a HPLC system as follows.

The enzyme activity was determined by measuring the enzymatic formation of hippuric acid from HHL using a reverse-phase HPLC system with a UV-VIS detector. Briefly, hippuric acid in 20 μl of the mixture was separated on a CAPCELL PAK C18 MG2 column (2 x 150 mm) with a mobile phase containing 20 % methanol and 0.1 % TFA for 15 min at a flow rate of 0.1 ml/min, and detected by measuring the absorbance at 228 nm. The column was refreshed by washing with 100 % methanol-0.1 % TFA in the intervals of assays.

Determination of polyphenolic compounds

Total phenolic compounds contained in the extracts were determined using Folin-Ciocalteu's phenol reagent according to the method described previously (Chen, et al., 2007). The sample solution (100 μl) containing different amounts of the ext-ract was mixed with 400 μl of diluted Folin-Ciocalteu reagent (diluted with water at 1 : 10), and then mixed with 500 μl of 5% (w/v) sodium carbonate solution. The assay mixture was kept for 20 min at room temperature, and the absorbance was measured at 765 nm. Gallic acid was used as an authentic sample to confirm the reliability of the method.

Statistical analysis

Results were presented as the mean ± SEM, and the statistical analyses were carried out using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The difference between two values with $p < 0.05$ was regarded as indicating a statistically significant.

Results and discussion

The inhibition of ACE activity is generally recognized to result in the reduction of blood pressure, thereby being considered to be one of the effective measures to improve the conditions of patients with hypertension. In the present study, the aqueous extracts were prepared from different parts of lotus root, the edible and joint parts, and the effects of these extracts on ACE activity were then examined. As shown in Fig. 1, both edible and joint part extracts caused the inhibition of ACE activity in a concentration-dependent manner, and the inhibitory effect of the joint part extract (Fig. 1A) was shown to be approximately 5-times as potent as that of the edible part extract (Fig. 1B). Furthermore, to characterize the inhibitory effects of these extracts on ACE activity, the kinetic properties of the enzyme inhibition were

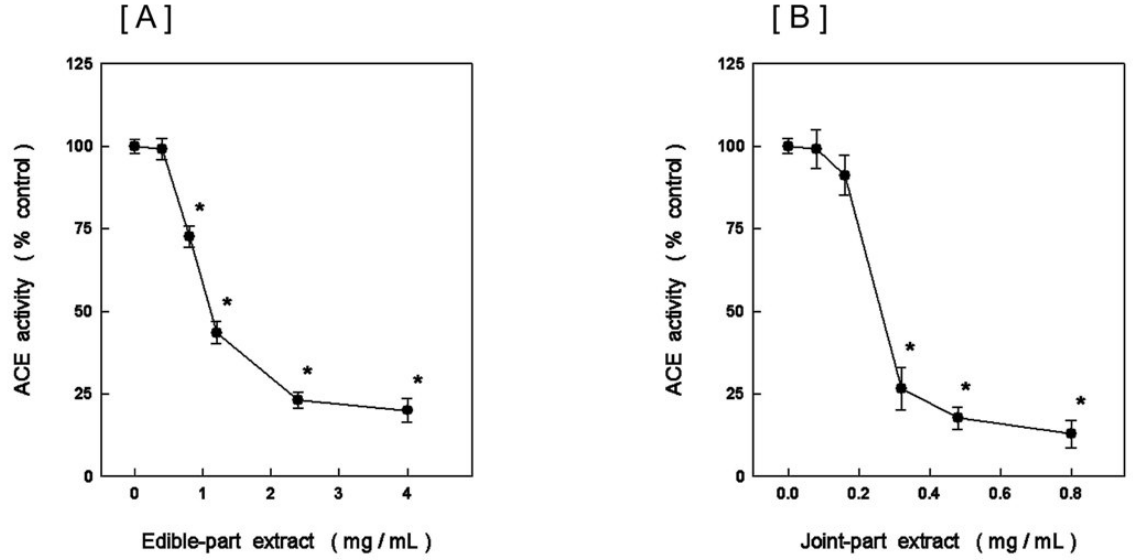

Figure 1. Inhibitory effects of lotus root extracts on ACE activity. The enzyme activity in the reaction mixture containing various concentrations of the edible part extract [A] or the joint part extract [B] was determined as described in the text, and the results were expressed as a percent of the control. Values are the mean ± SEM (*$p < 0.01$ *vs*. no extract, n = 6).

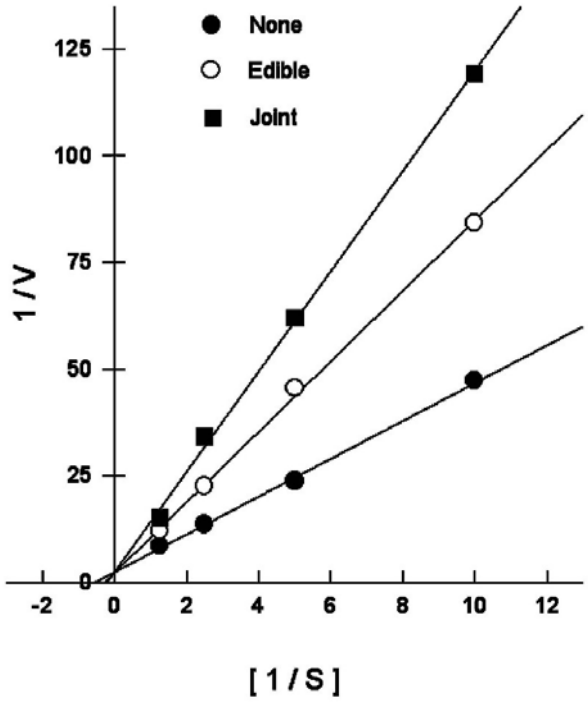

Figure 2. Lineweaver-Burk analyses of inhibitory effects of lotus root extracts on ACE activity. The extracts were added to the assay mixture containing different concentrations of the substrate, and the enzyme activity was determined as described in the text. The enzyme kinetics was graphically represented as the double-reciprocal plot.

analyzed using a Lineweaver-Burk plot, and the extracts of both edible and joint parts were shown to similarly cause the competitive inhibition of ACE activity under the assay conditions used here (Fig. 2). In preliminary study, the amounts of polyphenolic compounds contained in the extracts of lotus root were determined, and the contents of polyphenolic compounds in the joint part extract were estimated to be several times higher than those in the edible part extract. Therefore, it seems possible to speculate that the difference in the potencies of the inhibitory effects between the edible and joint part extracts may be associated with the difference in the contents of polyphenolic compounds in these extracts, but this speculation is obviously necessary to be further confirmed.

In general, many substances derived from a variety of foods, plants and other natural sources have previously been reported to cause the inhibitory effects on ACE activity, and most of them have been shown to inhibit this enzyme in a competitive manner. Therefore, it seems meaningful to characterize the properties of putative inhibitory substances contained in the extract. First, the aqueous extracts of both edible and joint parts were subjected to the dialysis for overnight, and the inhibitory effects of these dialyzed extracts on ACE activity were then examined to estimate the molecular size of the inhibitory substances contained in the extracts. Consequently, the inhibitory effects of these extracts were markedly reduced by dialyzing the extract against distilled water for overnight (Fig. 3). Therefore, it seems possible to predict that potential ACE inhibitory substances contained in these extracts may be relatively small molecule, thereby being mostly removed during the dialysis.

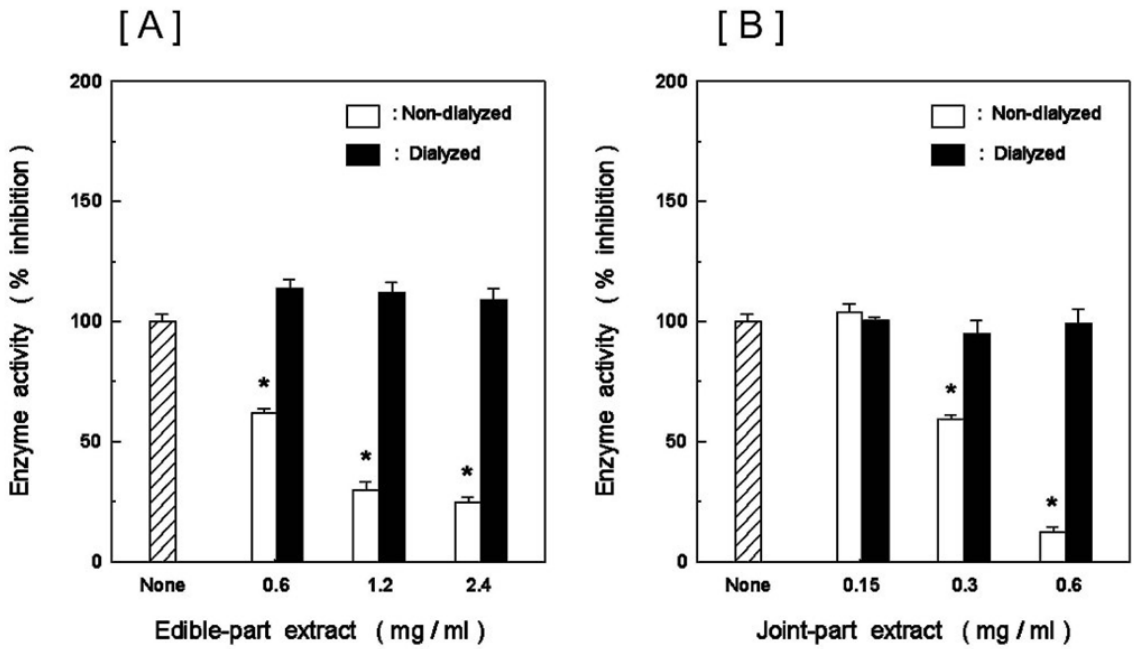

Figure 3. Inhibitory effects of dialyzed lotus root extracts on ACE activity. The extracts were dialyzed against distilled water (50-volume) at 4oC for overnight (exchanged 3-times), and the inhibitory effect of dialyzed edible part extract [A] or dialyzed joint part extract [B] on ACE activity was examined as described in the text. Results were expressed as a percent of the control. Values are the mean ± SEM (*$p < 0.01$ *vs*. no extract, n = 6).

To further elucidate the properties of potential inhibitory substances contained in lotus root, the extracts were incubated at 95°C for different periods, and the inhibitory effects of the heat-treated extracts on ACE activity were examined. As shown in Fig. 4, the heat expos-

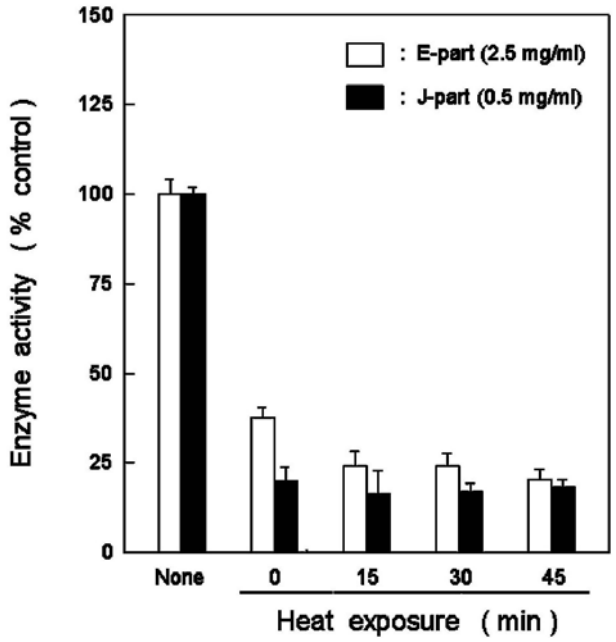

Fig. 4. Effects of heat-treated lotus root extracts on ACE activity. The extracts were heated at 95°C for different periods, and the direct effects of heat-treated edible part extract [A] and heat-treated joint part extract [B] on the enzyme activities were then determined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (*$p < 0.01$ *vs*. non-treated, n = 6).

ure of both edible and joint part extracts failed to cause any significant change in the inhibitory effects of the extracts on ACE activity, and the inhibitory potencies of these extracts was not altered by incubating them at 95°C for 45 min. Therefore, it seems conceivable that ACE inhibitory substances contained in the lotus root extracts may be thermostable, thereby maintaining their inhibitory potencies even after subjecting them to the drastic heat treatment. However, although potential thermostable substances contained in the extracts were shown to contribute to their inhibitory effects on the enzyme activity observed here, the properties of these inhibitory substances still remained entirely unidentified.

Polyphenolic compounds derived from various plants and foods have previously been shown to have the antioxidant and radical scavenging activities, and also suggested to cause their inhibitory effects on ACE activity, thereby effectively improving the conditions of patients with hypertension as a consequence of reducing the blood pressure (Actis-Goretta, et al., 2006; Ahmed, et al., 2010; Dong, et al., 2011; Liu, et al., 2010; Liu, et al., 2003). In previous study, the aqueous extracts of lotus root have been found to have both antioxidant and radical scavenging activities, and also shown to contain considerable amounts of polyphenolic compounds (Sagara, et al., 2012). Therefore, it seems reasonable to assume that polyphenolic compounds may be the most likely candidate to contribute to the inhibition of ACE activity observed here. Because the inhibitory effects of the extracts were shown to be thermostable, and therefore potentially active substances contained in the extracts might be resistant to heat (Fig. 4), it seemed worth testing whether polyphenolic compounds contained in these lotus root extracts might be resistant to the heat exposure, and therefore their concentrations in the extracts might also be unchanged after the heat treatment under the conditions in which the inhibitory effects of the extracts on the enzyme activity were unchanged. As shown in Fig. 5, the heat treatment of the extracts failed to cause any notable alteration in the contents of pol-

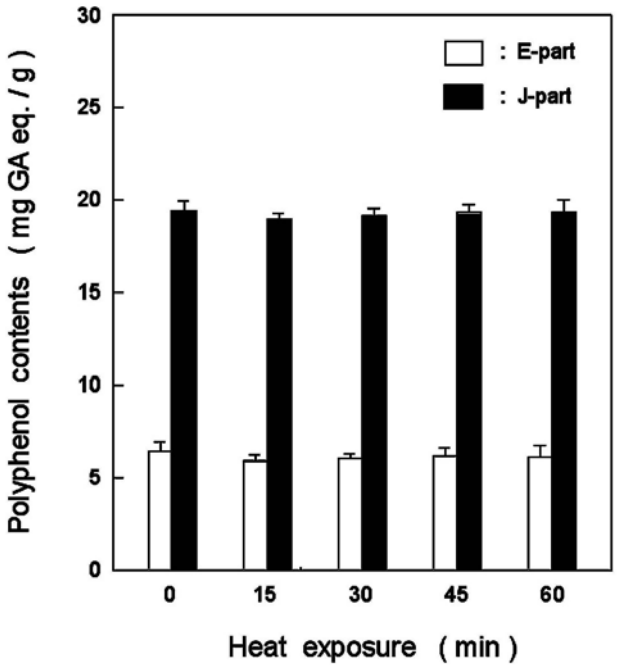

Figure 5. Effects of heat-treatment on polyphenolic compound contents in lotus root extracts. The extracts were heated at 95°C for different periods, and the amounts of polyphenolic compounds in heat-treated edible part extract (E-part) and heat-treated joint part extract (J-part) were then determined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (n = 6).

yphenolic compounds after incubating them at 95°C for 60 min. Therefore, it seems possible to speculate that polyphenolic compounds may be the most possible substances responsible for their inhibitory effects on ACE activity in vitro.

In conclusion, the active substances inhibiting ACE activity have been found in a variety of natural materials, such as fruits, vegetables and other plants, thereby suggesting the potential activities of these materials to be beneficial in the improvement of hypertensive conditions. Particularly, these natural materials have been reported to contain considerable amounts of polyphenolic compounds, which may be responsible for the inhibitory effects of these materials on ACE activity as well as their radical scavenging and antioxidant activities. On the other hand, the lotus root extracts have previously been reported to contain considerable amounts of polyphenolic compounds, and also suggested that these polyphenolic compounds may be responsible for their radical scavenging and antioxidant activities (Sagara, et al., 2012). Furthermore, the aqueous extract prepared from the joint part of lotus root has been shown to inhibit α-amylase and α-glucosidase activities *in vitro*, thus proposing its potential effect to prevent the postprandial elevation of blood glucose levels in diabetic patients (Nishibori, et al., 2012). On the other hand, the present study provides evidence for suggesting that the aqueous extracts of lotus root may be able to effectively reduce the blood pressure probably through the inhibition of ACE activity, which may be attributed to polyphenolic compounds contained in the extracts. Thus, lotus root is clearly shown to be not only an excellent low calorie foodstuff but also one of the prominent medicinal plants, but the chemical properties of the lotus root extracts should be more precisely investigated to identify the potential bioactive substances contained in this plant. Until today, the extensive studies on the potentially active substances inhibiting ACE activity have been carried out, and considerable evidence for suggesting various natural materials including foodstuffs as the effective enzyme inhibitors has been summarized. Practically, various polyphenolic compounds have been suggested as the active inhibitory substances (Actis-Goretta, et al., 2006; Dong, et al., 2011), and oligopeptides have been proposed as the useful inhibitory substances contained in these natural materials (Bhuyan and Mugesh, 2012; De Leo, et al., 2009; Geleijnse and Engberink, 2010; Guang and Phillips, 2009; Hernandez-Ledesma, et al., 2011; Ryan, et al., 2011). Based on these previous studies, two groups of compounds, polyphenols and oligopeptides, are considered to be most promising as the potential ACE inhibitors contained in the natural materials. On the other hand, nicotianamine, a nonprotein amino acid contained in higher plants, has recently been shown to inhibit ACE activity *in vitro* and *in vivo*, and proposed as a potential candidate for the plant-derived antihypertensive substance (Shimizu, et al., 1999; Hayashi and Kimoto, 2007; Hayashi and Kimoto, 2010). Therefore, the present study seems to propose polyphenolic compounds as the active substances contained in the lotus root extracts, it seems still necessary to more precisely analyze the extracts to identify the true active component contained in lotus root.

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Conflict of Interest statement

There is no conflict of interest associated with the authors of this paper, and the fund sponsors did not cause any inappropriate influence on this work.

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