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RESEARCH NOTE

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Tachioside, an Antioxidative Phenolic Glycoside from Bamboo Species

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Abstract Tachioside (4-hydroxy-3-methoxy-phenyl-1-*O*-glucoside), a known phenolic glycoside, was isolated from various bamboo species. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and Trolox equivalent antioxidant capacity determined a significant antioxidant activity of tachioside which was comparable to L-ascorbic acid. Each culm and leaf extracts were tested and the culm of *Phyllostachys bambusoides* appeared to contain the highest amount of tachioside.

Keywords: tachioside, *Phyllostachys bambusoides*, bamboo culm, antioxidant activity, 4-hydroxy-3-methoxy-phenyl-1-*O*-glucoside

Introduction

A great deal of attention has been paid to develop natural antioxidants to improve the properties of products such as foods and cosmetics without any adverse side effects (1,2). Among the various natural antioxidants, phenolics, and derivatives are reported to actively quench oxygen-derived free radicals and are abundant in plants (3). The functional and medicinal properties of bamboo such as antioxidant, antimicrobial, antimutagenic activities have been studied for decades. 5-*O*-Caffeoyl-4-methylquinic acid and isoorientin are among the antioxidant compounds isolated from bamboo leaves (4,5). Most of the efforts, however, have spotlighted the leaves of bamboo while elaborate work identifying functional phytochemical from the culms of this plant has been sparse in the literature. Recently, researchers reported that the culms exhibited a comparable or even higher antioxidant activity than the leaves of bamboo plant (6,7). Furthermore, Suga *et al.* (8) isolated an antioxidative phyllostadimer from *Phyllostachys edulis*, a common bamboo species. However, compounds responsible for antioxidant activity of bamboo culm are largely remained undiscovered. In this study, *Phyllostachys bambusoides* culm was used in our investigation to find antioxidant compounds from bamboo, based on our previous study in which the culm of this bamboo exhibited the highest antioxidant activity among the tested bamboo species and compartments. This note demonstrated the process of identification of tachioside, an antioxidative principle, from bamboo and the concentration of tachioside in various bamboo species using the high performance liquid chromatography (HPLC) technique in this note.

Materials and Methods

Extraction process Bamboo culms were received from south branch of Korea Forest Research Institute (Jinju, Gyongnam, Korea). The powder of dried *P. bambusoides* culm (100 g) was extracted with 70% ethanol (20-fold) 3 times followed by rotary evaporation (Buchi B-490; Buchi Labortechnik AG, Flawil, Switzerland) and lyophilization until dryness. The concentrated extract powder (4.40 g) was further partitioned sequentially to give *n*-hexane (0.14 g), dichloromethane (0.15 g), ethyl acetate (0.39 g), butanol (0.91 g), and water (2.81 g) fractions in powder.

Analysis and structural identification of antioxidative compounds The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay provided evidence on that the ethyl acetate fraction exhibited the highest DPPH radical scavenging activity than the other fractions. Therefore, the ethyl acetate extract of *P. bambusoides* culm (EA extract) was subjected to further analysis for structural identification of antioxidative elements. LH-20 chromatography was employed for initiative component separation. The EA extract (0.6 g) was dissolved in 2 mL of 10% methanol and loaded on the column (30 ×25 cm) (SR 25; Amersham Bioscience, Uppsala, Sweden). Mobile phase was 10% methanol. The elutes showing high ultraviolet (UV) absorbencies on 280 nm were collected and assayed by an analytical HPLC (Agilent1100 series; GMI, Inc., Ramsey, MN, USA) on a SB-C_{18} column (250 ×4.6 mm). The HPLC conditions were as follows: mobile phases were water and acetonitrile with 0.05%(v/v) trifluoroacetic acid (TFA), respectively; injection rate of 10 μL/time; flow rate of 1 mL/min. With a diode array detector (DAD), 205, 254, 280, and 320 nm wavelengths were selected to analyze elutes simultaneously. The purified fractions were freeze-dried to make powder for structural identification. Mass spectra were obtained on an Agilent LC/MSD 1100 series and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a JNM-ECX 600 Instrument (Jeol, Akishima, Japan).

Antioxidant activity measurement The antioxidant activities of the 70% ethanol extract, the ethyl acetate extract and a purified compound were determined by the DPPH and Trolox equivalent antioxidant capacity (TEAC)

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methods. The usefulness of these methods on measuring antioxidant activities of fruit and vegetable juice or extract has been well documented (9). For DPPH assay, 0.5 mL test samples were added to 2.5 mL, 0.08% EtOH solution of DPPH. Absorbance at 518 nm was determined after 30 min, and the % inhibition activity was calculated as $[(A_0-A_1)/A_0]\times100$ (where A_0 was the absorbance without sample and A_1 was the absorbance with sample). For TEAC assay, the $ABTS^{\bullet+}$ radicals were generated by mixing 20 mL ABTS solution with 70 mL $K_2S_2O_8$ in the dark for 16 hr, at room temperature. Before use, the $ABTS^{\bullet+}$ solution was diluted to get an absorbance of 0.700 ±0.020 at 734 nm with phosphate buffered saline (PBS) at pH 7.4. Changes in absorbance were observed every minute for 6 min. The TEAC values were obtained from the capacity of a sample to inhibit the $ABTS^{\bullet+}$ at a defined time point, relative to Trolox, which is used as a standard. L-Ascorbic acid was used as a positive control.

Table 1. NMR data of the purified compounds isolated from *Phyllostachys bambusoides* culm

600 MHz NMR TMS as int. standard			
^{1}H NMR		^{13}C NMR	
Benzene ring-2 (d)	6.6829	Benzene ring-1	150.723
Benzene ring-5 (d)	6.6450	Benzene ring-2	102.508
Benzene ring-6 (dd)	6.6453	Benzene ring-3	147.777
Glc-1 (d)	4.6588	Benzene ring-4	141.316
Glc-2 (d)	3.1832	Benzene ring-5	115.166
Glc-3 (t)	3.2535	Benzene ring-6	107.930
Glc-4 (t)	3.1121	Glc-1	101.703
Glc-5 (d)	3.265	Glc-2	77.056
Glc-6a (dd)	3.6969	Glc-3	73.295
Glc-6b (dd)	3.4393	Glc-4	69.945
-OCH_3 (s)	3.7273	Glc-5	76.713
		Glc-6	60.84

Results and Discussion

Identification of antioxidant compounds The initiative separation of compounds using the LH-20 chromatography yielded 96.65 mg of compound 1 and 5 mg of compound 2 out of 24 g loaded. UV absorbance and mass spectra determined molecular weight (Mw) of compound 1 to be 302.5 and 351 for tentative weight of compound 2. Interestingly, compound 2 failed to scavenge the DPPH radicals, suggesting its lack of antioxidant activity and its structure could not be characterized due to a low yield (data not shown). Table 1 summarized the ^{1}H and ^{13}C NMR spectrometry data for the compound 1. Data from mass and ^{1}H and ^{13}C NMR spectrometry confirmed the structure of compound 1 as 4-hydroxy-3-methoxy-phenyl-1-*O*-glucoside. This compound was discovered earlier from sugarcane molasses (10) and *Berchemia racemosa* (11), and was named 'tachioside'. Recent studies addressed some biofunctional activities of tachioside, including tyrosinase inhibition, antioxidant activity, and 15-lipoxygenase inhibition (12,13). It is noticeable that tachioside was isolated from bamboo species for the first time. Bamboo-culm derived tachioside was highly hygroscopic, pale-violate floccules powder. Water solubility of the compound seemed probably resulted from the glucoside moiety.

Antioxidant activities of tachioside IC_{50} (μg/μL) values of samples for the DPPH radical were 3.13, 1.22, 0.25, and 0.19 for 70% ethanol extract, ethyl acetate extract, tachioside, and ascorbic acid, respectively. The results of TEAC assay corroborated the high antioxidant activity of tachioside from the DPPH method. The values of mM Trolox/g of dried sample weight were 5.25, 15.82, 46.30, and 54.24 for 70% ethanol extract, ethyl acetate extract, tachioside, and ascorbic acid, respectively (Table 2). These results suggested that PB culm-derived tachioside may find its use as an antioxidant. According to a study done by Cai *et al.* (14), radical scavenging activity of phenolic compounds appeared to be strongly correlated with the number and position of hydroxyl (-OH) groups and methoxy (-OCH_3) substituents in the molecules, suggesting that the hydroxyl groups were potent H-donators and the methoxy groups enhanced the hydrogen-donating capacity and radical scavenging activity. Wei *et al.* (15) have reported that 4-hydroxy-3-methoxy phenyl groups borne by curcumin and its analogues played an important role in the compounds' antioxidant activities. It was shown that polysaccharides moiety, which widely dispersed in bamboo, possessed antioxidant effect to some extent (16).

Concentrations of tachioside in bamboo species The concentrations of tachioside in various bamboo species and

Table 2. Antioxidant activities of *Phyllostachys bambusoides* culm extracts, tachioside, and L-ascorbic acid measured by the stable DPPH and $ABTS^{\bullet+}$ radical scavenging capacities

Sample	DPPH	TEAC	
	IC_{50} (μg/μL)	IC_{50} (μg/μL)	mM Trolox/g d.w.
PB ethanol extract[1)]	3.131±0.006[4)]	1.850±0.026	5.251±0.074
PB ethylacetate extract[2)]	1.224±0.005	0.614±0.001	15.818±0.037
Tachioside[3)]	0.253±0.004	0.210±0.001	46.298±0.087
L-Ascorbic acid	0.185±0.003	0.179±0.002	54.241±0.523

[1)]Seventy % of ethanol extracts from *P. bambusoides* culm.
[2)]Ethyl acetate extracts fractionated from the 70% ethanol extract of *P. bambusoides* culm.
[3)]4-Hydroxy-3-methoxy-phenyl-1-*O*-glucoside (Mw: 305.2, pale-violate floccules powder), a new compound isolated and identified from the ethyl acetate extract via antioxidant activity-directed purification process.
[4)]Values are expressed as mean±SD of 3 independent determinations.

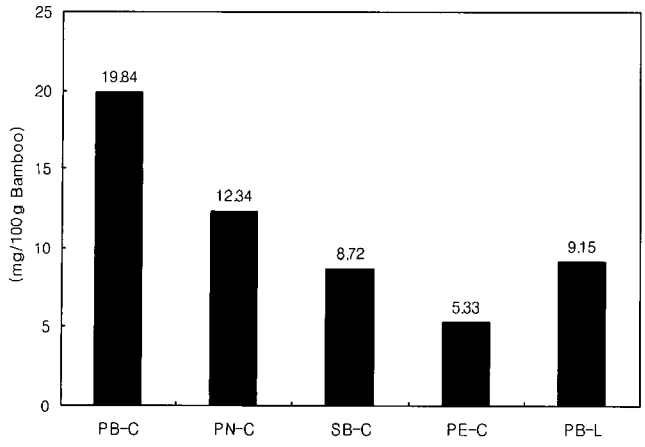

Fig. 1. Concentrations of tachioside in 70% ethanol extracted culm and leaf of various bamboo species. PB, *P. bambusoides*; PN, *P. nigra* var. *henonis*; SB, *Sasa borealis*; PE, *P. edulis*; -C and -L stand for culm and leaf, respectively.

compartments are summarized in Fig. 1. In general, culms indicated a higher amount of tachioside than the leave of bamboos tested. The culm of *P. bambusoides* possessed the highest amount of tachioside followed by the culms of *P. nigra* var. *henonis*, *Sasa borealis*, *P. edulis*, and leaf extracts of *P. bambusoides*, and *P. edulis* in descending order. The leaf extracts of the other two bamboo species contained very little amount of tachioside.

In summary, antioxidant activity-guided fractionation method resulted in the isolation of tachioside and the culms of *P. bambusoides* contained the highest amount of this compound. It is worthy to note that tachioside was isolated from bamboo species for the first time. Given that studies exploring biofunctionality of bamboo culm are relatively sparse in the literature, findings of this study should encourage researchers to broaden our knowledge on the usefulness of bamboo culm. Isolation of other bamboo culm-derived biofunctional compounds and their application to the prevention and treatment of various diseases may be the necessary steps for next.

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