Extraction of Antioxidant Compounds from *Polygonum cuspidatum* Roots in Sub-Critical Water

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ABSTRACT

Antioxidant compounds of the roots of traditional Chinese medicinal herm *Polygonum cuspidatum* has been extracted at hydrothermal condition. The antioxidant activity of the extract was also investigated in order to analyze the effect of temperature, pressure and water flow rate. Extraction was conducted at water flow rates of 1 mL/min, various temperatures of 150 – 200 ^{o}C, pressures of 5 – 15 MPa. Antioxidant compounds were identified as polyphenolic compounds of resveratrol, rutin and quercetin. The polyphenolic compounds were analyzed quantitatively by using HPLC with diode array detector. Based on the result, extraction yields of resveratrol, rutin and quercetin significantly increased with increasing temperature and pressure. To investigate reaction occurred on the extract, water soluble organic carbon was also analyzed by TOC, while the remaining solid was analyzed by FTIR. In addition, the effect of extraction time on the antioxidant activity of the extract was also investigated by using DPPH assay. The highest antioxidant activity was obtained at 90 min of extraction time.

INTRODUCTION

Polygonum cuspidatum, called Japanese knotweed or bamboo, is a famous Chinese traditional medicinal herb. The dried root of *P. cuspidatum* is well-known used for folk medicine in Korea and Japan. It is used as an analgesic, antipyretic, diuretic, expectorant, and anti-tussive agent and also used for treatment of chronic bronchitis, infectious hepatitis, diarrhea, cancer, hypertension, atherosclerosis, hyperlipidemia, leucorrhoea, dysmenorrhea, trauma with blood stasis, burn, snake bites, and allergic inflammatory diseases [1].

One of the most important bioactive compound in the *P. cuspidatum* roots is resveratrol (3, 5, 4-trihydroxystilbene), which is a polyphenolic compound with multiple therapeutic effects and pharmacological activities such as antibacterial, lipotropic, hepato-protective, and anti-tumor function [2]. Other important polyphenolic compounds contained in the *P. cuspidatum* roots are quercetine and rutin. Quercetin is a flavonol that occurs widely in plants. Several biological actions of quercetin including protection of LDL cholesterol against oxidation and promotion of endothelial vasorelaxation have been reported [3]. Rutin is a quercetin-3-rutinoside with antioxidant, anti-inflammatory and anticarcinogenic effects, and can also reduce the fragility of blood vessels related to haemorrhagic disease and hypertension in humans [4].

Traditionally, abundant volatile organic solvents, including methanol, ethanol, acetone, chloroform, ethyl acetate and some mixed solvents were utilized to extract polyphenolic compounds from Chinese herb by maceration at room temperature [5], heating reflux extraction [6], Soxhlet extraction [7] and microwave-assisted extraction (MAE) [2]. Supercritical CO_2 extraction technology with modifier has also been applied for extraction of polyphenolic compounds from *P. cuspidatum* [8, 9]. Water under sub-critical condition, a "natural and green" way for product extraction, has received increased attention as an important alternative to conventional separation methods, such as hot water extraction conducted at boiling point temperature and atmospheric pressure. Water in sub-critical condition can be applied to extract polar organic compounds or to decompose lignocellulosic materials to produce valuable compounds such as saccharides and aromatic organic acids. The method has been applied to recover protein and amino acids [10], and phenolic compounds [11]. The hydrothermal treatment has also been demonstrated by several studies to effectively convert cellulosic [12] and lignocellulosic biomass [13] into useful products.

As reported in previous work [9], small amount of polyphenolic compounds from *P. cuspidatum* could be extracted. To obtain higher amount of the polyphenolic compounds, such as reseveratrol, quercetin and rutin, the application of sub-critical water for the extraction was investigated. Water is considered generally safe and environmentally benign and can be used in foods and pharmaceutical-related extraction process. In this work, extraction using sub-critical water was carried out in a semi-batch extractor at various temperatures, pressures and water flow rate. In addition, antioxidant activity of extract was also examined.

MATERIALS AND METHODS

Materials and Chemicals

Dried roots of *P. cuspidatum* were provided by Futaba Chinese medicine pharmacy (Okayama, Japan). Prior to extraction, the roots were ground with a grinder into certain particle size. Resveratrol with minimum purity of 98.0 %, rutin, quercetin, and acetic acid with minimum purity of 99.9 %, BHA and DPPH were obtained from Wako Pure Chemical Industries Inc. (Tokyo, Japan).

Sub-critical Water Extraction

Sub-critical water extraction was carried out in a semi-batch extractor. **Figure 1** shows a schematic diagram of a continuous-flow pressurized hot water extraction apparatus. About 4 grams of *P. cuspidatum* roots was loaded into a semi-batch type extraction cell (Thar Tech, Inc., USA, 10 ml in volume), and set up in the heater. Experiment was started after the operating conditions were reached. Extraction was conducted at water flow rate of 1 mL/min, various pressures of 5 to 15 MPa and temperatures (T_2 in **Figure 1**) of 150 to 200 ^oC for 180 minutes. Extracted solution was collected every 30 minutes. A part of extraction solution was freeze-dried and weighted to obtain the extraction yield.

Analysis

Extracted solution was analyzed by HPLC LC-10AD equipped with diode array detector SPD-M10A (Shimadzu, Japan). 10 μl of extract dissolved in ethanol was injected by SIL-10AF auto-sampler (Shimadzu, Japan) and separated with an STR ODS II column (5μm; 4.6x250 mm; Shinwa Chemical Industries, Ltd., Japan) at room temperature. Ethanol/water/acetic acid (40/58/2 v/v) were used as mobile phases at flow rate of 0.5 mL/min. Resveratrol was detected at wavelength of 306 nm, and rutin and quercetin were detected at wavelength of 254 nm [14]. The extracted solution was also analyzed by TOC and MALDI-TOF-MS to determine organic carbon dissolved in water and molecular weight of compounds in the extract, respectively. In order to observe decomposition occurred in the

roots, remaining roots in the extractor was dried and analyzed by FT-IR. Antioxidant activity of freeze-dried extract was also analyzed by using DPPH assay.

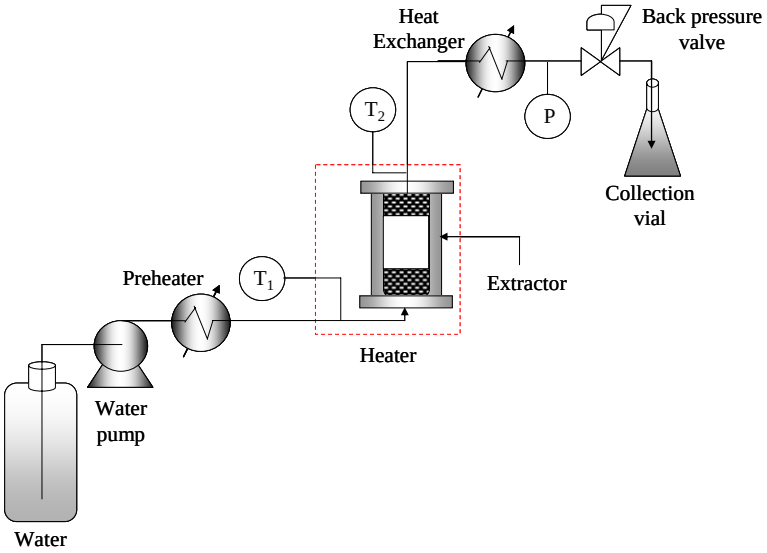
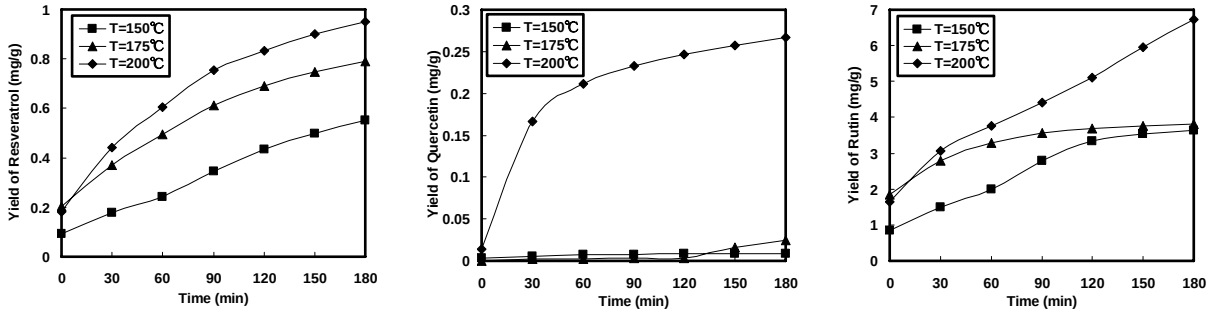


Figure 1 Schematic diagram of continuous-flow pressurized hot water extraction apparatus

RESULT AND DISCUSSION

Polyphenolic Compounds Extraction

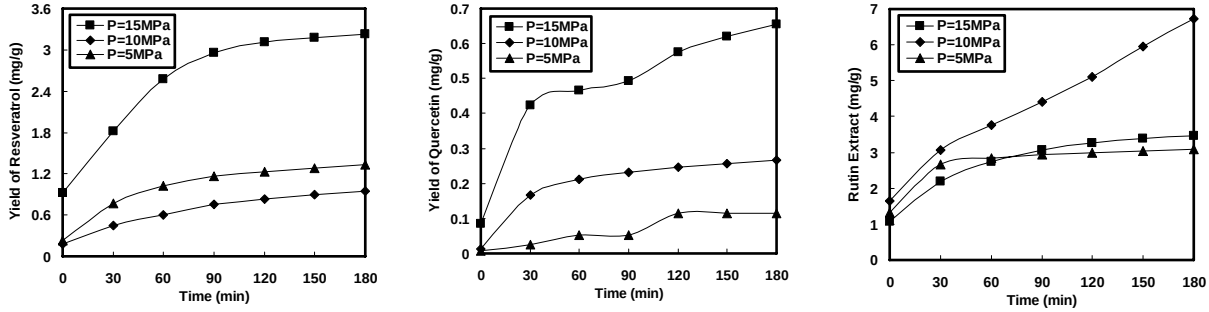


(a) (b) (c)

Figure 2 Effect of temperatures on polyphenolic compounds yield at 10 MPa and 1 mL/min, (a) Resveratrol, (b) Quercetin, (c) Rutin.

Polyphenolic compounds extraction was studied at various temperatures and pressures. **Figure 2(a)**, **(b)** and **(c)** show the effect of temperatures on the yield of resveratrol, quercetin and rutin, respectively. The effect of temperatures was determined at 10 MPa of pressure and 1 mL/min of water flow rate. The yield of polyphenolic compounds is defined as mg of extracted polyphenolic compounds divided by weight of dry roots. As shown in **Figure 2**, yield of resveratrol, quercetin and rutin dramatically increased with increasing temperature. The increasing temperature causes the increasing ion product of water that result in high penetration of water into *P. cuspidatum* roots to extract the polyphenolic compounds.

The effect of pressure on the yield of resveratrol, quercetin and rutin are shown in **Figure 3(a)**, **(b)** and **(c)**, respectively. Generally, the increasing pressure increased yield of resveratrol, quercetin and rutin, respectively. It can be explained that higher pressure promoted higher penetration of water in to *P. cuspidatum* roots. However, for resveratrol from 5 to 10 MPa, the yield of resveratrol significantly decreased with increasing pressure. This result might be due to small change of water properties in the range of 5 to 10 MPa at 200^{o}C.



(a) (b) (c)

Figure 3 Effect of pressures on polyphenolic compounds yield at 200°C and 1 mL/min, (a) Resveratrol, (b) Quercetin, (c) Rutin.

Accumulation Yield of Extract

Accumulation yield of extract was obtained after treatment of extracted solution using freeze-dried for 24 h. Accumulation yield of extract was determined by weight of dried extract divided by weight of dry roots. **Figure 4** shows the accumulation yield of extract at various temperatures. As expected, the increasing temperature caused increasing extraction yield. This result was caused by the increasing ion product of water due to the increasing temperature, and as the result the increasing yield of extract.

The accumulation yield of extract at various pressures is shown in **Figure 5**. Accumulation yield of extract decreased with increasing pressure. It might be caused the decomposition of extracted compounds into small molecular weight components that have been vaporized together with water.

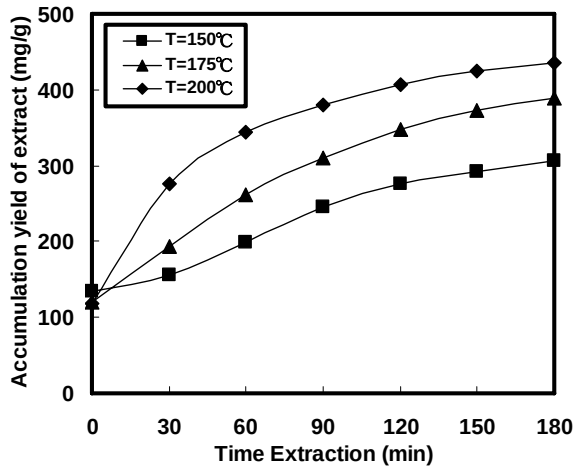


Figure 4 Accumulation yield of extract at various temperatures, 10 MPa and 1 mL/min

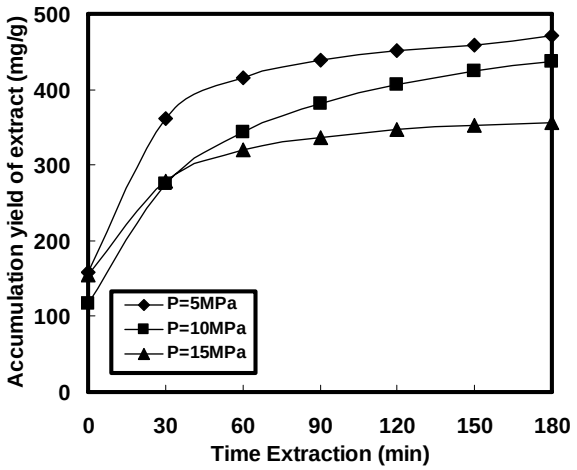


Figure 5 Accumulation yield of extract at various pressures, 200°C and 1 mL/min

Water Soluble Organic Carbon (WSOC)

In order to determine organic compounds in the extract, water soluble organic carbon (WSOC) of extracted solution was analyzed by TOC. Accumulation of WSOC is defined as weight of WSOC divided by weight of dry roots. In **Figure 6** and **7**, the accumulation of WSOC increased with increasing temperature and pressure, respectively. As expected, amount of ion product of water resulted by increasing temperature causes increasing organic carbon dissolved in water. While the increasing pressure caused higher water penetration into cells of

the roots, and resulted in higher organic carbon dissolved in water.

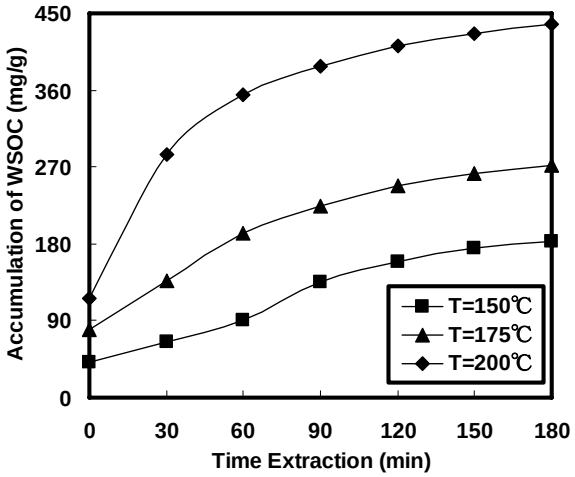


Figure 6 Water soluble organic carbon at various temperatures, 10 MPa and 1 mL/min

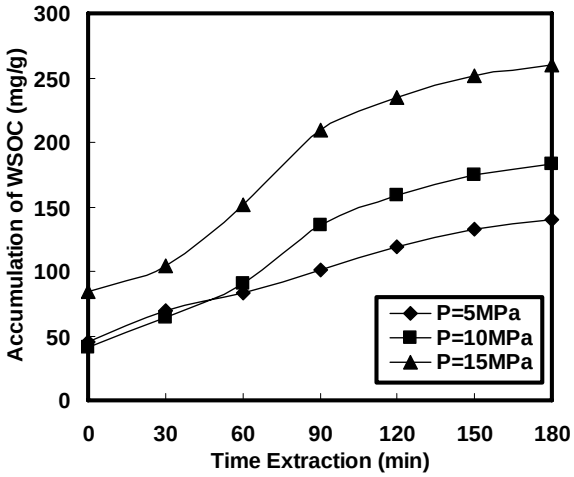


Figure 7 Water soluble organic carbon at various pressures, 150°C and 1 mL/min

FT-IR Analysis

FT-IR analysis was carried out in order to observe the decomposition of *P. cuspidatum* roots after extraction process. The effect of extraction pressure on the decomposition of the roots is shown in **Figure 8**. As shown in the figure, intensity of absorbance due to O-H and C-H stretching appeared and it decreased with increasing pressure. This spectrum could explain the effect of pressure on the yield and WSOC. The organic carbon dissolved in water increased due to the broken of C-H bonding.

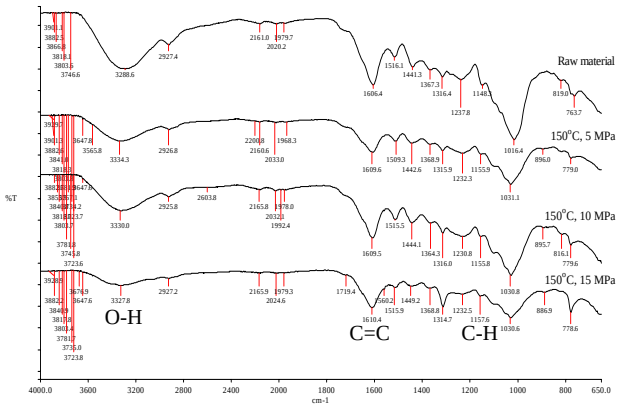


Figure 8 FT-IR spectrum of *P. cuspidatum* roots after extraction at various pressures, 150°C and 1 mL/min

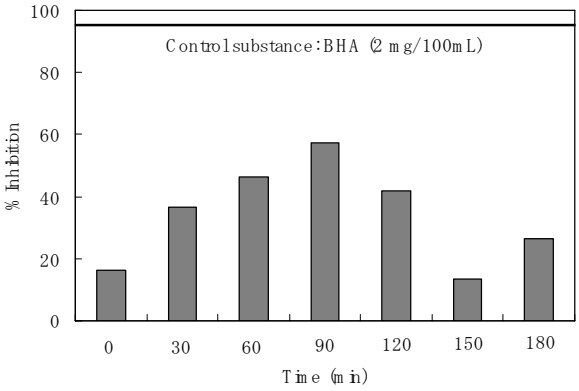


Figure 9 Antioxidant activity of extracted compounds at various extraction times, 200°C, 10 MPa and 1 mL/min

Antioxidant Activity of Extract

In this work, the effect of extraction time on the antioxidant activity of extracted compounds at 200°C, 10 MPa and 1 mL/min was examined. Butylated hydroxyl anisole (BHA) with concentration of 2 mg/100 mL was used as control of antioxidant component. The antioxidant activity was described as % inhibition of methanol solution of DPPH as free radical source. **Figure 9** shows the antioxidant activity of extract at various extraction times. The antioxidant activities of extracted compounds were lower than that of control substance.

At this condition, the antioxidant activity of extract initially increased with increasing extraction time up to 90 min, and then significantly decreased as increasing extraction time. The highest antioxidant activity was 57% inhibition obtained at 90 min.

CONCLUSION

Extraction of antioxidant compounds from *P. cuspidatum* has been conducted using sub-critical water. Polyphenolic compounds, such as resveratrol, quercetin and rutin, have been extracted at various pressures and temperatures. The yield of polyphenolic compounds increased with increasing temperature and pressure. The antioxidant activity of extract has also been examined using DPPH assay. The highest antioxidant activity of extract was obtained at 90 min of extraction time.

ACKNOWLEGMENT

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