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Short communication

Novel extraction method of antioxidant compounds from *Sasa palmata* (Bean) Nakai using steam explosion

Akihiro Kurosumi [a], Chizuru Sasaki [a], Kentaro Kumada [b], Fumihisa Kobayashi [b], Godliving Mtui [c], Yoshitoshi Nakamura [a,*]

[a] *Department of Life System, Institute of Technology and Science, The University of Tokushima, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan*
[b] *Graduate School of Natural Science & Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan*
[c] *Department of Molecular Biology and Biotechnology, University of Dar es Salaam, PO Box 35179, Dar es salaam, Tanzania*

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Abstract

Antioxidant compounds were extracted from various parts of *Sasa palmata* (Bean) Nakai, a bamboo plant whose leaves are commonly used to wrap foodstuffs such as *Sushi* in Japan. Highest concentrations of antioxidant compounds existed in the leaf part of *S. palmata*. Steam explosion treatment followed by hot water and methanol extractions was used for separating the antioxidant compounds from *S. palmata* leaf. The steam explosion treatment is the physical–chemical treatment which crushes a sample by sudden reduction of the pressure in reactor to atmospheric pressure after steaming the sample at high temperature and pressures. *Sasa palmata* leaf was hydrolyzed by steaming and crushed by the rapid decompression. The optimal condition of steam explosion for the effective extraction of antioxidant compounds from *S. palmata* was determined as a steam of temperature of 250 °C and a steaming time of 1 min. In these conditions 217.41 mg/(g-*Sasa* leaf) of phenolic compounds and 142.81 mg/(g-*Sasa* leaf) of radical scavenging activity, that was expressed as butylated hydroxyanisole (BHA), were obtained.

Keywords: Antioxidant compound; Phenolic compound; Radical scavenging activity; *Sasa palmata*; Steam explosion; DPPH

1. Introduction

The awareness has been raised in order to prevent and control the aging and age-related diseases. Attention has been focused on plant resources that contain physiologically active phenolic compounds that show deletion and antioxidative effects of the radicals responsible for the aging process [1–3]. Antioxidant compounds are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. They are able to intercept free radicals and protect cells from the oxidative damage that leads to aging and age-related diseases [4]. They also prevent injury to blood vessel membranes, help to optimize blood flow to the heart and brain, defence against cancer-causing DNA damage, and help to lower the risk of cardiovascular disease and dementia including Alzheimer's disease [5,6]. The discovery of natural antioxidant compounds such as carotenoids, flavonoids, isoflavonoids, isothiocyanates and organosulfur compounds have been one of the major areas of recent scientific research [7–9]. On the other hand, it has been reported that antioxidant compounds were abundant included in leaves of bamboo plants [10,11]. However, the effective extraction and separation method of antioxidant compounds from plants such as bamboo is not yet established. Recently, the steam explosion treatment has been attractive for the degradation and separation of not only structural components, i.e. cellulose, hemicellulose, and lignin, but also antineoplastic constituents from plant biomass [12]. The principle of the steam explosion treatment is the steam hydrolysis at high temperature and pressure, followed by sudden reduction of the pressure for physical treatment of the hydrolyzed product to produce low-molecular weight substances.

In this work, the efficient extraction and separation method of antioxidant compounds was investigated by using various parts of *Sasa palmata*. In order to increase the amount of antioxidant compounds extracted, steam explosion treatment followed by hot water and methanol extractions was attempted, and then the optimal steam explosion conditions such as steam temperature and steaming time were determined.

* Corresponding author. Tel.: +81 88 656 7518; fax: +81 88 656 9071.
E-mail address: ynakamu@bio.tokushima-u.ac.jp (Y. Nakamura).

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2. Materials and methods

2.1. Plant material

S. palmata (Bean) Nakai collected in the forest area of Kanazawa University, Japan, was used as a plant material sample in this study. The leaf, stem, rhizome, and root of *S. palmata* were air-dried to a constant weight immediately after collection and then cut into about 1 cm in length.

2.2. Steam explosion

Steam explosion apparatus (Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan) consisted of a high pressurized reactor, a steam generator, a receiver, and a condenser with a silencing action [13,14]. The reactor was maintained at a constant temperature. The capacity of the reactor was 1.2 dm^3 and the highest temperature was 275 °C (5.5 MPa). About 50 g of *S. palmata* leaf was put into the reactor and then steam heated at a steam temperature of 180–260 °C (1.0–4.9 MPa) for a steaming time of 0.5–20 min. A ball valve at the bottom of the reactor was then suddenly opened to bring the reactor rapidly to the atmospheric pressure. The product containing solid and liquid materials was recovered in the receiver.

2.3. Extraction and separation method

Extraction and separation method for obtaining the antioxidant compounds from *S. palmata* consisted of two stages, i.e. hot water and methanol extractions, as shown in Fig. 1. Sample A are leaf, stem, rhizome, and root of untreated *S. palmata* and sample B is *S. palmata* leaf exploded at a steam temperature of 180–260 °C and a steaming time of 0.5–20 min. Initially, 1 g of dry sample was extracted in a 300 ml Erlenmeyer flask with 100 ml distilled water at 98 °C for 2 h. The resulting residue I was further extracted in a 300 ml Erlenmeyer flask with 100 ml methanol at 25 °C for 2 h.

2.4. Determination of phenolic compounds

The phenolic compounds in the extract were determined according to the Folin–Ciocalteu method [15]. The extract (200 μl) was added to the test tube containing 4 ml of water, followed by addition of 1 ml phenol reagent (diluted five times with water). The mixture was thoroughly stirred. In addition, 1 ml of 10% (w/v) sodium carbonate was added to this solution. The absorbance of reaction was measured at 760 nm after 1 h of incubation at 30 °C. Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Phenolic compounds were expressed as a gallic acid equivalent (mg/(g sample)).

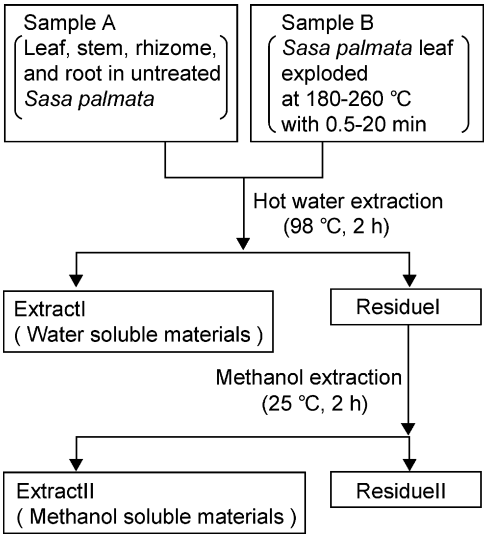

Fig. 1. Flow chart of extraction method from *Sasa palmata*.

2.5. Determination of radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl radical) is a stable nitrogen-centred free radical whose color changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation [16]. Radical scavenging activities were calculated based on the change of absorbance due to the decrease in DPPH in relation to the control value [17,18]. The extract (2 ml), ethanol (2 ml), and 0.5 mM DPPH in ethanol solution (1 ml) were mixed in the test tube, and the decrease in absorbance at 517 nm was measured after 30 min of reaction. Considering the color of extract, the ethanol solution (1 ml) instead of 0.5 mM DPPH in ethanol (1 ml) was used as a color blank. As a control water or methanol (1 ml) was added instead of the extract. The evaluation of radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity } (\%) = \frac{A_0 - (A - A_b)}{A_0} \times 100 \quad (1)$$

where A is the absorbance of the extract and DPPH at 517 nm after 30 min of reaction, A_0 the absorbance of DPPH at 517 nm as a control, and A_b is the absorbance of the extract at 517 nm as a blank. In addition, the radical scavenging activity of extract was expressed as a BHA (butylated hydroxyanisole) equivalent (mg/(g sample)) by the calibration curve showing the relationship between BHA equivalent and radical scavenging activity.

2.6. Statistical analysis

Analyses of at least three samples were carried out in triplicate. Results were processed by the following computer programs: Excel 2003 (Microsoft Corporation, Washington, USA).

3. Results and discussion

3.1. Antioxidant compounds in each part of S. palmata

Table 1 shows the amounts of phenolic compounds and BHA equivalent in extract I, extract II, and total extract (extract I and II) from various parts, i.e. leaf, stem, rhizome, and root, of *S. palmata.* The amount of phenolic compounds varied remarkably depending on the part of *S. palmata*. The leaf contained the highest value of phenolic compounds, 12.08 mg/(g-sample A), as a gallic acid equivalent. The amounts of phenolic compounds extracted from the stem and the rhizomes were 3.47 and 4.49 mg/(g-sample A), respectively, but little phenolic compounds were extracted from the root. The BHA equivalent of total extract from leaf was 8.63 mg/(g-sample A). The BHA equivalent per unit amount of phenolic compounds in extract II were higher than those in extract I. It was evident that the phenolic compounds with high radical scavenging activity were separated by methanol extraction. Previous work reported that ascorbic acid and low-molecular weight polyphenols were separated from wild ginseng leaves by hot water extraction, but not separated by methanol extraction [19]. Therefore, it is thought that the main antioxidant compounds were low-molecular weight polyphenols and ascorbic acid in extract I, and high-molecular weight polyphenols such as lignin in extract II. Since the amount of phenolic compounds and BHA equivalent in leaf sample were much higher than those in other parts, the leaves of *S. palmata* were used in the following experiments.