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% aqueous ethanol, followed by H103 macroporous 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 82±2 mg/g (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 a food supplement for obesity controlling.

1 **Chemical Profile and Pancreatic Lipase Inhibitory Activity of**

2 ***Sinobambusa tootsik* (Sieb.) Makino leaves**

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13 **ABSTRACT**

14 **Background.** *Sinobambusa tootsik* (Sieb.) Makino (*S. tootsik*) is one species of bamboo

15 distributed in China, Japan and Vietnam. The chemical profile of its leaves and its potential

16 application was unknown yet.

17 **Methods.** The chemical profile of *S. tootsik* was studied by HPLC and UPLC-DAD-QTOF-MS.

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19 macroporous resins adsorption and desorption processes. Pancreatic lipase inhibitory activity

20 was determined using *p*-nitrophenyl palmitate as the substance, which was hydrolyzed by lipase

21 to form coloured *p*-nitrophenol.

22 **Results.** Eighteen compounds were identified in *S. tootsik*. Most of them were the C-

23 glycosylated derivatives of luteolin and apigenin, such as isoorientin, isoorientin-2"-O-

24 rhamnoside and isovitexin. Isoorientin-2"-*O*-rhamnoside was the most dominant flavonoid in the

25 sample. *S. tootsik* extract was prepared through resin adsorption/desorption with yield of

26 1.12±015% and total flavonoids content of 82±2 mg/g (in term of isoorientin). The extract

27 exhibited pancreatic lipase inhibitory activity with IC50 value of 0.93 mg/mL.

28 **Conclusion.** The chemical profile of *S. tootsik* leaves was uncovered for the first time. C-

29 glycosyl flavonoids were the main constituents in the plant. The extract exhibited pancreatic

30 lipase inhibitory activity and may have potential to be used as a food supplement for obesity

31 controlling.

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

33 pancreatic lipase; inhibition

34

INTRODUCTION

35

36   Bamboo is a valuable plant distributed all over the world with more than 1500 species. The

37   bamboo shoots of some species, e.g. *Phyllostachys heterocycla* cv. *pubescens* (*P. heterocycla*),

38   were eaten as vegetable, while the leaves were used as herbal material in China. The flavonoids

39   extract of some bamboo species (*Phyllostachys* genus) were approved as the food antioxidant

40   and food resources in China (Wang et al., 2012). The pharmacological activities of bamboo

41   leaves were arise from the presence of phytochemicals. For instance, five C-Glycosyl flavones

42   were isolated from *Fargesia robusta* (Van Hoyweghen et al., 2010). Three chlorogenic acid

43   derivatives were isolated from *Phyllostachys edulis* and the antioxidant activity was evaluated

44   (Kweon et al., 2001). Wang et al. isolated three isoorientin derivatives from *Bambusa. textilis*

45   McClure (Wang et al., 2012). Previous, we identified twelve compounds in the leaves of

46   *Bambusa multiplex* cv. *Fernleaf* (*B. multiplex*), and found that C-glycosyl flavonoids including

47   vitexin, isovitexin, isoorientin and its derivatives, are the main chemical constitutes of the plant

48   (Qiu & Zhang, 2019). *Sinobambusa tootsik* (Sieb.) Makino (*S. tootsik*) is one species of bamboo

49   distributed in China, Japan and Vietnam. To the best of our knowledge, the chemical profile of

50   its leaves has not been studied yet. To further uncover its potential application, the chemical

51   composition of *S. tootsik* was studied by HPLC and UPLC-QTOF-MS in the present study.

52   Furthermore, the pancreatic lipase inhibition activity of its extract was studied.

MATERIALS AND METHODS

53

Chemicals and Plant materials

54

55 Leaves of *S. tootsik* were collected in Jiangxi Agricultural University (with east longitude of

56 115º50' and northern latitude of 28º46') on Mar. 2019. The plant material was authenticated

57 based on morphological characters by Prof. Qing-Pei Yang (Jiangxi Agricultural University),

58 and the voucher specimen was deposited in Jiangxi Key Laboratory of Natural Product and

59 Functional Food. The leaves was dried at 60 ºC and smashed to filter through 40 mesh sieve.

60 Isoorientin standard (>98%) was purchased from Beijing Solarbio Science & Technology Co.,

61 Ltd (Beijing, China). HPLC grade acetonitrile was purchased Anhui Tedia High Purity Solvents

62 Co., Ltd (Anqin, China). Porcine pancreatic lipase (extract powder, 15-35 units/mg, catalog

63 number L111237) was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China;

64 http://www.aladdin-e.com). Milli-Q water was used throughout the study. All other reagents

65 used were analytical grade.

66 **Sample extraction**

67 A 0.1 g aliquot of *S. tootsik* powder was mixed with 5.0 mL of 50% aqueous ethanol. After

68 sonicating for 30 min in a bath sonicator (100 W, 45 kHz, Kunshan, China), the mixture was

69 centrifuged at 1100*g* for 5 min. The supernatant was filtered by 0.22 mm pore size filter and then

70 used for HPLC and UPLC-DAD-QTOF-MS analysis.

71 For *S. Tootsik* extract preparation, 50 g of *S. tootsik* sample was extracted for twice with 500 mL

72 of 50% aqueous ethanol each time. After centrifugation at 1100*g* for 5 min, the supernatant was

73 combined together. The extract was condensed to about 500 mL by vacuum rotavapor at 50 ◦C.

74 The concentrates was two times diluted by water. Then, the extract was pumped through a fixed

75   bed of H103 macroporous resin with diameter of 1.5 cm and height of 40 cm in a glass column.

76   The flow rate was 10 mL/min. After adsorption, the fixed bed was desorbed with 4 BV of 90%

77   ethanol with flow rate of 5 mL/min. The eluent was concentrated by vacuum rotavapor at 50 ◦C

78   and then lyophilized to obtain the extract.

79   **UPLC-DAD-QTOF-MS analysis**

80   The chemical identification was performed on a QTOF 5600-plus mass spectrometer equipped

81   with Turbo V sources and a Turbolonspray interface (AB Sciex Corporation, Foster City, CA,

82   USA) coupled to a Shimadzu LC-30A UPLC-DAD system (Shimadzu Corporation, Kyoto,

83   Japan). Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters)was used. The

84   flow rate was 0.3 mL/min with injection volume of 1 μL and column temperature of 40 °C. The

85   mobile phase was acetonitrile (A) and 0.1% formic acid aqueous solution (B) using a linear

86   gradient program of   0-30min, 5-40% (A). The mass spectrometer was operated in the negative

87   ion mode. Ultrapure nitrogen was used as the ion source gas 1 (50 psi), ion source gas 2 (50 psi),

88   and curtain gas (40 psi). The Turbo Ion Spray voltage and temperature were set at -4500 V and

89   500 °C, respectively. Declustering potential, collision energy, and collision energy spread were

90   set at 100 V, -40 V, and 10 V, respectively. Data acquisition was performed with Analyst 1.6

91   software (AB Sciex).

92   **HPLC quantification Analysis**

93   The HPLC Analysis was performed on an Agilent 1260 HPLC system equipped with an

94   autosampler and DAD detector. A Symmetry C18 column (250 mm × 4.6 mm i.d., 5 μm; Waters,

95   USA) was used as the stationary phase. The mobile phase consisted of acetonitrile (A) and 0.1%

96   acetic acid aqueous solution (B). The flow rate was 1 mL/min with linear gradient program of 0-

97   30 min, 1-40% A; 30-35 min, 40% A. Detected wavelength was 349 nm with injection volume

98   of 10 μL and column temperature of 40 °C.

99   **Pancreatic lipase inhibitory activity assay**

100   Pancreatic lipase inhibitory activity was determined using *p*-nitrophenyl palmitate (*p*-NPP) as the

101   substance, which was hydrolyzed by lipase to form *p*-nitrophenol with maximum absorption

102   around 405 nm. Lipase (10 mg) was dissolved in 5 mL Tris-buffer (50 mM, pH 8, containing

103   0.1% gum arabic powder and 0.2% sodium deoxycholate). The mixture was stirred for 15 min

104   and centrifuged at 1800*g* for 10 min. The clear supernatant was used for the assay. Briefly, in a

105   96-well microplate, 30 μL Tris-buffer, 150 μL enzyme and 10 μL *S. tootsik* extract (dissolved in

106   50% ethanol) were mixed together. The mixture was incubated at 37 °C in the microplate reader

107   for 20 minutes. Then, 10 μL of 10 mM *p*-NPP pre-incubated at 37 °C was added to start the

108   reaction. The absorbance was determined under 405 nm for 20 min with interval of 1 min. The

109   absorbance growth slope (V) which represented the enzyme activity was calculated by linear

110   regression.

111   Lipase inhibition activity (%) $= \dfrac{V_b - V_s}{V_b} \times 100$

112   Where V_b and V_s were the enzyme activity in the absence and presence of *S. tootsik* extract,

113   respectively. Orlistat was used as the positive control. The same reaction mixture but without

114   lipase was used as the negative control, in which no absorbance change was found.

Fluorescence quench measurements

116   A 1.0 mL aliquot of the lipase solution was mixed with 4 mL of Tris-buffer. Subsequently, 0, 5,

117   10, 15, 20, 25 and 30 μL of *S. tootsik* extract (10 mg/mL in 50% aqueous ethanol) was added,

118   respectively. The fluorescence spectra of the mixture was recorded between 300 to 450 nm under

119   the excitation wavelength of 280 nm. A 970 CRT spectrofluophotometer (Shanghai Scientific

120   Instruments Limited Company, Shanghai, China) was used, and the excitation and emission

121   bandwidths were set at 10 nm.

Statistical Analysis

123   Data were expressed as the mean ± standard deviation (SD) of triplicates. Statistical analysis,

124   plotting, and curve fitting were performed by Origin 7.0 (Origin Lab Co., Northampton, MA,

125   USA).

Result and discussion

127   Figure 1 corresponds to the chromatograms of *S. tootsik* detected by QTOF-MS and DAD after

128   UPLC separation. By the QTOF-MS detector, the molecular mass of each peak and its further

129   MS^2 spectrum was obtained. The chemical identification was accomplished by comparing these

130   information with published literature. The details were listed in Table 1. A total of 18

131   components were identified. Most of them were the C-glycosylated derivatives of luteolin and

132 apigenin, such as isoorientin, isoorientin-2"-*O*-rhamnoside and isovitexin. Besides, some other

133 C-glycosyl and *O*-glycosyl flavonoids were found, such as isoscoparin-*O*-deoxyhexoside and

134 kaempferol-*O*-glucoside. Two non-flavonoid compounds, feruloylquinic acid and roseoside,

135 were also found. *S. tootsik* belongs to the family of *Poaceae*. Many studied showed that the main

136 secondary metabolite found in the leaves of *Poaceae* plants were C-glycosyl flavonoids, for

137 instance, barley, maize, wheat, rice, etc (Brazier-Hicks et al., 2009, Ferreres et al., 2008).

138 Previously, we have studied the chemical constituents in the leaves of *Bambusa multiplex cv.*

139 *Fernleaf* (*B. multiplex*), another bamboo species (Qiu & Zhang, 2019). It was found that C-

140 glycosylated derivatives of luteolin and apigenin were the main components of both species.

141 However, the specific flavonoids between the two plants were different. Only apigenin 6-C-

142 pentoside-8-C-glucoside, isoorientin and isovitexin were found in the both species.

143 Figure 2A was the HPLC chromatogram of *S. tootsik* detected at 349 nm. With the results of

144 UPLC-DAD-Q-TOF-MS analysis (Figure 1), the five main peaks in the HPLC chromatogram

145 were identified. The peak of isoorientin (peak 2) was further validated by comparing the

146 retention time and UV spectra with standard marker. Form the peak area, it was found that

147 isoorientin-2"-*O*-rhamnoside (peak 1) was the most dominant flavonoid in *S. tootsik*, followed by

148 isoscoparin-*O*-deoxyhexoside (peak 4) and apigenin 6-C-[2"-*O*-deoxyhexoside]-pentoside (peak

149 5). This was also different from *B. multiplex*, in which isoorientin was the most dominant

150 flavonoid, followed by isovitexin (Qiu & Zhang, 2019). The UV spectra of the five peaks were

151 presented in Figure 2B. It was found that the UV spectra of peaks 1, 2 and 4 were very similar

152   with maxmium absorption around 348 nm, while the maxmium absorption of peaks 3 and 5 was

153   around 338 nm.

154   **_3.2 S. tootsik extract preparation_**

155   Through 50% aqueous ethanol extraction, followed by H103 macroporous resins adsorption and

156   desorption processes, the yield of _S. tootsik_ extract was 1.12±015%. HPLC analysis showed that

157   the chemical profile was unchanged (Figure 2). However, the chemical content reflected by peak

158   area were about 29.8 times increased. Besides isoorientin, most of the other flavonoids identified

159   in _S. tootsik_ were market unavailable. The calibration curves of isoorientin were Y = 24.82X,

160   with correlation coefficient of 0.999, where Y was the peak area and X was concentration of

161   astilbin (5-200 μg/ml). By submitted the area sum of peak 1 to 5 to the calibration curves, the

162   total flavonoids content in _S. tootsik_ extract was calculated as 82±2 mg/g in term of isoorientin.

163   **_3.3 Pancreatic lipase inhibitory activity of S. tootsik extract_**

164   Obesity is becoming one of the biggest threats to human health around the world. Before being

165   absorbed by the small intestine, food fats need first be hydrolyzed by lipase into monoglycerol

166   and free fatty acids. Thus, the inhibition of lipase,   especially pancreatic lipase, could

167   effectively reduce the absorption of fat in the diet, thereby controlling and treating obesity

168   (Bialecka-Florjanczyk et al., 2018; Birari & Bhutani, 2007; Buchholz & Melzig, 2015; Yun,

169   2010). Thus, the finding of lipase inhibitor from natural source is getting more and more

170   attention. Many flavonoids from plant source show pancreatic lipase inhibitory activity, such as

171   luteolin, genistein, hyperin, kaempferol, etc (Buchholz & Melzig, 2015). Lee et al. found that the

172   C-glycosylated derivatives of luteolin on A-ring exhibited much stronger pancreatic lipase

173   inhibitory activity than luteolin (Lee et al., 2010). The main identified flavonoids in *S. tootsik*

174   were the C-glycosylated derivatives of luteolin and apigenin. Thus, the pancreatic lipase

175   inhibitory activity of *S. tootsik* extract was studied in the present study. The result showed that

176   the pancreatic lipase inhibitory activity of *S. tootsik* extract steadily increased with the

177   concentration, and the IC50 value was about 0.93 mg/mL (Figure 3). In comparison, the IC50

178   value of Orlistat, the clinically approved pancreatic lipase inhibitor, was 74 ng/mL. Many plant

179   extracts with components of saponins, phenolic acids, and/or flavonoids, possess pancreatic

180   lipase inhibitory effects (Buchholz & Melzig, 2015;de la Garza et al., 2011). For instance,

181   *Crocus cancellatus* subsp. *damascenus* extract with main constituents of catechin, ferulic and

182   caffeic acids, induced 50.39% of inhibition of lipase activity at 5 mg/mL (Loizzo et al., 2016).

183   The acetic extracts of *Aronia melanocarpa* L. and its cyanidin-3-glucoside fraction exhibited

184   pancreatic lipase inhibitory activities with IC50 values of 83.45 and 1.74 mg/mL, respectively

185   (Worsztynowicz et al., 2014). The IC50 value of *Moricandia arvensis* (L.) DC methanolic

186   extract with main constituents of flavonoid glycosides was 2.06 mg/mL, while the IC50 value of

187   Orlistat was 0.018 mg/mL (Marrelli et al., 2018).

188   Fluorometric analysis showed that the addition of *S. tootsik* extract could gradually quench the

189   endogenous fluorescence of pancreatic lipase (Figure 4). Furthermore, it also caused the red shift

190   of maximum emission wavelength. These phenomenons implied that the flavonoids in *S. tootsik*

191 extract could bind on the enzyme. If the ligand is a monomeric compound, the fluorescence

192 titration results can be further used to calculated the binding constant and binding site of the

193 complexes using Stern-Volmer equations. However, in the present study, the *S. tootsik* extract is

194 a mixture without definite molecular weight, and Stern-Volmer equations can't be applied.

195   Although the lipase inhibitory activity of *S. tootsik* extract was far weaker than Orlistat, as an

196 abundant and safe natural product, it may also have potential to be used as a food supplement for

197 obesity controlling. The in *vivo* study of its anti-obesity is in progress in our Lab.

198 **CONCLUSION**

199 The chemical profile of *S. tootsik* was studied by HPLC and UPLC-DAD-QTOF-MS. Eighteen

200 compounds were identified, most of them were the C-glycosylated derivatives of luteolin and

201 apigenin, such as isoorientin, isoorientin-2"-O-rhamnoside and isovitexin. Isoorientin-2"-O-

202 rhamnoside was the most dominant flavonoid in the sample. *S. tootsik* extract was prepared

203 through resin adsorption/desorption with yield of 1.12±0.15% and total flavonoids content of

204 82±2 mg/g (in term of isoorientin). The extract exhibited pancreatic lipase inhibitory activity

205 with IC50 value of about 0.93 mg/mL.

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209 **Competing Interests**

210   The authors declare there are no competing interests

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

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	Han et al., 2007

				-7-O-rutinoside flavone	
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

The chromatograms of *S. tootsik* detected by QTOF-MS (a) and DAD (b) after UPLC separation.

Line a: base peak chromatogram of QTOF-MS; Line b: detected at 349 nm.
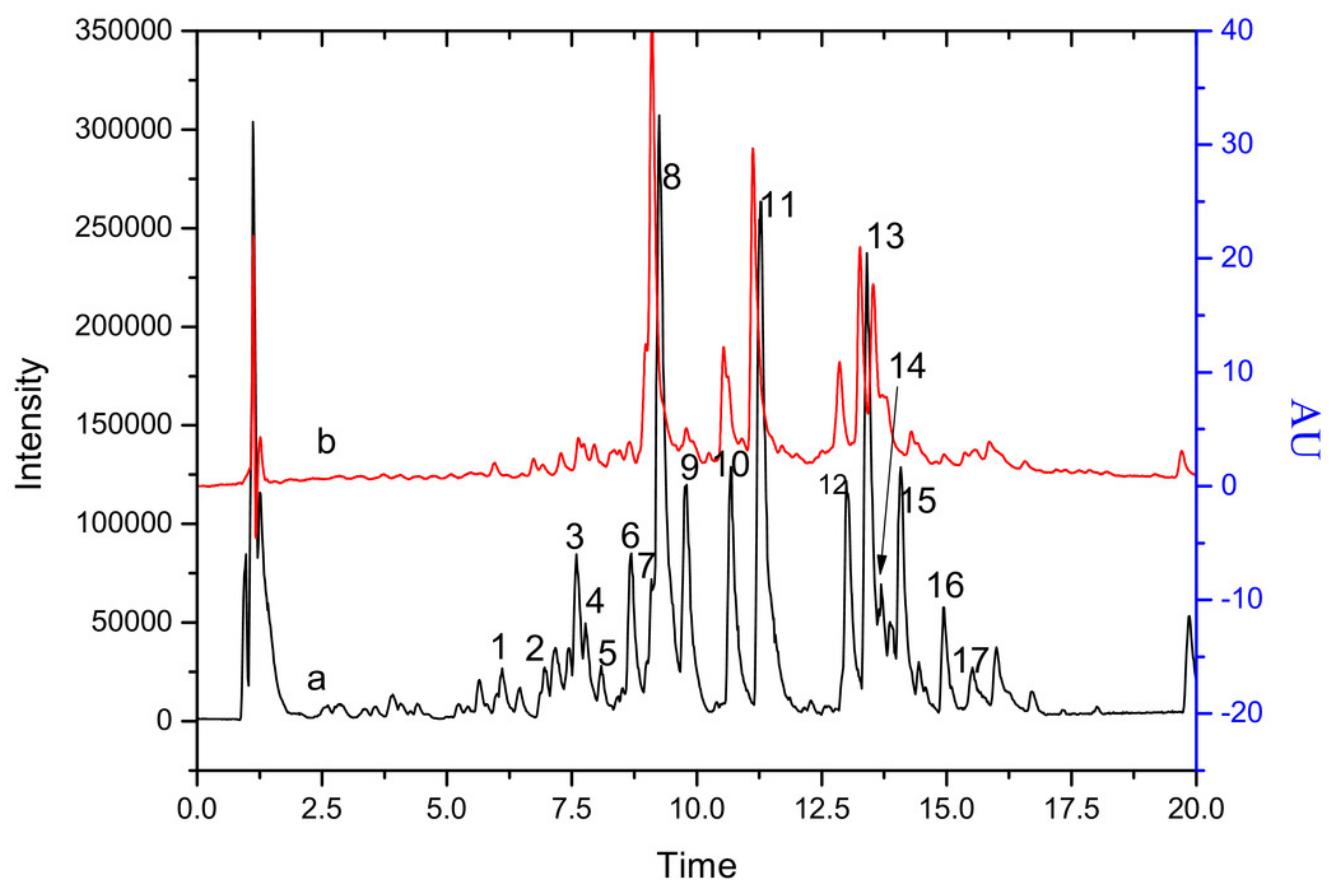
Figure 2

A: HPLC chromatogram of *S. tootsik* extract before (a) and after (b) resin purification; B: The UV spectra of peak 1-5.

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.
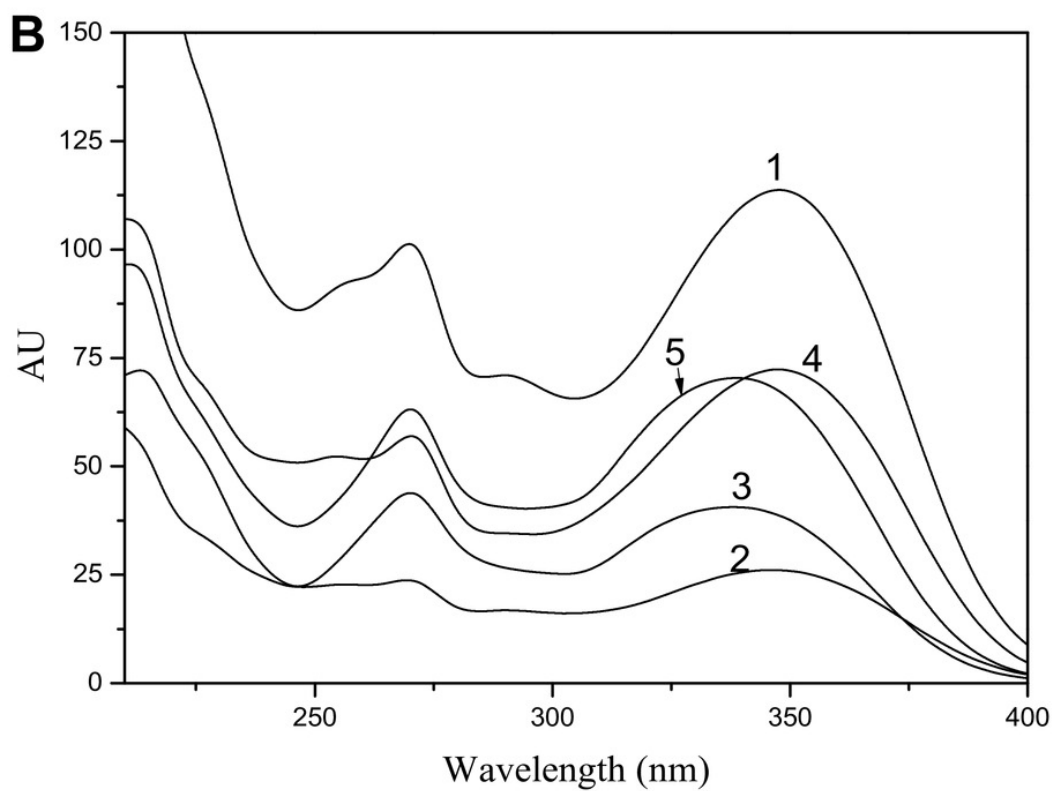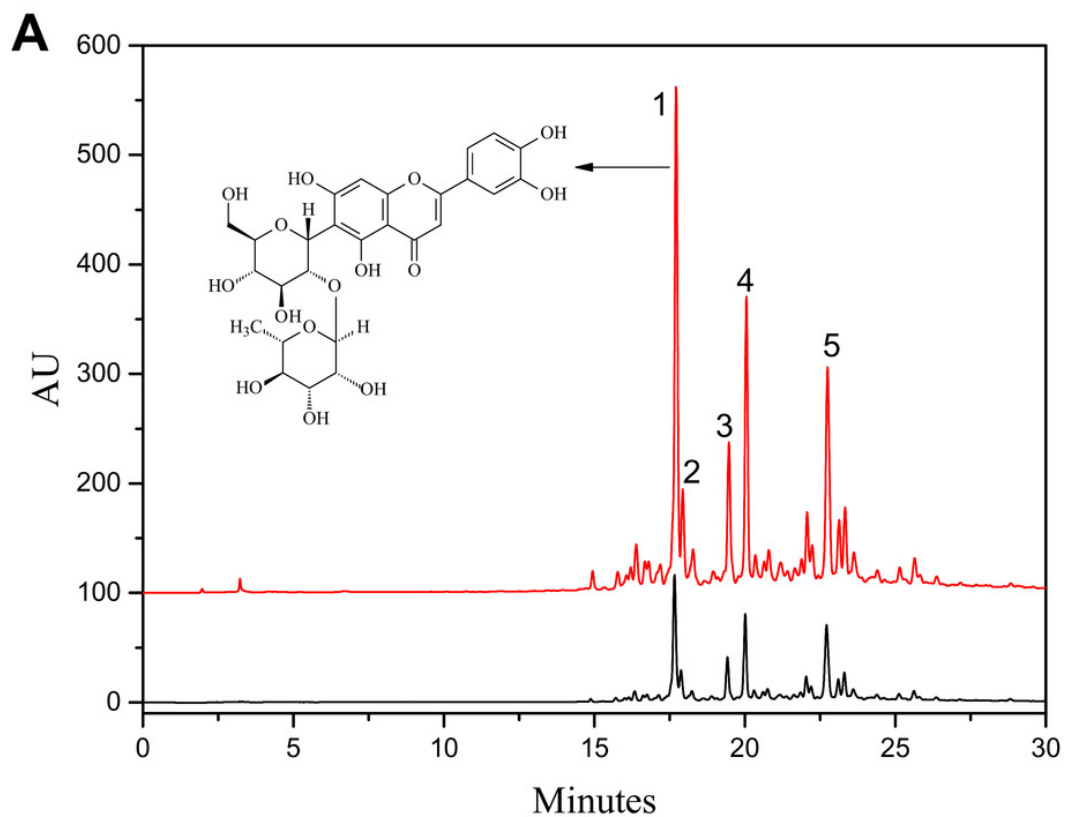
Figure 3

The lipase inhibitory activity of *S. tootsik* extract.
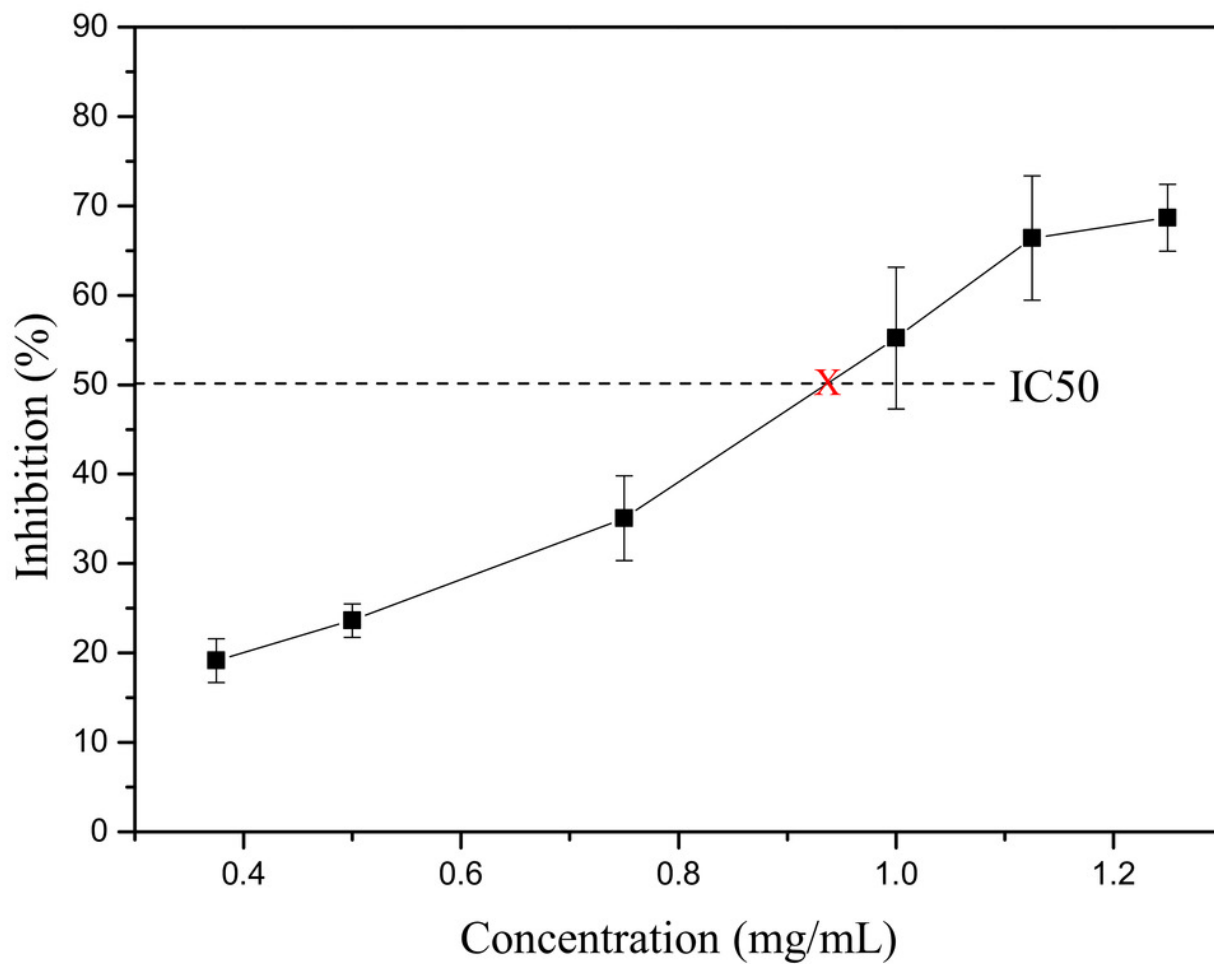
Figure 4

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.