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Original Research Article

Isolation, Identification and Determination of Six Nucleosides and Two Amino Acids from Bamboo Shoots of Gramineae *Phyllostachys prominens* (W Y Xiong)

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Abstract

Purpose: *To develop a method to identify and quantify the compounds in the shoots of four Phyllostachys bamboo species (Gramineae Phyllostachys prominens W. Y. Xiong, Gramineae Phyllostachys iridescins C. Y. Yao Gramineae Phyllostachys pubescens (Carr.) Mitford, Gramineae Phyllostachys praecox C. D. Chu et C. S. Chao.).*
Methods: *The compounds in bamboo shoots were isolated and identified by ultraviolet (UV) spectroscopy, mass spectrometry (MS), and nuclear magnetic resonance (NMR). Quantitative analysis was performed by reversed-phase high performance liquid chromatography (RP-HPLC) using a C18 column and a mixture (1:1ratio) of acetonitrile and 15 mM ammonium acetate (pH 6.0) as mobile phase. This method was validated for its reproducibility, chemical stability, and recovery.*
Results: *Six nucleosides and two amino acids were isolated from bamboo shoots, including guanosine, 2'-deoxyguanosine, adenosine, thymidine, uridine, cytidine, tryptophan, and phenylalanine. The HPLC method was rapid and reproducible. The intraday and interday concentrations of the eight identified compounds showed good linearity in the range of 0.22 - 60.00 µg/mL. The relative standard deviation (RSD) for intraday and interday precision for reproducibility and stability was < 3 %. The validated method was successfully applied to determine the content of the eight compounds in four different Phyllostachys species.*
Conclusion: *Adenosine was isolated from bamboo shoots previously, but the isolation of the other seven compounds are reported here for the first time. The method proposed is sensitive and reproducible, and would facilitate studies of nutritional/medicinal compounds in bamboo shoot.*

Keywords: *Bamboo shoots, Phyllostachys prominens, Guanosine, 2'-Deoxyguanosine, Adenosine, Thymidine, Uridine, Cytidine, Tryptophan, Phenylalanine*

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INTRODUCTION

Bamboo shoot is an important traditional food and medicinal material. The Compendium of Materia Medica in China records many medical uses of bamboo shoot. The chemical composition and active properties of bamboo shoots are attracting the attention of health advocates and scientists [1]. Bamboo shoot has a low fat content and is a rich source of nutrients such as vitamins, amino acids, and antioxidants such as flavones, phenols, and steroids [2]. Bamboo shoot is reported to possess anti-aging, antioxidant, anticancer, antibacterial, and antiviral activities [3]. However, few studies have been performed on bamboo shoots of

Phyllostachys prominens, which is an important crop species for bamboo shoots [4].

Nucleosides and their bases are involved in the regulation of many physiological processes in the human body and exhibit active functional properties such as antiplatelet aggregation, antiarrhythmic, antioxidant, antiseizure, and antitumor [5,6]. Nucleotides and amino acids can be used as indicators to evaluate the quantity of food and medicine. High performance liquid chromatography (HPLC) has been established as a reliable method to identify nucleosides in herbs and food such as Xiaochaihu, edible fungi, *Ganoderma*, and *Ziziphus jujuba* [7-10]. A major aim of this study was to extract, isolate, and identify compounds from bamboo shoots of four *Phyllostachys* species using ultraviolet (UV) spectroscopy, mass spectrometry (MS), nuclear magnetic resonance (NMR), and reversed-phase high performance liquid chromatography (RP-HPLC). Another aim was to develop and validate an HPLC-UV method for the identification of the important compounds in bamboo shoot.

EXPERIMENTAL

Materials and chemicals

Bamboo shoot samples were collected from *Phyllostachys prominens, Phyllostachys iridescens, Phyllostachys pubescens,* and *Phyllostachys praecox* in Hangzhou City (Zhejiang Province, China) in April, 2014. The plants were identified by Professor Chen Shuang-Lin of the Research Institute of Subtropical Forestry (Fuyang, Zhejiang, China).

Bamboo shoots were harvested, shells were removed, and the shoots were washed with distilled water, dried in the shade, and stored in glass bottles at 4 ^{o}C until used. Voucher specimens were deposited in the herbarium of State Forestry Administration Key Open Laboratory, International Center for Bamboo and Rattan (Beijing, China).

Chemical standards

Guanosine, 2'-deoxyguanosine, adenosine, thymidine, uridine, cytidine, tryptophan, and phenylalanine were obtained from J & K Scientific Company (Beijing, China). The purities of the standard chemicals were all > 98 %.

Equipment

Preparative HPLC was performed using a Shimadzu LC-6AD instrument with an SPD-20A detector and a YMC-Pack ODS-AQ column (250 × 4.6 mm, 5 μm). NMR was performed using a Bruker 300 spectrometer. Electrospray ionization (ESI)-MS spectra were obtained using an Agilent 6540 high resolution quadrupole time-of-flight (Q-TOF) mass spectrometer.

Extraction and isolation of compounds from bamboo shoots

Dried bamboo shoots (3.0 kg) were extracted three times with a 10 L volume of 75 % aqueous ethanol at room temperature. Each extraction was performed for 1 day. The aqueous ethanol extract was evaporated under reduced pressure to yield the residue; the final residue weight was 90 g. The residue components were separated on a macroporous resin column (AB-8, 10 × 80 cm) and eluted with an ethanol gradient (0, 15, 30 and 100 %) in water. Four fractions (F1 - F4) were collected based on HPLC analysis. Fraction 2 (F2, 12.0 g) was separated repeatedly on a Sephadex LH-20 column to obtain 25 fractions (E1 □ E25), which were further separated and identified using preparative HPLC analysis. The final analysis identified Compounds 1 - 8. The structures of these compounds were identified using UV spectroscopy, MS, and NMR.

HPLC analysis and determination of compounds 1 - 8

Preparation of standard marker stock solutions

Each of the eight standard compounds (10.0 mg) was dissolved in 10 ml methanol-water (1:1) in a 10 mL volumetric flask to make the individual standard stock solutions. The working stock solution (designated as the mixed standard solution) containing the eight identified compounds was formulated by mixing 2.0 mL of the phenylalanine stock solution and 0.6 mL of the remaining stock solutions (guanosine, 2'-deoxyguanosine, adenosine, thymidine, uridine, cytidine, and tryptophan) in a 10 mL volumetric flask.

Sample preparation

Bamboo shoots (1.0 g) were mixed with 30 mL of ditilled water, and then ultrasonic extraction was performed at room temperature for 20 min for three times. The resulting extract was centrifuged at 4,000 × g for 30 min, and the supernatant was transferred to a new tube. The supernatant was evaporated under reduced pressure and the residue was resuspended in 5 mL of distilled water, which was transferred to a 10 mL volumetric flask. This sample was used for HPLC

analysis; 1 mL was removed from the flask and filtered through a 0.45 µm membrane filter before injection onto the HPLC column.

HPLC analysis

An XTerra RP C18 column (5 µm, 250 × 4.6 mm) and a YMC Hydrosphere C18 column (5 µm, 250 × 4.6 mm) were used for separation. The mobile phase consisted of Solvent A (acetonitrile) and Solvent B (15 mM ammonium acetate in water, pH 6.0). The following gradient was used: 0 - 11 min, 5 - 10 % A; 11 - 13 min, 10 - 15 % A; 13 - 15 min, 15 - 5 % A; 15 - 18 min, 5 % A. The flow rate was 1.0 mL/min. The compounds were identified based on their retention times and spectrum against known standards. The external standard method was used for the determinations each compound.

Validation of the method

A concentration series of the mixed standard solution was prepared to assess the concentrations of the isolated samples. The limit of detection (LOD) and limit of quantitation (LOQ) were measured by duplicate injections of the mixed standard solution based on signal-to-noise ratios (S/Ns) of 3 and 10, respectively.

The precision of inter-day and intra-day measurements was determined by repeating the mixed standard solution measurement five times in one day and for five consecutive days. Precision was expressed as relative standard deviation (RSD). To evaluate reproducibility, six independent *Phyllostachys prominens* samples were analyzed and the results were expressed in terms of RSD.

To confirm the stability, one *Phyllostachys prominens* sample was analyzed after the sample was left at room temperature for different times (1, 2, 3, 4, 5, and 6 d), and the results were expressed in terms of RSD. A recovery test was performed by adding three different volumes of the mixed standard solution to three different 1.0 g samples of *Phyllostachys prominens*. The three replicate samples spiked with different standard contents were extracted and analyzed by HPLC. The recovery rates of the mixed standards were calculated. A control also was analyzed.

Statistical analysis

All bamboo shoot samples were analyzed in quadruplicate, and experiments were repeated three times. Statistical analysis was conducted using Excel and RSD computed.

RESULTS

Structures of the identified compounds

Eight compounds were isolated from bamboo shoot samples and identified as guanosine, 2′-deoxyguanosine, adenosine, thymidine, uridine, cytidine, tryptophan, and phenylalanine. Data for structural identifications are presented in the following paragraphs.

Compound 1

The high resolution electron ionization mass spectrometric (HREIMS) analysis of Compound 1 displayed a molecular ion peak at *m*/*z* 282.2407 $[M-H]^-$($C_{10}H_{13}N_5O_5$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [11]. The ^{1}H-NMR (*δ*, CD_3OD, 300 MHz) spectra revealed the following peaks: 10.60 (1H, br. s, NH); 7.94 (1H, s, H-8); 6.48 (2H, br. s, -NH_2); 5.71 (1H, t, *J* = 8.0 Hz, H-1′); 5.45 (1H, d, *J* = 6.0 Hz, 2′-OH); 5.26 (1H, d, *J* = 4.6 Hz, 3′-OH); 4.95 (1H, m, 5′-OH); 4.41 (1H, d, *J* = 5.2 Hz, H-2′); 4.06 (1H, m, H-3′); 3.78 (1H, m, H-4′); 3.59 (1H, m, H-5′a); and 3.51 (1H, m, H-5′b). The ^{13}C-NMR (*δ*, CD_3OD, 300 MHz) spectra revealed the following peaks: 157.9 (C-6); 154.1 (C-2); 151.7 (C-4); 136.0 (C-8); 117.2 (C-5); 86.8 (C-1′); 85.6 (C-4′); 74.1 (C-2′); 70.8 (C-3′); and 61.8 (C-5). Based on these data, Compound 1 was identified as guanosine.

Compound 2

The HREIMS analysis of Compound 2 displayed a molecular ion peak at *m*/*z* 266.2435 $[M-H]^-$($C_{10}H_{13}N_5O_4$). One of the hydroxy signals disappeared in the ^{1}H-NMR analysis of Compound 2 compared with Compound 1. Methylene signals appeared at *δ* 40.0 (C-2′) in the ^{13}C-NMR analysis of Compound 2. Based on these data, Compound 2 was identified as 2′-deoxyguanosine.

Compound 3

The HREIMS analysis of Compound 3 displayed a molecular ion peak at *m*/*z* 266.2419 $[M-H]^-$($C_{10}H_{13}N_5O_4$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [11]. The ^{1}H-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 8.34 (1H, s, H-2); 8.12 (1H, s, H-8); 7.36 (2H, s, -NH_2); 5.86 (1H, d, *J* = 8.0 Hz, H-1′); 5.40 (2H, m, 2′, 5′-OH); 5.16 (1H, m, 3′-OH); 4.59 (1H, q, *J* = 5.6 Hz, H-2′); 4.12 (1H, q, *J* = 3.0 Hz, H-3′); 3.94 (1H, q, *J* = 3.2 Hz, H-4′); 3.67 (1H, m, H-5′a); and 3.55 (1H, m, H-5′b). The ^{13}C-NMR (*δ*,

DMSO-d_6, 300 MHz) spectra revealed the following peaks: 157.18 (C-6); 154.09 (C-2); 151.74 (C-4); 136.01 (C-8); 117.29 (C-5); 86.87 (C-1′); 85.66 (C-4′); 74.15 (C-2′); 70.82 (C-3′); and 61.87 (C-5′). Based on these data, Compound 3 was identified as adenosine.

Compound 4

The HREIMS analysis of Compound 4 displayed a molecular ion peak at *m*/*z* 241.2231 [M-H]$^-$($C_{10}H_{14}N_2O_5$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [12]. The ^{1}H-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 11.25 (1H, s, NH); 7.69 (1H, s, H-6); 6.16 (1H, m, OH-3′); 5.21 (1H, m, 3′-OH); 5.00 (1H, m, 5′-OH); 4.23 (1H, d, *J* = 2.2 Hz, H-3′); 3.75 (1H, m, H-4′); 3.56 (1H, d, *J* = 11.6 Hz, 5′b); 1.92 (2H, m, H-2′); and 1.68 (3H, d, *J* = 1.1 Hz, 5-CH_3). The ^{13}C-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 163.8 (C-4); 150.5 (C-2); 136.2 (C-6); 109.5 (C-5); 87.3 (C-4′); 83.9 (C-1′); 70.5 (C-3′); 61.4 (C-5′); 39.4 (C-2′); and 12.4 (CH_3). Based on these data, Compound 4 was identified as thymidine.

Compound 5

The HREIMS analysis of Compound 5 displayed a molecular ion peak at *m*/*z* 243.2031 [M-H]$^-$($C_9H_{12}N_2O_6$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [13]. The ^{1}H-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 7.85 (1 H, d, *J* = 7.8 Hz, H-6); 5.68 (1 H, d, *J* = 7.8 Hz, H-5); 11.28 (1H, s, NH); 5.76 (1H, d, *J* =5.2 Hz, H-1); 3.99 (1H, m, H-2′); 3.95 (1H, m, H-3′); 3.82 (1H, m, H-4); 3.53 (1H, m, H-5′a); 3.59 (1H, m, H-5′b); and 5.07 – 5.35 (2H, m, 2′,3′,5′-OH). The ^{13}C-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 152.5 (C-2); 166.9 (C-4); 102.7 (C-5); 142.2 (C-6); 90.0 (C-1); 74.0 (C-2); 84.6 (C-3); 69.8 (C-4); and 61.2 (C-5). Based on these data, Compound 5 was identified as uridine.

Compound 6

The HREIMS analysis of Compound 6 displayed a molecular ion peak at *m*/*z* 242.2131 [M-H]$^-$($C_9H_{13}N_3O_5$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [14]. The ^{1}H-NMR (*δ*, 300 MHz, DMSO-d_6) spectra revealed the following peaks: 5. 81 (1H, d, *J* =7.2 Hz, H-5); 7.45 (1H, d, *J* =7.2 Hz, H-6); 5.91 (1H, d, *J* = 5.2 Hz, H-1′); and 3.52 – 5.00 (5H, m, H-2′, 5′). The ^{13}C-NMR (300 MHz, DMSO-d_6) spectra revealed the following peaks: 158.2 (C-2); 166.8 (C-4); 96.9 (C-5); 142.4 (C-6); 91.2 (C-1′); 74.8 (C-2′); 70.1 (C-3′); 84.6 (C-4′); and 61.6 (C-5′). Based on these data, Compound 6 was identified as cytidine.

Compound 7

The HREIMS analysis of Compound 7 displayed a molecular ion peak at *m*/*z* 203.2242 [M-H]$^-$($C_{11}H_{12}N_2O_2$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [15]. The ^{1}H-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 10.99 (1H, s, NH); 7.15 (1H, s, H-2); 7.05 (2H, NH_2); 6.81 (2H, d, *J* = 8.3 Hz, H-4, 7); and 6.66 (2H, d, *J* = 8.2 Hz, H-5, 6). The ^{13}C-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 170.7 (COOH); 135.7 (C-9); 127.1 (C-8); 124.4 (C-2); 121.2 (C-4); 118.8 (C-5); 118.6 (C-6); 111.7 (C-7); 110.15 (C-3); 54.9 (C-11); and 26.5 (C-10). Based on these data, Compound 7 was identified as tryptophan.

Compound 8

The HREIMS analysis of Compound 8 displayed a molecular ion peak at *m*/*z* 164.1862 [M-H]$^-$($C_9H_{11}NO_2$). The ^{1}H- and ^{13}C-NMR spectra were in close agreement with those of a previous report [15]. The ^{1}H-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 7.36 (5H, m, C_6H_5); 4.88 (5H, m, H-8); 3.81 (1H, s, H-8); 3.06 (1H, m, -CH_2); and 3.01 (1H, m, -CH_2). The ^{13}C-NMR (*δ*, DMSO-d_6, 300 MHz) spectra revealed the following peaks: 173.9 (C-9); 135.2 (C-1); 129.4 (C-3, 5); 129.2 (C-2, 6); 127.7 (C-4); 56.1 (C-8); and 36.4 (C-7). Based on these data, Compound 8 was identified as phenylalanine.

HPLC method validation

Optimization of the protocol

Nucleosides are polar compounds; therefore, the initial concentration of Solvent A (acetonitrile) in the mobile phase was kept low to promote retention on the column. Nucleosides and amino acids are both weak acids; therefore, the retention time and peak shape on a reversed-phase column can differ depending on the mobile phase [12]. Solvent B (15 mM ammonium acetate in water, pH = 6.0) was selected for its suitable chemical properties for these experiments. In the present study, two reversed-phase columns (XTerra RP C18 and YMC Hydrosphere C18) were tested for the separation of nucleosides and amino acids isolated from bamboo shoot. The results indicated that the YMC Hydrosphere C18 column retained and

separated the compounds better than the XTerra column. Figure 1 presents the HPLC chromatogram of six nucleosides and two amino acids and resolved on a YMC Hydrosphere C18 column at 254 nm; phenylalanine was analyzed at 215 nm.

Linearity and sensitivity

The linear relationships observed from the standard solutions of the compounds in the samples are presented in Table 1. The standard curves in the corresponding ranges had good linearity. The LOD and LOQ for the chromatographic experiments were determined separately at S/Ns of 3 and 10, respectively. LOD values ranged from 0.008 to 0.500 µg/mL. LOQ values ranged from 0.025 to 1.600 µg/mL. The linear range of phenylalanine was 3.12 to 200.00 µg/mL, whereas the linear range of the other seven compounds was 0.22 to 60.00 µg/mL.

Precision, reproducibility, and stability

Table 2 presents the RSD values for intra-day and inter-day precision, reproducibility, and stability for the eight identified compounds they were all < 2.8, which suggests that the developed method is reproducible and stable.

Recovery

The compound recoveries were tested in bamboo shoot samples spiked with different amounts of mixed standard markers, and determined to be within 85.98 − 102.04 %. This indicated that the developed extraction method was reliable. Taken together with other control tests, all results indicated that the method performance was satisfactory.

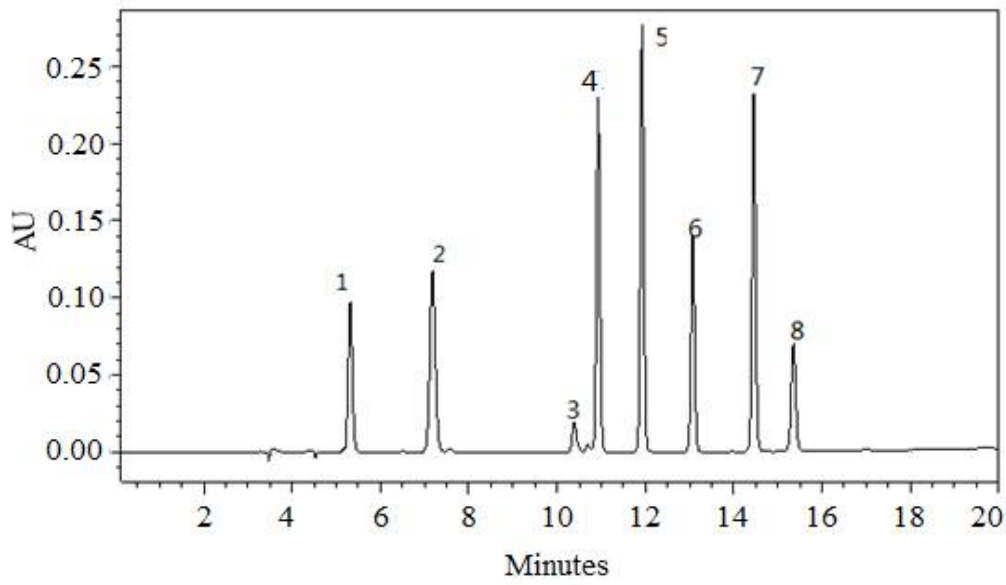

Figure 1: HPLC chromatogram and identification eight compounds: (1) cytidine, (2) uridine, (3) phenylalanine, (4) guanosine, (5) 2′-deoxyguanosine, (6) thymidine, (7) adenosine, and (8) tryptophan

Table1: Linear equations, LOD, LOQ, and linear ranges of eight compounds isolated from bamboo shoot

Compound	λ (nm)	Regression equation	R^2	LOD (µg/mL)	LOQ (µg/mL)	Linear range (µg/mL)
Cytidine	254	y =12,043x+548	0.9989	0.015	0.047	0.22–60.00
Adenosine	254	y = 25,613x–622	0.9992	0.010	0.031	0.22–60.00
Uridine	254	y = 19,484x–497	0.9999	0.008	0.025	0.22–60.00
Guanosine	254	y = 23,866x–216	0.9998	0.009	0.027	0.22–60.00
2′-deoxy-guanosine	254	y = 25,655x+25	0.9997	0.008	0.025	0.22–60.00
Thymidine	254	y = 16,338x–60	0.9997	0.010	0.031	0.22–60.00
Tryptophan	254	y = 9,083x–971	0.9996	0.024	0.075	0.22–60.00
Phenylalanine	215	y = 17,608x–326	0.9997	0.500	1.600	3.12–200.00

y is the peak area value; x is the reference compound's concentration (µg/mL)

Table 2: Experimental precision, reproducibility, and stability of eight compounds isolated from bamboo shoot

Compound	Intraday precision (RSD %, *n* = 5)	Interday precision (RSD %, *n* = 5)	Reproducibility (RSD %, *n* = 6)	Stability (RSD %, *n* = 6)
Cytidine	1.02	2.23	1.22	1.74
Adenosine	0.67	2.18	0.87	2.31
Uridine	1.44	1.55	1.43	1.02
Guanosine	0.57	1.34	0.78	1.98
2′-deoxyguanosine	0.45	0.89	0.81	1.80
Thymidine	1.11	2.45	1.21	2.68
Tryptophan	1.74	2.04	0.68	2.78
Phenylalanine	0.50	1.67	2.70	2.25

Table 3: Recovery of samples spiked with standard markers of eight compounds isolated from bamboo shoot

Compound	Spiked quantity (μg/g)	Recovery (%)	RSD (%)
Cytidine	60	102.35	3.33
	120	103.21	3.12
	240	104.1	2.13
Adenosine	60	91.34	1.77
	120	89.45	1.89
	240	92.94	3.13
Uridine	60	95.5	2.33
	120	93.48	1.87
	240	96.06	2.05
Guanosine	60	99.26	0.98
	120	96.68	0.67
	240	97.9	0.88
2′-deoxyguanosine	60	95.24	4.11
	120	94.64	2.22
	240	93.48	1.97
Thymidine	60	111.22	0.46
	120	112.49	1.25
	240	109.39	0.53
Tryptophan	60	88.89	0.43
	120	90.74	0.56
	240	91.67	1.48
Phenylalanine	100	89.43	1.88
	200	97.88	1.32
	400	85.72	0.81

Table 4: Contents (mg/kg) ± RSD of six nucleosides and two amino acids in shoots of four *Phyllostachys* bamboo species

Compound	*Phyllostachys prominens*	*Phyllostachys iridescens*	*Phyllostachys pubescens*	*Phyllostachys praecox*
Cytidine	32.56±0.54	139.34±1.26	294.69±1.17	221.67±2.07
Adenosine	22.41±0.43	9.88±0.11	ND	ND
Uridine	167.77±1.35	511.57±3.40	482.82±2.97	105.62±1.67
Guanosine	435.25±1.59	49.54±0.78	225.08±1.73	472.11±3.51
2′-deoxyguanosine	43.38±0.43	ND	78.23±0.88	95.16±1.05
Thymidine	59.88±0.42	15.37±0.06	112.45±1.38	96.67±1.21
Tryptophan	547.48±3.15	699.11±4.16	530.32±3.80	367.76±4.12
Phenylalanine	1,218.33±7.88	858.57±4.62	1,460.93±8.93	1,344.45±6.27

ND, not detected

Application of the method

The identification of investigated compounds was carried out by comparison of their retention time and their UV spectra with those obtained by injecting standards in the same conditions. The results of qualitative and quantitative analyses of six nucleosides and two amino acids isolated from bamboo shoots of four *Phyllostachys* species are presented in Table 4. Cytidine,

uridine, guanosine, thymidine, tryptophan, and phenylalanine were all detected in *Phyllostachys prominens, Phyllostachys iridescens, Phyllostachys pubescens*, and *Phyllostachys praecox*. Adenosine was not detected in *Phyllostachys pubescens* and *Phyllostachys praecox*. Phenylalanine had the highest content in bamboo shoots of all four species.

DISCUSSION

Nucleosides are involved in the regulation of many physiological processes in the human body and exhibit multiple activities. There have been few studies of nucleosides in bamboo shoots. Chen *et al* isolated adenosine from *Pleioblastus amarus* bamboo shoots [16]. The current study developed a method for the extraction, isolation, and identification of six nucleosides and two amino acids from bamboo shoots. The method was validated and found to be reproducible. This study will facilitate further research on bioactive, nutritional, and medicinal compounds in bamboo shoots.

Bamboo shoot consumption is relatively modest in most countries outside Asia. The production of bamboo shoots is generally based on non-standardized, traditional customs [2]. The development of this HPLC method for determination of cytidine, uridine, phenylalanine, guanosine, 2'-deoxyguanosine, thymidine, adenosine, and tryptophan from bamboo shoots could be applied for quality control of bamboo shoot production. This would add value to the final product. The guanosine contents of *Phyllostachys prominens* and *Phyllostachys praecox* were 435.25 and 472.11 mg/kg respectively. These values are higher than the guanosine contents in *Ziziphus jujube* (16.62 - 185.72 mg/kg) and *Ganoderma* (4.36 - 25.47 mg/kg) [7,10]. Food is one of the major resources of nucleosides and nucleotides for humans. Our study showed that bamboo shoots are rich sources of nucleosides. Therefore, bamboo shoots should be considered as excellent sources of nucleoside dietary supplements.

CONCLUSION

This study developed an HPLC method for the identification and quantitative analysis of six nucleosides and two amino acids from shoots of four *Phyllostachys* bamboo species. The method was tested and validated as reliable. Adenosine was previously identified in bamboo shoot, but this study identified guanosine, 2'-deoxyguanosine, thymidine, uridine, cytidine, tryptophan, and phenylalanine for the first time. The method is rapid, sensitive, and accurate, and can be applied for the determination of nucleosides in bamboo shoots.

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