SUPPLEMENTARY MATERIAL

A New Anti-proliferative Acylated Flavonol Glycoside from Fuzhuan Brick-Tea

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Abstract:

Fuzhuan Brick-tea (FBT) is unique for a fungal fermentation stage in its manufacture process and is classified in dark tea. A new acylated flavonol glycoside, kaempferol 3-*O*-[*E*-*p*-coumaroyl-($\rightarrow$ 2)][α -L-arabinopyranosyl-(1 $\rightarrow$ 3)][α -L-rhamnopyranosyl(1 $\rightarrow$ 6)]- β -D-glucopyranoside, which was trivially named as camellikaempferoside A (**1**), was isolated from the Fuzhuan brick-tea along with camelliquercetiside C (**2**). Their structures were unambiguously elucidated by combination of spectroscopic and chemical methods. Compound **1** showed anti-proliferative activity against MCF-7 and MDA-MB-231 cells with IC₅₀ values of 7.83 μ M and 19.16 μ M, respectively.

Keywords:

Fuzhuan brick-tea; dark tea; camellikaempferoside A; anti-proliferative activity; MCF-7 cells; MDA-MB-231 cells

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HPLC analysis of Some FGs in FBT

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Experimental

1. General

UV spectrum was obtained with a U-5100 spectrophotometer (Hitachi, Japan). IR spectrum was obtained with an iS50 FI-IR spectrometer (Thermo, the USA). Optical rotation was measured on a P-1020 Polarimeter (Jasco, Tokyo, Japan). ^1H and ^{13}C NMR, ^1H - ^1H COSY, HSQC, HMBC and ROESY spectra were recorded with Bruker AM-400 spectrometers operating at 400 MHz for ^1H , and 100 MHz for ^{13}C . Coupling constants were expressed in Hz and chemical shifts were given on a δ (ppm) scale with TMS as an internal standard. The HRESI-MS was obtained with a Thermo LTQ Orbitrap XL LC-MS system by direct inlet. The GC-MS analysis was conducted on a GCMS-QP2010S (Shimadzu, Japan) with DB-5MS column (30 m $\times$ 0.25 μm i.d., Agilent Technologies, the USA), using the following conditions: injection temperature at 280 $^{\circ}\text{C}$, initial temperature was 160 $^{\circ}\text{C}$ for 1 min, then raised to 200 $^{\circ}\text{C}$ at 6 $^{\circ}\text{C}/\text{min}$, and further raised to 280 $^{\circ}\text{C}$ at 3 $^{\circ}\text{C}/\text{min}$, final temperature kept for 5 min. HPLC analyses were performed on a Waters Alliance 2695 HPLC system equipped with a binary solvent manager, sample manager, column heating compartment, photodiode array (PDA) detector and controlled by Waters Empower software (Waters, Milford, MA, the USA). A XP ODS-A C18 column (4.6 $\times$ 250 mm i.d., 5 μL , H&E Co. Ltd., China) was employed with column temperature maintained at 30 $^{\circ}\text{C}$. All solvents and samples were filtered through a 0.22 μm membrane prior to chromatography analysis. The mobile phases A and B were 0.17% aqueous acetic acid and acetonitrile, respectively. The gradient of solvent B was as follows: 0–4 min, 6%; 4–16 min, from 6% to 14%; 16–18 min, from 14% to 15%; 18–32 min, from 15% to 18%; 32–34 min, 18%; 34–37 min, from 18% to 29%; 37–45 min, from 29% to 45%; 45–50 min, 45%; 50–51 min, from 45% to 6%; then kept at 6% for 10 min. Solvent flow rate was 1.0 mL/min and a 10 μL injection of each sample was made onto the column. MCI-gel CHP20P (20–100 μm , Mitsubishi Chemical Co. Ltd., Japan), Sephadex LH-20 (25–100 μm , Pharmacia Fine Chemical Co. Ltd., Sweden), ODS-A C18 silica gel (50 μm , YMC Co. Ltd., Japan), and polyamide (100–200 mesh, Luqiaosijia Biochemical Co. Ltd., Zhejiang, China) were used for CC. Polyamide CC was carried out with the eluant of 40% aqueous MeOH. Aqueous MeOH from 0 to 100% (v/v) in increments of 10% was used as eluant for all Sephadex LH-20, and ODS-A CC. Silica gel (GF254, Liangchen Chemical Co. Ltd., Huoshan, China) thin layer chromatography experiments were performed with chloroform/methanol/ H_2O (7:3:0.5, v/v), or ethyl acetate/methanol/ H_2O (100:17:13, v/v), and spots were detected by UV illumination followed by spraying with 2% ethanolic FeCl_3 . The standard sugar of L-rhamnose was purchased from Shanghai Hushi Laboratorial Equipment Co. Ltd., D-glucose and L-arabinose were purchased from Sigma, the USA. 5-FU, MTT, and DMSO

for the cytotoxic activity assay were purchased from Sigma Chemical Co. Ltd. Media and serum were purchased from Gibco (Invitrogen Co. Ltd., the USA). Standard FGs of camellikaempferoside A (**1**), camelliquercetiside C (**2**), myricetin-3-rutinoside (**3**), quercetin-3-*O*-[β -D-glucopyranosyl-(1 $\rightarrow$ 3)-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)-*O*- β -D-glucopyranoside] (**4**), vitexin-2''- α -L-rhamnopyranoside (**5**), rutin (**6**), kaempferol-3-*O*-[β -D-glucopyranosyl-(1 $\rightarrow$ 3)-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)-*O*- β -D-glucopyranoside] (**7**) were isolated from FBT in our laboratory (Luo et al. 2013). The purity was not less than 95% by HPLC analysis. Myricetin (**8**), quercetin (**9**), kaempferol (**10**) were purchased from Shanghai Ronghe Science Technology Development Co., Ltd. Trimethylsilylimidazole for sugar derivation was purchased from Adamas Reagent Co., Ltd., Switzerland.

2. Materials

FBT (produced in December, 2006) was purchased from Yiyang Fu Cha Industry Development Co. Ltd. (Yiyang, Hunan Province, PR China). The tea samples for HPLC analysis (BFFT and AFFT, respectively produced in March and April 2012 as the same production batch) were supplied by the same company. The samples of AFFT and BFFT were stored in -20°C before being prepared for HPLC analysis.

3. Extraction and Isolation

The 70% aqueous acetone extract of commercial FBT (3.6 kg) was separated successively by partitioning with petroleum ether, CHCl_3 , *n*-BuOH and Water. The *n*-BuOH solution was concentrated under reduced pressure to afford a brown residue (500 g) (Luo et al. 2013). This residue was applied to a Sephadex LH-20 CC and eluted by MeOH- H_2O (from 0:1 to 1:0, 10% stepwise gradient, each 1.5 L), yielding three fractions (S1-S3). The fraction S2 was further purified by MCI-gel CC with MeOH- H_2O (from 0:1 to 1:0, 10% stepwise gradient, each 0.5 L) as the mobile phase, obtaining eleven fractions (S2-a to S2-k). The fraction S2-h was purified using polyamide CC with acetone- H_2O (1:2, v/v), giving seven fractions (S2-h-1 to S2-h-7). The fraction S2-h-5 was purified using ODS CC with MeOH- H_2O (5:4, v/v) to afford compounds **1** (39.5 mg) and **2** (14 mg).

4. Spectroscopic Data

Camellikaempferoside A: Kaempferol 3-*O*-[*E*-*p*-coumaroyl-($\rightarrow$ 2)][α -L-arabinopyranosyl-

(1→3)][α -L-rhamnopyranosyl(1→6)]- β -D-glucopyranoside (**1**), yellow amorphous powder; $[\alpha]_D^{25} - 88.9383$ (c 0.0027, MeOH). IR (KBr) cm^{-1} : 3428, 2923, 2853, 1655, 1606, 1514, 1448, 1362, 1261, 1208, 1171, 1081, 834, 581, 519. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 215 (4.22), 268 (3.95), 315 (4.10). ESI-MS: m/z 895 $[\text{M} + \text{Na}]^+$, 1767 $[2\text{M} + \text{Na}]^+$ in positive mode. HRESI-MS: m/z 895.2252 (calcd. for $\text{C}_{41}\text{H}_{44}\text{O}_{21}\text{Na}^+$, 895.2267) in positive mode. ^1H and ^{13}C NMR data, see Table S1.

5. Determination of Sugar Components

The determination of monosaccharides in **1** was established by a slightly modified method reported in the references (Li et al. 2014; Zong et al. 2015). Compound **1** (0.8 mg) was dissolved in 2 M HCl (0.8 mL) and heated at 80°C for 4 h. The reaction mixture was extracted with chloroform (0.8 mL $\times$ 2), and then the aqueous layer was freeze dried to give a mixture of monosaccharides. The residue was dissolved in a pyridine solution (0.2 mL) of L-cysteine methyl ester hydrochloride (10 mg/mL) and reacted at 60°C for 2 h, the solvent was evaporated *in vacuo*, and 0.2 mL trimethylsilylimidazole was added. Then the mixture was heated at 70 °C for 1.5 h. After partition between hexane (0.5 mL) and H_2O , the hexane extract was analysed by GC-MS. The standard sugars were prepared by the same method. The retention times of D-glucose, L-rhamnose and L-arabinose were 21.67 min, 18.33min and 16.76 min, respectively (Figure S11).

6. Cell Culture

MCF-7 and MDA-MB-231 cells, were generously donated by Prof. Zhijie Chang (Tsinghua University School of Medicine, Beijing, PR China), and were kept in our lab and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). All the cells were kept at 37°C in a 5% CO_2 containing atmosphere. Media and serum were purchased from Gibco (Invitrogen Corporation, NY).

7. MTT Assay

Anti-proliferative activity assay of **1** was carried out by MTT colorimetric method with slight modification (Yang et al. 2010). MCF-7 and MDA-MB-231 cells were seeded on 96-well plates at a density of 1×10^3 cell/well overnight. Adherent cells were antiseptically treated with 5-FU or different concentrations of **1** for 72 h. Then 20 μL 5 mg/ml MTT was added into each well of the 96-well plates. After incubation for 4 h, the cells culture medium was discarded and the purple precipitate attached in the bottom of the plates were dissolved in DMSO for 10 min in the dark.

Bio-Rad model 680 microplate reader was used to obtain the absorbance at 570 nm/630 nm.

HPLC Analysis of Some FGs in FBT

The FGs **2-7** were isolated from FBT in our experiments and were applied for quantitation (Luo et al. 2013). The content of **1** was not able to be calculated for its low concentration, though it could be detected in HPLC-PDA of BFFT and AFFT (Figure S15).

1. Preparation of Standard Solutions for HPLC Analysis

Compounds **1-10** were respectively weighed and dissolved in 70% aqueous methanol by ultrasonic treatment [with the concentrations of 0.00875 (**1**), 0.029575 (**2**), 0.1 (**3**), 0.32 (**4**), 0.101 (**5**), 0.122 (**6**), 0.27 (**7**), 0.02 (**8**), 0.020625 (**9**), 0.016625 (**10**) mg/mL, respectively]. The linear calibration curves contained six different concentrations of each standard compound diluted with 70% aqueous methanol. All samples were stored in $-20\text{ }^{\circ}\text{C}$ until analysis.

2. Preparation of Tea Extracts for HPLC Analysis

The whole bricks of BFFT and AFFT were milled into powder and dehydrated, respectively. Each of tea samples (2.500 g) was saturated in 70% aqueous methanol (100 mL) for 12 h at room temperature, during which an ultrasonic bath was carried out twice for 15 min. A 2 mL-aliquot of the liquid extract was centrifuged at 10,000 rpm for 10 min and then the supernatant was passed through a $0.22\text{ }\mu\text{m}$ filter disk for HPLC analysis. The experiments were performed in triplicate, and the results of quantitation analysis were expressed as mean values with standard deviations.

3. Statistical Analysis

The data in Figure S16 were presented as means $\pm$ SD. The values were evaluated by the Student's *t*-test using Excel 2007 Software (Microsoft Software Inc.). Differences were considered as * ($p < 0.05$) and ** ($p < 0.01$).

4. Identification of 2-10 in BFFT and AFFT

The peaks were identified by the retention times of authentic samples, which were 43.096 (**1**), 42.335 (**2**), 27.210 (**3**), 30.763 (**4**), 32.074 (**5**), 33.497 (**6**), 36.812 (**7**), 41.723 (**8**), 45.013 (**9**), and 47.926 (**10**) min, respectively. In addition, the peaks of **1** in chromatography of BFFT and

AFFT were confirmed by HPLC-PDA method, in which the UV spectra (200–400 nm) were compared with that of **1** (Figure S14). Compound **1** was not indicated as a product of fungal fermentation as it was detected in both BFFT and AFFT.

5. Quantitation of 2-10 in BFFT and AFFT

The concentration of **1** was too low to be quantitated by the current method. The contents of **2-10** were calculated and compared between BFFT and AFFT. The contents of quercetin and its *O*-glycosides (**4**, **6** and **9**) were decreased significantly after fungal fermentation procedure. While the contents of vitexin-2''- α -L-rhamnopyranoside (**5**) and camelliquercetiside C (**2**) were increased (Figure S16, Table S5).

6. Method Validation for the Quantitation of Some FGs (2-10) in FBT

6.1. Linearity

A series of solutions of **2-10** were prepared to establish the linearity of the proposed method. Calibration curves showed good linearity over the concentration range in all samples with correlation coefficients (R) larger than 0.999 (Table S2).

6.2. Precision, Repeatability and Stability

The precision were assessed by determining standard samples of stock solution at medium concentration levels continuously on the same day (n=5). The repeatability were determined by assaying parallel FBT samples repeatedly on the same day (n=5). The stability was determined by tea samples on 0, 4, 8, 12 and 24h. Relative standard deviations (RSDs) of the precision, repeatability and stability were all less than 7% (Table S3).

6.3. Limit of Detection and the Lower Limit of Quantification

The limits of detection (LOD, S/N=3) for **2-10** were 0.45, 0.38, 0.42, 0.45, 0.71, 0.54, 0.2, 0.43, and 0.61 $\mu\text{g/mL}$, respectively. The lower limits of quantification (LOQ, S/N=10) for **2-10** were 1.44, 1.08, 1.24, 1.36, 2.31, 1.56, 0.53, 1.26, and 1.95 $\mu\text{g/mL}$, respectively.

6.4. Recoveries

A certain amount of reference substances (low, medium, and high concentrations) were added into a certain amount of BFFT or AFFT samples (precisely weighted 0.25 g), respectively, and were then extracted and analysed under the proposed method. Three replicate extractives at each level were used to determine the extraction recovery rates and the RSDs were calculated. The recoveries of the investigated FGs were between 90-110% with RSD values less than 5.0% (Table S4).

In addition to camellikaempferoside A, the present work discovered that the contents of **4**, **6** and **9** were decreased by fungal fermentation, though no significant change was observed on variety of FGs between BFFT and AFFT (Figure S12). Therefore, the metabolic mechanism of the microorganisms on these FGs may be interesting research areas in future (Figure S12), which will help to understand the generation mechanism of the special flavor and functions of Fuzhuan brick-tea.

References

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Figure S1: UV spectrum of **1**

Figure S2: IR spectrum of **1**

Optical rotation measurement								
Model : P-1020 (A060460638)								
No.	Sample	Mode	Data	Monitor Blank	Temp. Cell Temp Point	Date Comment Sample Name	Light Filter Operator	Cycle Time Integ Time
No.1	12 (1/3)	Sp.Rot	-89.4810	-0.1208 0.0000	15.0 50.00 Cell	Wed Jan 16 16:45:06 2013 0.00270g/mlMeOH CSC-28	Na 589nm	2 sec 10 sec
No.2	12 (2/3)	Sp.Rot	-88.8890	-0.1200 0.0000	15.0 50.00 Cell	Wed Jan 16 16:45:19 2013 0.00270g/mlMeOH CSC-28	Na 589nm	2 sec 10 sec
No.3	12 (3/3)	Sp.Rot	-88.4440	-0.1194 0.0000	15.0 50.00 Cell	Wed Jan 16 16:45:33 2013 0.00270g/mlMeOH CSC-28	Na 589nm	2 sec 10 sec

Figure S3: Optical rotation of **1**

Figure S4: HRESI-MS spectrum of **1**

Figure S5: ^1H -NMR of **1** (in Methanol- d_4)

Figure S6: ^{13}C -NMR of **1** (in Methanol- d_4)

Figure S7: ^1H - ^1H COSY of **1** (in Methanol- d_4)

Figure S8: HSQC of **1** (in Methanol- d_4)

Figure S9: HMBC of **1** (in Methanol- d_4)

Figure S10: NOESY of **1** (in Methanol- d_4)

Figure S11: GC spectral comparison of the sugar units

Figure S11A: The retention time of *D*-glucose (21.67 min)

Figure S11B: The retention time of *L*-rhamnose (18.33 min)

Figure S11C: The retention time of *L*-arabinose (16.76 min)

Figure S11D: The retention times of the sugar units of **1**

Figure S12: HPLC (350 nm) analysis for UF-1 to UF-28 in BFFT and AFFT

[A: HPLC (350 nm) of UF-1 to UF-28; B: the chromatographic area comparison of each UFs between BFFT and AFFT]

The chromatographic peaks of UF (unidentified FGs)-1 to UF-28 were nevertheless characterized as FGs preliminary by their UV spectra (see Figure S-13), though the exact structures of them were unknown yet.

The mystery of fungal metabolism on FGs in FBT

Figure S13: UV spectra of UF-1 to UF-28 in HPLC

Figure S14: UV spectra of the chromatography peaks (t_R 43.096) in HPLC of **1**, AFFT and BFFT

Figure S15: HPLC (350 nm) of the BFFT, AFFT and standard compounds **1-10**

Figure S16: Quantitation analysis of **2-10** in BFFT and AFFT

Table S1: NMR data of **1** and partial ¹³C NMR data of **2**

Positions	1 ^a			2 ^b
	δ _H (<i>J</i> in Hz)	δ _C	HMBC (¹ H to ¹³ C)	δ _C
2	–	158.98		158.7
3	–	134.72		134.8
4	–	179.05		178.8
5	–	163.06		162.9
6	6.050 d (2.0)	99.84	C-5, 7, 8, 10	99.8
7	–	165.73		165.6
8	6.245 d (2.0)	94.80	C-6, 7, 9, 10	94.8
9	–	158.43		158.2
10	–	105.85		105.8
1'	–	122.85		
2', 6'	7.887 d (8.8)	132.26	C-2, 4'	
3', 5'	6.796 d (8.8)	116.25	C-1', 4'	
4'	–	161.39		
Glc				
1	5.505 d (8.0)	100.73	C-3, Glc-6	100.8
2	5.077 dd (9.2, 8.4)	74.50	C-Glc-(1, 3), Cou-1	74.6
3	3.762 c	84.34	C-Glc-4, Ara-1	84.5
4	3.364 e	70.26	C-Glc-(3, 5)	70.3
5	3.406 m	76.96		77.0
6a	3.772 c	68.25	C-Glc-5, Rha-1	68.3
6b	3.356 e		C-Glc-5, Rha-1	
Ara				
1	4.238 d (6.8)	105.33	C-Glc-3, Ara-(3, 5)	105.3
2	3.431 d	72.22	C-Ara-1	72.3
3	3.380 e	73.91		74.0
4	3.674 m	69.54		69.9
5 ^a	3.772 c	67.23	C-Ara-1	67.3
5b	3.465 m		C-Ara-1	
Rha				
1	4.455 d (1.6)	102.42	C-Glc-6, Rha-(2, 3, 6)	102.4
2	3.565 dd (3.2, 1.6)	72.09	C-Rha-(1, 3, 4)	72.2
3	3.431 d	72.29	C-Rha-4	72.3
4	3.179 t (9.2)	73.89	C-Rha-(2, 3, 5, 6)	74.0
5	3.364 e	69.80	C-Rha-(1, 6)	69.9
6	1.033 d (6.0)	17.91	C-Rha-(4, 5)	18.1
<i>p</i> -Cou				
C=O	–	168.62		168.6
α	6.282 d (16.0)	115.18	C- <i>p</i> -Cou-(1, 1')	115.1
β	7.584 d (16.0)	147.26	C- <i>p</i> -Cou-(1, 2, 2', 6')	147.2
1	–	127.34		127.3
2, 6	7.362 d (8.4)	131.31	C- <i>p</i> -Cou-(3, 4')	131.3
3, 5	6.705 d (8.4)	116.79	C- <i>p</i> -Cou-(1, 2', 4', 6')	116.1
4	–	161.27		161.1

^{a, b} measured in methanol-*d*₄^{c, d, e} signals were overlapped.Glc: glucopyranosyl, Ara: arabinopyranosyl, Rha: rhamnopyranosyl, *p*-Cou: *E-p*-hydroxycoumaroyl

Table S2: Linear calibration information for **2-10**

compounds	calibration curves	R
2	$Y = 1.88\text{e}+007 X - 2.11\text{e}+004$	0.999209
3	$Y = 1.38\text{e}+007 X - 1.42\text{e}+004$	0.999486
4	$Y = 8.41\text{e}+006 X - 2.03\text{e}+004$	0.999612
5	$Y = 1.55\text{e}+007 X - 1.30\text{e}+004$	0.999683
6	$Y = 1.77\text{e}+007 X - 1.59\text{e}+004$	0.999651
7	$Y = 6.95\text{e}+006 X - 9.31\text{e}+003$	0.999765
8	$Y = 1.23\text{e}+007 X - 1.64\text{e}+004$	0.999384
9	$Y = 7.31\text{e}+006 X - 1.02\text{e}+003$	0.999455
10	$Y = 2.48\text{e}+007 X - 1.44\text{e}+004$	0.999226

Table S3: Precision, repeatability and stability of 2-10

compounds	Precision (RSD/%)	Repeatability (RSD/%)	Stability (RSD/%)
2	2.6	2.7	5.6
3	0.8	2.6	3.3
4	1	1.5	1.4
5	0.9	2.1	3.6
6	0.4	1.6	1.9
7	0.1	3.6	1.6
8	3.6	3.3	6
9	1.1	3	6.2
10	1.1	5.5	4.5

Table S4: Recoveries of **2-10**

Compounds	Content in sample /mg	Added (mg)	Theoretical value (mg)	Measured quantity (mg)	Recovery rate (%)	Average recovery rate (%)	RSD (%)
2	0.0534	0.0015	0.0549	0.0513	93.43	98.45	4.88
	0.0533	0.0015	0.0548	0.0509	92.98		
	0.0528	0.0015	0.0543	0.0506	93.27		
	0.0534	0.0030	0.0564	0.0562	99.65		
	0.0535	0.0030	0.0565	0.0555	98.34		
	0.0531	0.0030	0.0561	0.0596	106.28		
	0.0534	0.0060	0.0594	0.0607	102.24		
	0.0541	0.0060	0.0601	0.0578	96.26		
	0.0538	0.0060	0.0598	0.0619	103.61		
3	0.1263	0.0050	0.1313	0.1257	95.71	100.51	3.68
	0.1269	0.0050	0.1319	0.1298	98.41		
	0.1267	0.0050	0.1317	0.1374	104.36		
	0.1265	0.0100	0.1365	0.1342	98.27		
	0.1264	0.0100	0.1364	0.1408	103.22		
	0.1266	0.0100	0.1366	0.1328	97.18		
	0.1268	0.0200	0.1468	0.1444	98.37		
	0.1262	0.0200	0.1462	0.1505	102.93		
	0.1270	0.0200	0.1470	0.1561	106.17		
4	0.9895	0.0160	1.0055	0.9939	98.85	101.60	4.13
	0.9868	0.0160	1.0028	0.9818	97.90		
	0.9845	0.0160	1.0005	0.9855	98.51		
	0.9869	0.0320	1.0189	1.0808	106.07		
	0.9852	0.0320	1.0172	1.0561	103.83		
	0.9845	0.0320	1.0165	1.0125	99.61		
	0.9873	0.0640	1.0513	1.0168	96.71		
	0.9890	0.0640	1.0530	1.1392	108.19		
	0.9885	0.0640	1.0525	1.1018	104.69		
5	0.0999	0.0051	0.1050	0.1091	103.91	103.33	2.81
	0.0997	0.0051	0.1047	0.1130	107.89		

	0.0993	0.0051	0.1043	0.1095	104.94		
	0.0992	0.0101	0.1093	0.1163	106.43		
	0.0996	0.0101	0.1097	0.1109	101.10		
	0.0988	0.0101	0.1089	0.1077	98.90		
	0.0999	0.0202	0.1201	0.1215	101.17		
	0.0997	0.0202	0.1199	0.1236	103.13		
	0.0998	0.0202	0.1200	0.1230	102.50		
6	0.2753	0.0061	0.2814	0.2807	99.72	103.02	3.66
	0.2803	0.0061	0.2864	0.2800	97.78		
	0.2768	0.0061	0.2829	0.2948	104.20		
	0.2758	0.0122	0.2880	0.2966	102.98		
	0.2754	0.0122	0.2876	0.2857	99.33		
	0.2750	0.0122	0.2872	0.3148	109.62		
	0.2766	0.0244	0.3010	0.3134	104.14		
	0.2761	0.0244	0.3005	0.3107	103.40		
	0.2788	0.0244	0.3032	0.3215	106.03		
7	0.3742	0.0135	0.3877	0.3972	102.44	101.42	3.56
	0.3746	0.0135	0.3881	0.3695	95.20		
	0.3756	0.0135	0.3891	0.3847	98.87		
	0.3768	0.0270	0.4038	0.4022	99.58		
	0.3785	0.0270	0.4055	0.4285	105.67		
	0.3782	0.0270	0.4052	0.4174	103.00		
	0.3799	0.0540	0.4339	0.4276	98.54		
	0.3756	0.0540	0.4296	0.4472	104.09		
	0.3751	0.0540	0.4291	0.4523	105.40		
8	0.1659	0.0010	0.1669	0.1585	94.94	97.79	3.08
	0.1663	0.0010	0.1673	0.1581	94.55		
	0.1610	0.0010	0.1620	0.1590	98.15		
	0.1659	0.0020	0.1679	0.1562	93.04		
	0.1663	0.0020	0.1683	0.1657	98.47		
	0.1659	0.0020	0.1679	0.1650	98.24		

	0.1669	0.0040	0.1709	0.1705	99.73		
	0.1663	0.0040	0.1703	0.1707	100.29		
	0.1650	0.0040	0.1690	0.1735	102.67		
9	0.1219	0.0010	0.1229	0.1208	98.27	101.06	4.02
	0.1232	0.0010	0.1242	0.1220	98.21		
	0.1235	0.0010	0.1245	0.1205	96.76		
	0.1219	0.0021	0.1239	0.1349	108.85		
	0.1232	0.0021	0.1252	0.1246	99.50		
	0.1235	0.0021	0.1255	0.1224	97.47		
	0.1219	0.0041	0.1260	0.1311	104.03		
	0.1232	0.0041	0.1273	0.1307	102.70		
	0.1235	0.0041	0.1276	0.1324	103.74		
10	0.0622	0.0008	0.0630	0.0603	95.71	102.35	3.89
	0.0626	0.0008	0.0634	0.0672	105.93		
	0.0622	0.0008	0.0630	0.0653	103.61		
	0.0621	0.0017	0.0638	0.0629	98.70		
	0.0625	0.0017	0.0642	0.0693	107.94		
	0.0622	0.0017	0.0638	0.0631	98.92		
	0.0621	0.0033	0.0654	0.0674	103.04		
	0.0622	0.0033	0.0655	0.0687	104.76		
	0.0622	0.0033	0.0656	0.0672	102.57		

Table S5: The contents^a of **2-10** in BFFT and AFFT

compounds	BFFT (mg/g)	AFFT (mg/g)
1 ^b	–	–
2	0.1996 ± 0.0085	0.2193 ± 0.0058
3	0.1784 ± 0.0017	0.1793 ± 0.0031
4	3.4575 ± 0.0145	3.2983 ± 0.0106
5	0.1395 ± 0.0052	0.1522 ± 0.0039
6	0.8128 ± 0.0027	0.7864 ± 0.0074
7	1.0438 ± 0.0041	1.0149 ± 0.0169
8	0.2511 ± 0.0059	0.2516 ± 0.0055
9	0.1089 ± 0.0008	0.0847 ± 0.0012
10	0.0403 ± 0.0010	0.0419 ± 0.0013

^a Mean ± SD (n = 3).

^b Less than the quantifiable limit.