Chemical profile and pancreatic lipase inhibitory activity of *Sinobambusa tootsik (Sieb.) Makino* leaves

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Background. *Sinobambusa tootsik (Sieb.) Makino* (*S. tootsik*) is one species of bamboo distributed in China, Japan and Vietnam. The chemical profile of its leaves and its potential application was unknown yet.

Methods. The chemical profile of *S. tootsik* was studied by HPLC and UPLC-DAD-QTOF-MS. The *S. tootsik* extract was prepared by extraction with 50% ethanol, followed by H103 resins adsorption and desorption processes. Pancreatic lipase inhibitory activity was determined using *p*-nitrophenyl palmitate as the substance, which was hydrolyzed by lipase to form coloured *p*-nitrophenol.

Results. Eighteen compounds were identified in *S. tootsik*. Most of them were the C-glycosylated derivatives of luteolin and apigenin, such as isoorientin, isoorientin-2''-O-rhamnoside and isovitexin. Isoorientin-2''-O-rhamnoside was the most dominant flavonoid in the sample. *S. tootsik* extract was prepared through resin adsorption/desorption with yield of 1.12±015% and total flavonoids content of 8.2±0.2% (in term of isoorientin). The extract exhibited pancreatic lipase inhibitory activity with IC50 value of 0.93 mg/ml.

Conclusion. The chemical profile of *S. tootsik* leaves was uncovered for the first time. C-glycosyl flavonoids were the main constituents in the plant. The extract exhibited pancreatic lipase inhibitory activity and may have potential to be used as food supplement for obesity controlling.

Chemical Profile and Pancreatic Lipase Inhibitory Activity of *Sinobambusa tootsik (Sieb.) Makino* leaves

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ABSTRACT

Background. *Sinobambusa tootsik (Sieb.) Makino* (*S. tootsik*) is one species of bamboo distributed in China, Japan and Vietnam. The chemical profile of its leaves and its potential application was unknown yet.

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Discussion. The chemical profile of *S. tootsik* leaves was uncovered for the first time. C-glycosyl flavonoids were the main constituents in the plant. The extract exhibited pancreatic lipase inhibitory activity and may have potential to be used as food supplement for obesity controlling.

Keywords: *Sinobambusa tootsik (Sieb.) Makino*; chemical profile; C-glycosyl flavonoids;

32 pancreatic lipase; inhibition

33

INTRODUCTION

Bamboo is a valuable plant distributed all over the world with more than 1500 species. The bamboo shoots of some species, e.g. *Phyllostachys heterocycla* cv. *pubescens* (*P. heterocycla*), were eaten as vegetable, while the leaves were used as herbal material in China. The flavonoids extract of some bamboo species were approved as the food antioxidant and food resources in China (Wang et al., 2012a). The pharmacological activities of bamboo leaves were arise from the presence of phytochemicals. For instance, five C-Glycosyl flavones were isolated from Fargesia robusta (Van Hoyweghen et al., 2010). Three chlorogenic acid derivatives were isolated from Phyllostachys edulis and the antioxidant activity was evaluated (Kweon et al., 2001). Wang et al. isolated three isoorientin derivatives from Bambusa. textilis McClure (Wang et al., 2012a). Previous, we identified twelve compounds in the leaves of *Bambusa multiplex cv. Fernleaf* (*B. multiplex*), and found that C-glycosyl flavonoids including vitexin, isovitexin, isoorientin and its derivatives, are the main chemical constitutes of the plant (Qiu & Zhang, 2019). *Sinobambusa tootsik (Sieb.) Makino* (*S. tootsik*) is one species of bamboo distributed in China, Japan and Vietnam. To the best of our knowledge, the chemical profile of its leaves has not been studied yet. To further uncover its potential application, the chemical composition of *S. tootsik* was studied by HPLC and UPLC-QTOF-MS in the present study. Furthermore, the pancreatic lipase inhibition activity of its extract was studied.

MATERIALS AND METHODS

Chemicals and Plant materials

Leaves of S. tootsik was collected in Jiangxi Agricultural University (with east longitude of 115°50' and northern latitude of 28°46') on Mar. 2019. The plant material was authenticated by Prof. Qing-Pei Yang (Jiangxi Agricultural University), and the voucher specimen was deposited in Jiangxi Key Laboratory of Natural Product and Functional Food. The leaves was dried at 60 °C and smashed to filter through 40 mesh sieve. Isoorientin (>98%) were purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). HPLC grade acetonitrile was purchased Anhui Tedia High Purity Solvents Co., Ltd (Anqin, China). Porcine pancreatic lipase was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China; http://www.aladdin-e.com). Milli-Q water was used throughout the study. All other reagents used were analytical grade.

Sample extraction

A 0.1 g aliquot of *S. tootsik* powder was mixed with 5.0 mL of 50% ethanol. After sonicating for 30 min in a bath sonicator (100 W, 45 kHz, Kunshan, China), the mixture was centrifuged at 3000 rpm for 5 min. The supernatant was filtered by 0.22 mm pore size filter and then used for HPLC and UPLC-DAD-QTOF-MS analysis.

For *S. Tootsik* extract preparation, 50 g of *S. tootsik* sample was extracted by 500 mL of 50% ethanol for twice. After centrifugation at 3000 rpm for 5 min, the supernatant was combined and condensed to about 500 mL. The concentrates was two times diluted by water. Then, the extract was pumped through a fix bed of H103 resin. After adsorption, the fix bed was desorbed with 4 BV of 90% ethanol. The eluent was concentrated and then lyophilized to obtain the extract.

73 **UPLC-DAD-QTOF-MS analysis**

74 The chemical identification was performed on a QTOF 5600-plus mass spectrometer equipped
75 with Turbo V sources and a Turbolonspray interface (AB Sciex Corporation, Foster City, CA,
76 USA) coupled to a Shimadzu LC-30A UPLC-DAD system (Shimadzu Corporation, Kyoto,
77 Japan). Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters） was used. The
78 flow rate was 0.3 mL/min with injection volume of 1 μL and column temperature of 40 °C. The
79 mobile phase was acetonitrile (A) and 0.1% formic acid aqueous solution (B) using a linear
80 gradient program of 0-30min, 5-40% (A). The mass spectrometer was operated in the negative
81 ion mode. Ultrapure nitrogen was used as the ion source gas 1 (50 psi), ion source gas 2 (50 psi),
82 and curtain gas (40 psi). The Turbo Ion Spray voltage and temperature were set at -4500 V and
83 500 °C, respectively. Declustering potential, collision energy, and collision energy spread were
84 set at 100 V, -40 V, and 10 V, respectively. Data acquisition was performed with Analyst 1.6
85 software (AB Sciex).

86 **HPLC quantification Analysis**

87 The HPLC Analysis was performed on an Agilent 1260 HPLC system equipped with an
88 autosampler and DAD detector. A Symmetry C18 column (250 mm × 4.6 mm i.d., 5 μm; Waters,
89 USA) was used as the stationary phase. The mobile phase consisted of acetonitrile (A) and 0.1%
90 acetic acid aqueous solution (B). The flow rate was 1 mL/min with linear gradient program of 0-
91 30 min, 1-40% A; 30-35 min, 40% A. Detected wavelength was 349 nm with injection volume
92 of 10 μL and column temperature of 40 °C.

93 **Pancreatic lipase inhibitory activity assay**

94 Pancreatic lipase inhibitory activity was determined using *p*-nitrophenyl palmitate (*p*-NPP) as the
95 substance, which was hydrolyzed by lipase to form *p*-nitrophenol with maximum absorption
96 around 405 nm. Lipase (10 mg) was dissolved in 5 mL Tris-buffer (50 mM, pH 8, containing
97 0.1% arabinus gum powder and 0.2% sodium deoxycholate). The mixture was stirred for 15 min
98 and centrifuged at 5000 rpm for 10 min. The clear supernatant was used for the assay. Briefly, in
99 a 96-well microplate, 30 μL Tris-buffer, 150 μL enzyme and 10 μL *S. tootsik* extract (dissolved
100 in 50% ethanol) were mixed together. The mixture was incubated at 37 ^{o}C in the microplate
101 reader for 20 minutes. Then, 10 μL of 10 mM *p*-NPP pre-incubated at 37 ^{o}C was added to start
102 the reaction. The absorbance was determined under 405 nm for 20 min with interval of 1 min.
103 The absorbance growth slope (V) which represented the enzyme activity was calculated by linear
104 regression.

105 $$\text{Lipase inhibition activity (\%)} = \frac{V_b - V_s}{V_b} \times 100$$

106 Where V_b and V_s were the enzyme activity in the absence and presence of *S. tootsik* extract,
107 respectively. Orlistat was used as the positive control.

108 **Fluorescence quench measurements**

109 A 1.0 mL aliquot of the lipase solution was mixed with 4 mL of Tris-buffer. Subsequently, 0, 5,
110 10, 15, 20, 25 and 30 μL of *S. tootsik* extract (10 mg/ml in 50% ethanol) was added, respectively.

The fluorescence spectra of the mixture was characterized under excitation wavelength of 280 nm.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD) of triplicates. Statistical analysis, plotting, and curve fitting were performed by Origin 7.0 (Origin Lab Co., Northampton, MA, USA).

Result and discussion

Figure S1was the chromatogram of *S. tootsik* after UPLC separation detected by QTOF-MS (b) and DAD (a, 349nm). By the QTOF-MS detector, the molecular mass of each peak and its further MS^2 spectrum was obtained. The chemical identification was accomplished by comparing these information with published literature. The details were listed in Table 1. A total of 18 components were identified. Most of them were the C-glycosylated derivatives of luteolin and apigenin, such as isoorientin, isoorientin-2"-*O*-rhamnoside and isovitexin. Besides, some other C-glycosyl and *O*-glycosyl flavonoids were found, such as isoscoparin-*O*-deoxyhexoside and kaempferol-*O*-glucoside. Two non-flavonoid compounds, feruloylquinic acid and roseoside, were also found. *S. tootsik* belongs to the family of *Poaceae*. Many studied showed that the main secondary metabolite found in the leaves of *Poaceae* plants were C-glycosyl flavonoids, for instance, barley, maize, wheat, rice, etc (Brazier-Hicks et al., 2009, Ferreres et al., 2008). Previously, we have studied the chemical constituents in the leaves of *Bambusa multiplex cv.*

130 *Fernleaf* (*B. multiplex*), one of the other bamboo species (Qiu & Zhang, 2019). It was found that
131 C-glycosylated derivatives of luteolin and apigenin were the main components of both species.
132 However, the specific flavonoids between the two plants were different. Only apigenin 6-C-
133 pentoside-8-C-glucoside, isoorientin and isovitexin were found in both species.

134 Figure 1 was the HPLC chromatogram of *S. tootsik* detected at 349 nm. With the result of
135 UPLC-DAD-Q-TOF-MS analysis (Figure S1), the main peaks in the HPLC chromatogram were
136 identified. The peak of isoorientin was further validated by comparing the retention time with
137 standard marker. Form the peak area, it was found that isoorientin-2"-*O*-rhamnoside was the
138 most dominant flavonoid in *S. tootsik,* followed by isoscoparin-*O*-deoxyhexoside and apigenin 6-
139 C-[2"-*O*-deoxyhexoside]-pentoside. This was also different from *B. multiplex,* in which
140 isoorientin was the most dominant flavonoid, followed by isovitexin (Qiu & Zhang, 2019).

141 ***3.2 S. tootsik extract preparation***

142 Through 50% ethanol extraction, followed by H103 resins adsorption and desorption processes,
143 the yield of *S. tootsik* extract was 1.12±015%. HPLC analysis showed that the chemical profile
144 was unchanged (Figure 1). However, the chemical content reflected by peak area were about
145 29.8 times increased. Besides isoorientin, most of the other flavonoids identified in *S. tootsik*
146 were market unavailable. The calibration curves of isoorientin were Y = 24.82X, with
147 correlation coefficient of 0.999, where Y was the peak area and X was concentration of astilbin
148 (5-200 μg/ml). By submitted the area sum of peak 1 to 4 to the calibration curves, the total
149 flavonoids content of *S. tootsik* extract was 8.2±0.2%.

3.3 Pancreatic lipase inhibitory activity of S. tootsik extract

Obesity is becoming one of the biggest threats to human health around the world. Before being absorbed by the small intestine, fats in the food was first hydrolyzed by lipase into monoglycerol and free fatty acids. Thus, the inhibition of lipase could effectively reduce the absorption of fat in the diet, thereby controlling and treating obesity (Birari & Bhutani, 2007; Buchholz & Melzig, 2015). Many flavonoids from plant source show pancreatic lipase inhibitory activity, such as luteolin, genistein, hyperin, kaempferol, etc (Buchholz & Melzig, 2015). Lee et al. found that the C-glycosylated derivatives of luteolin on A-ring exhibited much stronger pancreatic lipase inhibitory activity than luteolin (Lee et al., 2010). The main identified flavonoids in *S. tootsik* were the C-glycosylated derivatives of luteolin and apigenin. Thus, the pancreatic lipase inhibitory activity of *S. tootsik* extract was studied in the present study. The result showed that the pancreatic lipase inhibitory activity of *S. tootsik* extract steadily increased with the concentration with IC50 value of 0.93 mg/mL (Figure 2). In comparison, the IC50 value of Orlistat, the clinically approved pancreatic lipase inhibitor, was 74 ng/mL. Fluorometric analysis showed that the addition of *S. tootsik* extract could gradually quench the endogenous fluorescence of pancreatic lipase (Figure 3). Furthermore, it also caused the red shift of maximum emission wavelength. These phenomenons implied that the flavonoids in *S. tootsik* extract could bind on the enzyme. Although the lipase inhibitory activity of *S. tootsik* extract was far weaker than Orlistat, as an abundant and safe natural product, it may also have potential to be used as food supplement for obesity controlling. The in *vivo* study of its anti-obesity was in

progress in our Lab.

CONCLUSION

The chemical profile of *S. tootsik* was studied by HPLC and UPLC-DAD-QTOF-MS. Eightteen compounds were identified, most of them were the C-glycosylated derivatives of luteolin and apigenin, such as isoorientin, isoorientin-2"-O-rhamnoside and isovitexin. Isoorientin-2"-O-rhamnoside was the most dominant flavonoid in the sample. *S. tootsik* extract was prepared through resin adsorption/desorption with yield of 1.12±015% and total flavonoids content of 8.2±0.2% (in term of isoorientin). The extract exhibited pancreatic lipase inhibitory activity with IC50 value of 0.93 mg/ml.

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Competing Interests

The authors declare there are no competing interests

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Table 1(on next page)

Table 1 Mass characterizations of main peak in the chromatogram of *Sinobambusa tootsik (Sieb.) Makino* by UPLC-QTOF-MS.

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Peak No.	RT (min)	$[M–H]^-$ (m/z)	Fragment ions (m/z) (% base peak)	Proposed structure	Reference
1	6.10	367.1033	193(45), 134(100), 117(10)	Feruloylquinic acid	Qiu et al., 2018
2	6.97	609.1449	519(25), 489(60), 399(70), 369(100)	Quercetin-3-O-robinobioside	Iswaldi et al., 2011
3	7.60	431.1915	385(15), 205(35), 153(100)	Roseoside	Spínola et al., 2015
4	7.78	519.1707	325(10), 265(33), 223(60), 205(100), 190(55),	Unidentified	
5	8.09	489.159	223(40), 205(100),190(80), 164(27)	Unidentified	
6	8.69	371.098	121(100), 249(45), 231(10)	Unidentified	
7	9.14	563.1393	353(100), 383(65), 443(45), 473(32)	Apigenin 6-C-pentoside-8-C-glucoside	Ozarowski et al., 2018
	9.14	447.0924	327(100), 357(70), 297(55), 285(35)	Isoorientin (luteolin 6-C-glucoside)	Figueirinha et al., 2008
8	9.26	593.1504	298(100), 473(85), 327(55), 309(40), 357(35), 429(25)	Isoorientin-2"-O-rhamnoside	Ibrahim et al., 2015
9	9.78	613.213	181(100), 387(85), 166(30),205(25),399(20)	Unidentified	
10	10.68	533.128	353(100), 383(90), 443(50), 473(40), 365(25), 297(23)	Apigenin 6, 8-di-C-pentoside	Ozarowski et al., 2018
	10.68	577.1546	293(100), 413(35), 323(15), 311(15), 457(10),	Isovitexin-2"-O-rhamnoside	Ibrahim et al., 2015
	10.71	431.0986	311(100), 341(35), 283(75)	Isovitexin (apigenin 6-C-glucoside)	Ibrahim et al., 2015
11	11.27	607.1649	323(100), 443(40),308(20),341(15)	Isoscoparin-O-deoxyhexoside	Ozarowski et al., 2018
	11.29	447.091	285(100))	Kaempferol-O-glucoside	Singh et al., 2011
12	12.89	561.1595	561(100), 457(30), 399(14), 337(18), 295(40)	Chrysin 6-C-deoxyhexoside-7-O-glucoside	Ozarowski et al., 2018

	13.01	637.1759	329(100), 314(15), 299(10)	3 ,4 -Dihydroxy-5,6-dimethoxy -7-O-rutinoside flavone	Han et al., 2007
13	13.41	547.1446	293(100), 383(85),341(35), 311(28)	Apigenin 6-C-[2"-O -deoxyhexoside] - pentoside	Ozarowski et al., 2018
14	13.69	577.1546	311(100),415(50),397(15)	Apigenin-6-C-deoxyhexoside-7-O-glucoside	Ozarowski et al., 2018
15	14.08	575.1392	325(100), 297(100), 411(100), 337(70), 285(70), 367(55)	"X"-O-Rhamnosyl C-(6-deoxy -pento-hexos-ulosyl) luteolin	Figueirinha et al., 2008
16	14.45	577.1549	311(100), 298(70), 415(70), 473(50), 327(35)	Apigenin-8-C-deoxyhexoside-7 -O-glucoside	Ozarowski et al., 2018
17	15.52	559.1441	457(10), 395(95), 321(100), 309(25), 293(50), 281(30), 269(60)	Apigenin-8-C-[6-deoxy-2-O-rhamnosyl]-xylo-Hexos-3-uloside	Bezerra et al., 2016
18	16.00	589.1554	425(100),351(65),325(35),299(35)	Unidentified	

Figure 1(on next page)

Figure 1 HPLC chromatogram of *S. tootsik* extract before (a) and after (b) resin purification.

Peaks: 1, Isoorientin-2''-O-rhamnoside; 2, *Isoorientin; 3,* Isovitexin-2''-O-rhamnoside; 4, Isoscoparin-O-deoxyhexoside; 5, Apigenin 6-C-[2"-O-deoxyhexoside]-pentoside.

Figure 1

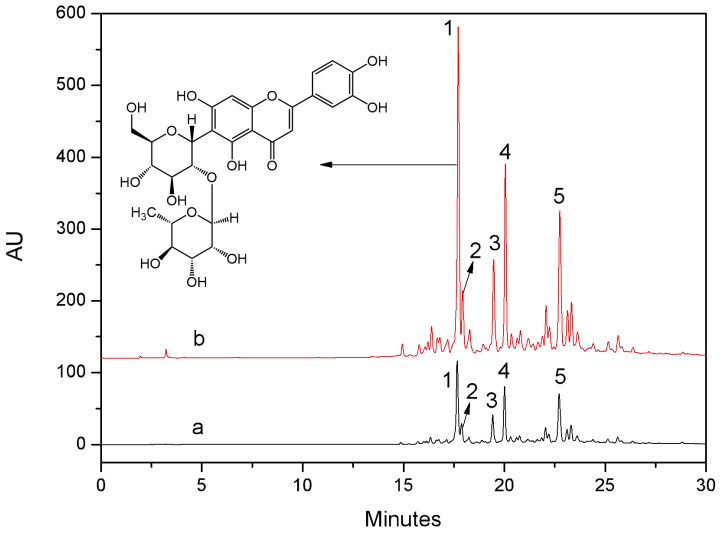

600
500
400
300
200
100
0
AU
0
5
10
15
20
25
30
Minutes
1
2
3
4
5
b
a
OH
HO
H
O
H3C

Figure 2(on next page)

Figure 2 The lipase inhibitory activity of *S. tootsik* extract.

Figure 2

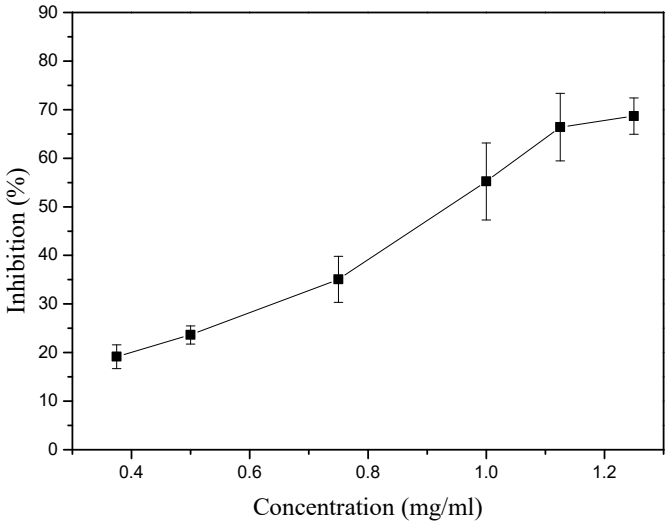

Inhibition (%)
Concentration (mg/ml)
0
10
20
30
40
50
60
70
80
90
0.4
0.6
0.8
1.0
1.2

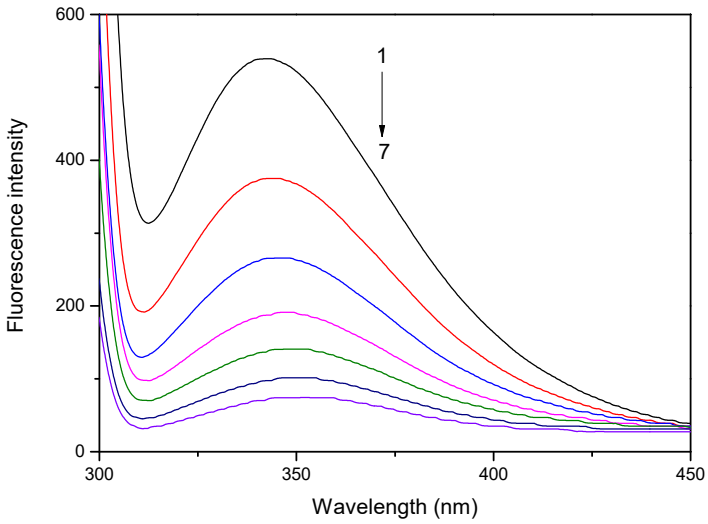
Figure 3(on next page)

Figure 3 The effect of *S. tootsik* extract on fluorescence emission spectra of pancreatic lipase.

The concentrations of *S. tootsik* extract from 1-7 were 0, 10, 20, 30, 40, 50, 60 μg/mL, respectively.

1 Figure 3

2



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