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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Study on the Functionality of *Polygonum Cuspidatum*
（イタドリの機能性に関する研究）

学位論文要約：
Dissertation Summary

Polygonum cuspidatum, commonly known as Japanese knotweed, is a rhizomatous perennial geophyte of family Polygonaceae and is originated in East Asia including Japan, Korea and China (Fig. 1) [1]. *P. cuspidatum* has distributed worldwide, and it is considered as one of the most invasive species [3]. Even though *P. cuspidatum* is viable and invasive, this plant is widely utilized by people in the East Asian countries. Its rhizome, Hu Zhang, is a traditional Chinese medicine and used to treat various illnesses which include infectious diseases as well as lifestyle-related diseases [7, 8]. Numbers of bioactive compounds have been identified to explain such effects. One of the notable compounds found in this plant is resveratrol (*trans*-3,5,4'-trihydroxystilbene) (**1**) (Fig. 2). Resveratrol with its various health promoting effects have become popular among people in modern society, and *P. cuspidatum* rhizomes which abundantly contain resveratrol are utilized as a source for resveratrol supplements [19]. On the other hand, the above ground parts such as stems and leave are recognized as food ingredients in Japan, especially in Kochi where the stems are traditionally pickled and cooked as a familiar food. Although its rhizomes have been well-researched for their effectiveness, the above ground, edible parts have rarely been the subject of study, so that there are few studies to evaluate the functionality of those parts of *P. cuspidatum*. In this study, the functionality of these edible parts of *P. cuspidatum* were investigated and the bioactive compounds related to such effects were elucidated.

Fig. 1. *Polygonum cuspidatum*

Quantification of resveratrol contents

To evaluate the functionality of the edible parts, the resveratrol content in each part of *P. cuspidatum* was first quantified. The fresh samples were collected from three different locations in Kochi prefecture (Shimanto, Kagami, and Muroto) in each season, and regional and seasonal differences in contents were observed. *P. cuspidatum* was separated into 7 different parts, and the extracts were prepared with 80% MeOH extraction, liquid-liquid partition, and octadecylsilane (ODS) Sep-pak® cartridge. High performance liquid chromatography (HPLC) analysis was performed to see the amount of free resveratrol and total resveratrol which was obtained by hydrolysis using β-glucosidase. As a result of the study, the edible part was found to contain small amount of

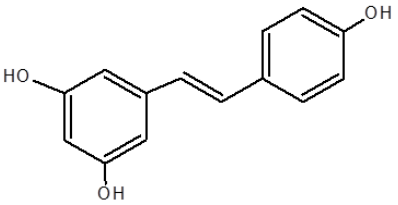

Fig. 2. Structure of trans-resveratrol (**1**)

resveratrol (Table 1). In the edible young stem collected in spring, a trace amount of resveratrol was detected (~0.40 μg/g f.w.eq.). The leaf part contained resveratrol up to 7.35 μg/g f.w.eq.. The underground parts, tuber and rhizome, contained significant amount of resveratrol (~5260 μg/g f.w.eq.). The content of resveratrol changed by seasons and the tendency was different depending on the parts and the grown place. The above-ground parts showed increased amount in fall whereas the subterranean parts tended to increase in spring. Among three different regions, Muroto had a different tendency compared with other regions, probably due to difference in its weather and climate. This result suggested that the environmental factors greatly affect to the content of resveratrol as they do for other polyphenols. The antioxidant capacity of resveratrol and its contribution were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay and superoxide anion scavenging activity assay. As an antioxidant, resveratrol contributed very little to the above-ground parts (< 0.01%). Even in the tuber and rhizome which contained significant amount of resveratrol, its contribution was found to be small (1.9% and 4.0%, respectively), indicating the presence of other antioxidants which are greatly contributing to each part of *P. cuspidatum*.

Table 1. Content of free *trans*-resveratrol and *trans*-resveratrol glycoside in each part of *P. cuspidatum*

		Content [μg/g f.w.eq.]							
		Spring		Summer		Fall		Winter	
		free	glycoside	free	glycoside	free	glycoside	free	glycoside
Shimanto									
Stem	Whole		-	0.36	0.19	0.48	tr		-
	Lower	tr	0.11		-		-		-
	Middle	tr	0.17		-		-		-
	Upper	tr	0.26		-		-		-
	Skin	0.18	0.10		-		-		-
Leaf		2.37	4.98	0.43	1.46	1.04	0.05		-
Subterranean	Tuber	744	2300	147	800	211	3036	360	2162
	Rhizome	1230	2690	61.5	1094	92.5	2036	97.8	780
Kagami									
Stem	Whole		-	tr	0.44	0.12	tr		-
	Lower	tr	0.25		-		-		-
	Middle	tr	tr		-		-		-
	Upper	tr	0.40		-		-		-
	Skin	0.21	0.24		-		-		-
Leaf		0.23	3.33	1.18	0.87	1.40	tr		-
Subterranean	Tuber	21.0	284	714	55.9	181	1420	53.7	937
	Rhizome	2120	3140	1010	84.1	302	1940	91.7	1450
Muroto									
Stem	Whole		-	tr	0.23	0.15	0.48		-
	Lower	tr	0.34		-		-		-
	Middle	tr	0.19		-		-		-
	Upper	tr	0.39		-		-		-
	Skin	tr	0.33		-		-		-
Leaf		1.95	0.42	2.24	3.65	2.79	2.44		-
Subterranean	Tuber		-	75.4	1230	39.2	1600	67.3	987
	Rhizome		-	177	2200	109	590	248	2270

Note: "-" indicates not determined. "Whole" indicates the combination of lower, middle, upper and skin parts. "tr" indicates the content less than 0.1 μg/g f.w.eq.

Antioxidant Activity of *P. cuspidatum*

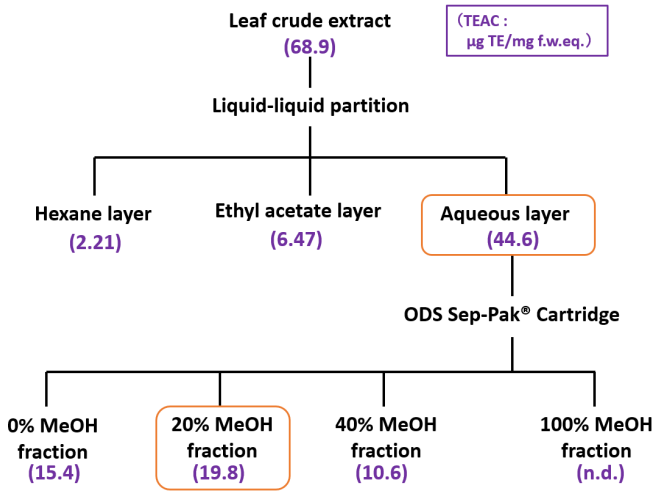

Fig. 3. Fractionation of leaf extract and TEAC of each fraction

Antioxidant capacity of the rhizome, stem and leaf of the plant was measured using DPPH radical scavenging activity assay, and the leaf showed the highest activity among three parts. Even well-known antioxidant, resveratrol, contributed less than 0.01% in the leaf; therefore, the edible leaf part was further fractionated in order to find out its predominant antioxidant. The predominant antioxidant was present in the ODS 20% MeOH fraction of the aqueous layer (Fig. 3, 4). The antioxidant was determined to be 3-caffeoylquinic acid (neochlorogenic acid) (**2**) which consisted of a quinic acid and caffeic acid ester as a result of nuclear magnetic resonance (NMR) and liquid chromatography-mass spectroscopy (LC-MS) analysis. In 1 g of the flesh *P. cuspidatum* leaves, 2.31 mg of neochlorogenic acid was contained which accounted for 12.8% of the total phenolic content of the leaves measured by Folin-Ciocalteu method. The antioxidant contribution was found to be 16.5% of trolox-equivalent (TE) antioxidant capacity (TEAC) and 36.5% of superoxide anion scavenging activity (SOSA) as examined by DPPH radical scavenging assay and superoxide anion scavenging assay, respectively. This result indicated that neochlorogenic acid works for a large part of SOSA among all other existing antioxidants. Neochlorogenic acid is a well functional compound, and *P. cuspidatum* leaves may be a good source for this compound.

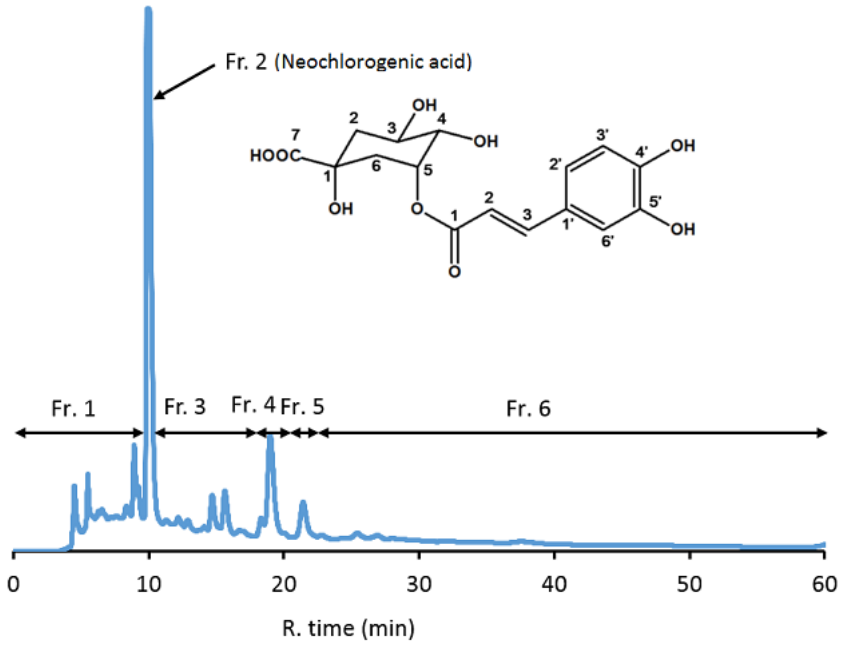

Fig. 4. HPLC profile for 20% MeOH fraction of aqueous layer of leaf extract

Tyrosinase Inhibitory Activity of *P. cuspidatum*

Tyrosinase inhibitory activity was tested using mushroom tyrosinase. The leaf crude extract of *P. cuspidatum* showed inhibitory activity at 0.1 g/g f.w.eq. (63.0%). According to Lineweaver-Burk plot, the inhibitors from the leaf extract were speculated to be non-competitive inhibitors (Fig. 5). The leaf crude extract was further fractionated to elucidate the tyrosinase inhibitors. Upon the liquid-liquid separation, the inhibitory activity was observed in both BtOH and aqueous layers; however, inhibitors in BtOH layer was elucidated due to the difficulty in fractionation of the aqueous layer. The inhibitory activity was seen in the ODS 20% MeOH fraction of the BtOH layer and found to be smaller than 10 kDa. In the structural analysis using NMR and LC-MS, the inhibitor found in this fraction was identified as luteolin 6-*C*-glucoside (isoorientin) (**4**) (Fig. 6), which has previously been reported to have melanogenesis. The content of isoorientin was 1.99 mg per 1 g of *P. cuspidatum* leaves, which is relatively high amount when compared with other plant leaves. Interestingly, the promoters of tyrosinase activity were also found in this fraction. These co-existing promoters also had flavone backbone in their structures and were identified as luteolin 8-*C*-β-glucopyranside (orientin) (**3**) and apigenin 8-*C*-β-glucopranside (vitexin) (**5**) (Fig. 6). These flavonoids were structurally similar to isoorientin. However, they had glycosidic linkage at C-6 while isoorientin had glycosidic linkage at C-8, indicating that glycoside position may be the key to promote or inhibit the activity. The presence of isoorientin as an inhibitor of tyrosinase activity along with orientin and vitexin as promoters adds a new finding to *P. cuspidatum* research.

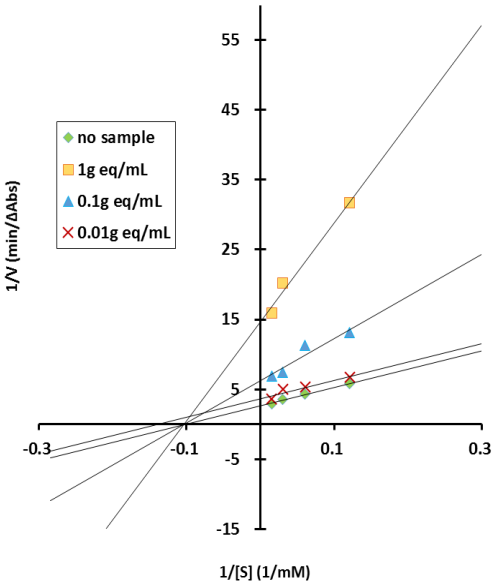

Fig. 5. Lineweaver-Burk plot of tyrosinase activity with different concentration of aqueous layer of leaf extract

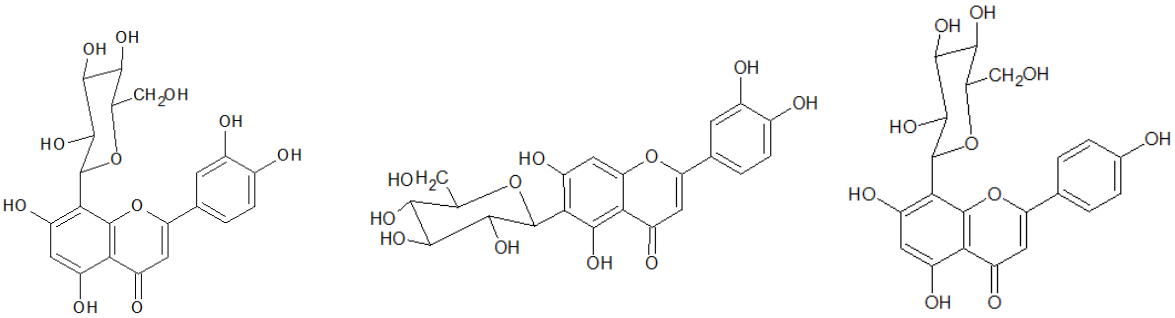

Fig. 6. Structures of orientin (**3**) (left), isoorientin (**4**) (middle), and vitexin (**5**) (right)

Hyaluronidase Inhibitory Activity and Anti-allergic Effect of *P. cuspidatum*

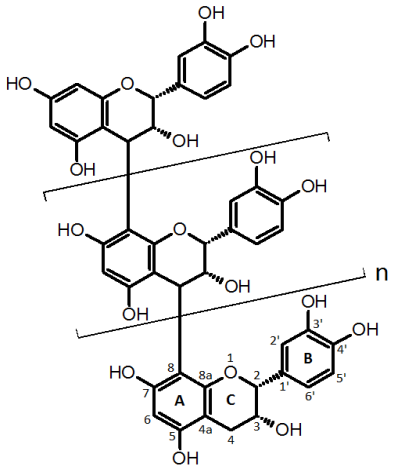

Fig. 7. Structure of proanthocyandin (**6**)

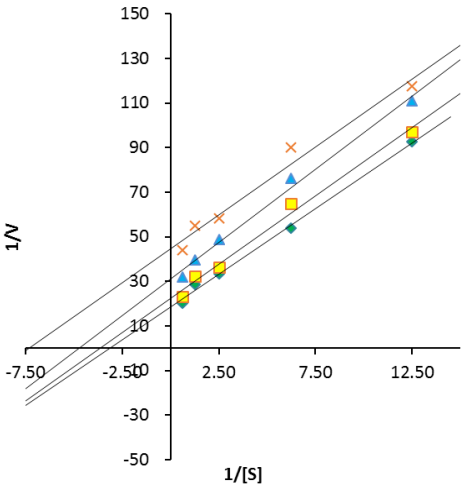

Fig. 8. Lineweaver-Burk plot for hyaluronidase activity with various concentrations of proanthocyanidins

The leaves of *P. cuspidatum* also exhibited hyaluronidase inhibitory activity. Strong activity (98.5%) was observed in the leaf crude extract at 50 mg f.w.eq./mL. To elucidate the active compound, the leaf crude extract was separated using liquid-liquid partitioning, ODS column chromatography and ultrafiltration. The inhibitor was found to be a > 10 kDa compound from ODS 50% MeOH fraction of the precipitate of the aqueous layer, and identified as proanthocyanidin (**6**) (Fig. 7), a polymerized polyphenol consisting of epicatechins with B type linkage of 4β→8 bonds. The number and weight average molecular weights of this compound were 2.82×10^4 and 6.97×10^4, respectively, and its mean degree of polymerization was determined to be 97.2. This highly polymerized compound exhibited uncompetitive inhibitory pattern, interfering with the enzyme-substrate complex in hyaluronidase activity (Fig. 8). The content of proanthocyanidins was 9.03 mg/g f.w.eq., and proanthocyanidins accounted for 24.6% of the entire inhibitory activity of *P. cuspidatum* leaves. Because hyaluronidase inhibitors often have suppressive effect on degranulation of mast cells or basophilic cells, the leaf crude extract and the isolated proanthocyanidins were tested for antigen-induced degranulation assay on RBL-2H3 cells. As a result, both the leaf crude extract and the isolated proanthocyanidins significantly reduced granulation of RBL-2H3 cells in a dose-dependent manner, indicating that they have alleviative effect on type I allergic symptoms (Fig. 9). This is the first study to report about the hyaluronidase inhibitory and anti-allergic effect of *P. cuspidatum* leaves and about proanthocyanidins as effective compounds.

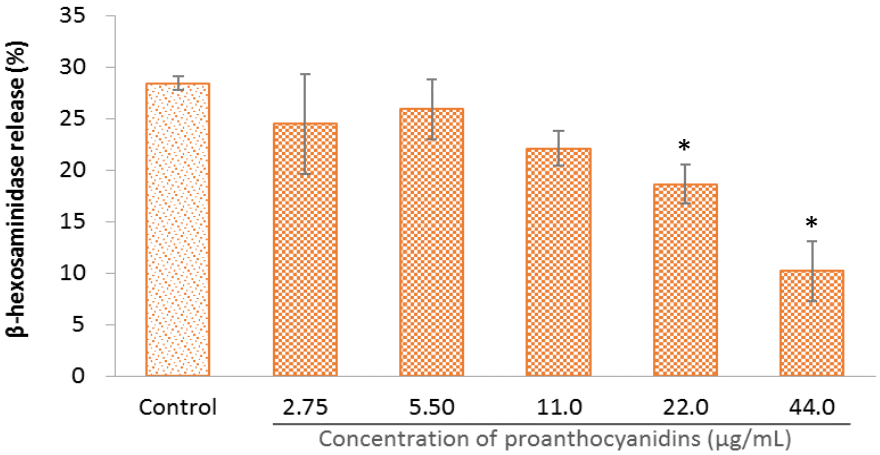

Fig. 9. Degranulation inhibitory effect of proanthocyanidins on sensitized RBL-2H3 cells (*p <0.05)

In conclusion, the functionality of the edible parts of *P. cuspidatum* was explored in this study, and especially the leaf part was found to possess various effects which can promote human health. Its antioxidant, tyrosinase inhibitory, hyaluronidase inhibitory activities as well as anti-allergic effect were evaluated, and the relating compounds were newly identified. Even though the leaves are not recognized as usable materials as the rhizomes, they can be as functional and valuable as the rhizomes. As a food ingredient or medicinal use, *P. cuspidatum* leaves potentially provides beneficial effects to our health in various ways.

References:

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