ORIGINAL ARTICLE

Flavanols from the *Camellia sinensis* var. *assamica* and their hypoglycemic and hypolipidemic activities

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KEY WORDS

*Camellia sinensis* var. *assamica*; Pu-erh tea; Flavanol; Hypoglycemic; Hypolipidemic

Abstract  
α-Glucosidase and lipase inhibitors play important roles in the treatment of hyperglycaemia and dyslipidemia. To identify novel naturally occurring inhibitors, a bioactivity-guided phytochemical research was performed on the pu-erh tea. One new flavanol, named (−)-epicatechin-3-O-(Z)-coumarate (1), and 16 known analogs (2−17) were isolated from the aqueous extract of the pu-erh tea. Their structures were determined by spectroscopic and chemical methods. Furthermore, the water extract of pu-erh tea and its fractions exhibited inhibitory activities against α-glucosidases and lipases *in vitro*; compound 15 showed moderate inhibitory effect against sucrase with an IC$_{50}$ value of 32.5 μmol/L and significant inhibitory effect against maltase with an IC$_{50}$ value of 1.3 μmol/L. Compounds 8, 10, 11 and 15 displayed moderate activity against a lipase with IC$_{50}$ values of 16.0, 13.6, 19.8, and 13.3 μmol/L, respectively.

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1. Introduction

Type 2 diabetes mellitus (DM) is a metabolic disorder that is characterized by hyperglycemia caused by insulin resistance. It has become the third largest chronic noninfectious disease threatening the world. The classic symptoms include excess thirst, frequent urination, and constant hunger. Long-term complications from high blood sugar include heart disease, strokes, diabetic retinopathy and kidney failure. The use of herbal remedies to treat DM has been practiced since ancient times. Currently, more than 1000 plant species are being used as folk medicines to treat DM throughout the world.

Tea is one of the most popular beverages consumed worldwide. According to the manufacturing process, Chinese commercial teas can be classified as green tea, yellow tea, white tea, oolong tea, black tea, and dark tea. Pu-erh tea is a kind of dark tea, which comes from “rough” Camellia sinensis (Linn.) var. assamica (Masters) Kitamura (mao cha) in Yunnan province in China. The key production process of pu-erh tea is secondary fermentation, in which microorganisms play a very important role in producing the taste, color, fragrance, and functional components. Pu-erh tea is not only a popular tea but also a traditional Chinese medicine, which has many biological and biochemical effects, such as antiobesity, antiviral, antioxidative, hepatoprotective, medicine, which has many biological and biochemical effects, Pu-erh tea is not only a popular tea, but also a traditional Chinese tea. The EtOAc section of the water extract from the pu-erh tea was purified by preparative HPLC, affording one new flavanol (1) and 16 known compounds (Fig. 1). These compounds were identified as (-)-epicatechin-3-O-(Z)-coumarate (1), (-)-epicatechin-3-O-(E)-coumarate (2), (-)-epicatechin-3-O-(E)-caffeate (3)\textsuperscript{10}, (-)-catechin (4)\textsuperscript{1}, amppelopin (5)\textsuperscript{10}, (-)-epicatechin (6)\textsuperscript{1}, (-)-epicatechin-3-O-gallate (7),\textsuperscript{11} (-)-epiafzelechin-3-O-gallate (8),\textsuperscript{11} (-)-epiafzelechin-3-O-gallate (9),\textsuperscript{11} (-)-catechin-3-O-gallate (10)\textsuperscript{12}, (+)-epiafzelechin-3-O-gallate (11)\textsuperscript{12}, epicatechin-3-O-p-hydroxybenzoate (12)\textsuperscript{12}, (-)-epigallocatechin (13)\textsuperscript{1}, (+)-gallocatechin (14)\textsuperscript{1}, (-)-epigallocatechin-3-O-gallate (15)\textsuperscript{1}, (+)-gallocatechin-3-O-gallate (16)\textsuperscript{1} and (-)-epicatechin-3-O-(3′-O-methyl)-gallate (17)\textsuperscript{1,16}.

Compound 1 was obtained as a pale amorphous powder and showed absorption bands for hydroxy group (3276 cm\textsuperscript{-1}), aromatic double bond (1623, 1604 and 1514 cm\textsuperscript{-1}), and carbonyl group (1709 cm\textsuperscript{-1}) in the IR spectrum. Its molecular formula, C\textsubscript{24}H\textsubscript{20}O\textsubscript{8}, was deduced from HR-ESI-MS (m/z 437.1242 [M+H]\textsuperscript{+}). In the \textsuperscript{1}H NMR spectrum (Table 1), an ABX system of protons were observed at δ\textsubscript{H} 6.88 (1H, d, J = 1.8 Hz), 6.66 (1H, d, J = 8.0 Hz) and 6.69 (1H, dd, J = 1.8, 8.0 Hz); 2 methine proton signals at δ\textsubscript{H} 5.79 (1H, d, J = 1.8 Hz, 1H, d, J = 1.8 Hz) were assigned to the H-8 and H-6 in the A ring, respectively; 2 oxygenated methine proton signals at δ\textsubscript{H} 4.99 (1H, br, s) and 5.32 (1H, br s), and 1 methylene proton signal at δ\textsubscript{H} 2.91 (1H, dd, J = 4.0, 17.5 Hz) and 2.64 (1H, br d, J = 17.5 Hz) were ascribable to the C ring in flavanol; 2 cis-olefinic protons at δ\textsubscript{H} 6.25 (1H, d, J = 12.5 Hz) and 7.40 (1H, d, J = 12.5 Hz) indicated the presence of cis-double bond. Four aromatic proton signals were assigned to a p-hydroxy benzene ring at δ\textsubscript{H} 7.49 (2H, d, J = 8.5 Hz, H-5″, -9″) and 6.75 (2H, d, J = 8.5 Hz, H-6″, -8″). In the \textsuperscript{13}C NMR spectrum (Table 1), 24 carbon signals, including 1 ketone carbon, 1 methylene carbon, 2 oxygenated methine carbons, 2 cis-olefinic carbons and 18 aromatic carbons, were also observed. In the HMBC spectrum (Fig. 2), correlations to a ketone carbon at δ\textsubscript{C} 165.2 (C-1′) from 2 cis-olefinic protons of H-2″ and H-3″ indicated the presence of a\textsubscript{β}-unsaturated ketone group. Correlations from H-3” to C-1′, C-5” and C-9” and from H-2′″ to C-1″ indicated the presence of a (Z)-coumaroyl moiety. The oxygenated methine proton H-3 was correlated with the carbonyl carbon signal at δ\textsubscript{C} 165.2 (C-1′), confirming that the (Z)-coumaroyl moiety was connected to the 3-OH. Based on these data, the planar structure of compound 1 can be deduced.

Compounds 1 has 2 chiral centers at C-2 and C-3. The 2-cis configuration of I was determined on the basis of a broad singlet at δ\textsubscript{H} 4.99 for H-2 and an upfield shift of C-2 to δ\textsubscript{C} 76.2\textsuperscript{19,20}. The optical rotation [(α\textsubscript{R})\textsubscript{D} = +66.8 (c 0.025, MeOH)] and CD data [217 (Δε = -9.96), 267 (Δε = +1.24) and 314 (Δε = -4.05) nm, MeOH] of 1 were found to be similar to those of (-)-epicatechin-3-O-(E)-caffeate [(α\textsubscript{R})\textsubscript{D} = +175.5 (c 0.21, MeOH); 233 (Δε = -4.13), 273 (Δε = +1.21) and 321 (Δε = -4.37) nm, MeOH]\textsuperscript{10}. Thus, the absolute configuration of compound 1 was determined to be 2R and 3R. The structure of compound 1 was determined as (-)-epicatechin-3-O-(Z)-coumarate.

2. Results and discussion

2.1. Compounds from pu-erh tea

The EtOAc section of the water extract from the pu-erh tea was subjected to multiple column chromatographic purification steps and further purified by preparative HPLC, affording one new flavanol (1) and 16 known compounds (Fig. 1). These compounds were identified as (-)-epicatechin-3-O-(Z)-coumarate (1), (-)-epicatechin-3-O-(E)-coumarate (2), (-)-epicatechin-3-O-(E)-caffeate (3), (+)-catechin (4), ammpelopin (5), (-)-epicatechin (6), (+)-epicatechin-3-O-gallate (7), (-)-epiafzelechin-3-O-gallate (8), (-)-epiafzelechin-3-O-gallate (9), (+)-catechin-3-O-gallate (10), (+)-epiafzelechin-3-O-gallate (11), epicatechin-3-O-p-hydroxybenzoate (12), (-)-epigallocatechin (13), (+)-gallocatechin (14), (-)-epigallocatechin-3-O-gallate (15), (+)-gallocatechin-3-O-gallate (16) and (-)-epicatechin-3-O-(3′-O-methyl)-gallate (17).

Figure 1 Chemical structure of compounds 1–17.
and 13
gallate (15 also measured using the same methods. (Table 3). In addition, compounds
μ32.5
moderate inhibitory activity with the IC50 values of 14.4
measured. The results showed that the EtOAc fraction had
extract of pu-erh tea and its fractions on sucrose and maltase was
from pu-erh tea. To that end, the inhibitory activity of the water
extract of pu-erh tea. The EtOAc fraction showed strong
activity, which indicated that the functional materials were
isolated from the EtOAc fraction. The compounds isolated from the EtOAc
fraction also showed inhibitory activity against \( \alpha \)-glucosidases and
lipase. Compounds 1, 8, 9, 10, 12, and 17 showed weak activities with
IC50 values ranging from 27.3 to 63.1 \( \mu \)mol/L (Table 3).

Lipase inhibitors could reduce the uptake of fat from food
intake. The inhibitory activity of water extract and compounds 1–
17 against a lipase was further evaluated. The results showed that
the EtOAc fraction displayed strong activity with an IC50 value of
7.52 \( \mu \)g/mL (Table 2), and compounds 8, 10, and 15 displayed
good activities with IC50 values of 16.0, 13.6, and 13.3 \( \mu \)mol/L,
respectively (Table 3). And compounds 4–6, 9, 11, 13, and 16
showed weak activities with IC50 values ranging from 19.8 to
62.6 \( \mu \)mol/L (Table 3).

3. Conclusions

One new flavanol and 16 analogs were isolated from the water
extract of pu-erh tea. The EtOAc fraction showed strong in vitro
activity, which indicated that the functional materials were
enriched in this fraction. The compounds isolated from the EtOAc
fraction also showed inhibitory activity against \( \alpha \)-glucosidases and
a lipase. Compounds 11 and 15 displayed good activity, which
may have the potential to be developed as therapeutic agents. The
current data suggested that the activity of catechin-type compounds
might be associated with the galloyl group.

4. Experimental section

4.1. General experimental procedures

The optical rotations were measured using a Jasco P2000
polarimeter, UV spectra were determined using a Jasco V650
spectrophotometer (JASCO, Corporation, Tokyo, Japan). IR spec-
tra were carried out on a Nicolet 5700 spectrophotometer with KBr
disks (Thermo Electron Scientific Instruments Corp.). \(^1\)H NMR
(500 MHz), \(^{13}\)C NMR (125 MHz), HSQC and HMBC spectra
were run on a Mercury-500 with TMS as an internal standard
(Varian, Palo Alto, CA, USA). HR-ESI-MS was performed on a
6520 Accurate-Mass Q-TOF LC/MS mass spectrometer. Sephadex
LH-20 (Pharmacia, Uppsala, Sweden), silica gel (Qingdao Marine
Institute).

| No. | \( \delta_\text{H} \) (s) | \( \delta_\text{C} \) | No. | \( \delta_\text{H} \) (dd, \( J = 1.8 \) Hz) | \( \delta_\text{C} \) |
|-----|----------------|--------|-----|----------------|--------|
| 2   | 4.99 (s)      | 76.2   | 4   | 6.66 (d, \( J = 8.0 \) Hz) | 144.9  |
| 3   | 5.32 (br s)   | 67.7   | 5   | 6.69 (dd, \( J = 1.8, 8.0 \) Hz) | 117.4  |
| 4   | 2.91 (dd, \( J = 4.0, 17.5 \) Hz, H-a), 2.64 (br d, \( J = 17.5 \) Hz, H-b) | 25.5   | 6   | 7.40 (d, \( J = 8.0 \) Hz) | 165.5  |
| 5   |                |        | 1   |                | 156.5  |
| 6   | 5.93 (d, \( J = 1.8 \) Hz) | 95.5   | 2   | 6.25 (d, \( J = 12.5 \) Hz) | 115.0  |
| 7   | 156.6          | 3     | 3   | 7.40 (d, \( J = 12.5 \) Hz) | 143.6  |
| 8   | 5.79 (d, \( J = 1.8 \) Hz) | 94.3   | 4   |                | 125.2  |
| 9   | 155.4          | 5     | 5   | 7.49 (d, \( J = 8.5 \) Hz) | 132.5  |
| 10  | 97.2           | 6     | 6   | 6.75 (d, \( J = 8.5 \) Hz) | 114.9  |
| 1'  | 129.2          | 7     | 7   |                | 158.9  |
| 2'  | 6.88 (d, \( J = 1.8 \) Hz) | 114.2  | 8   | 6.75 (d, \( J = 8.5 \) Hz) | 114.9  |
| 3   | 144.9          | 9     | 9   | 7.49 (d, \( J = 8.5 \) Hz) | 132.5  |

\(^a\)Data were measured in DMSO-\( d_6 \) for 1 (500 MHz for \(^1\)H NMR and 125 MHz for \(^{13}\)C NMR).
Chemical Factory, 200–300 mesh), and RP-18 (Merck, 40–60 μm) were used for CC and silica gel GF-254 (Qingdao Marine Chemical Factory, Qingdao, China) was used for TLC. The HPLC experiments were performed on a preparative YMC-Pack ODS-column (250 mm × 20 mm, 10 μm, YMC, Kyoto, Japan) equipped with a Shimadzu SPD-6A UV spectrophotometric detector and an SPD-6AD pumping system (Shimadzu, Japan).

4.2. Plant material

Dried pu-erh tea was purchased from “Da de sheng” tea-shop in Beijing, China, in August 2011 and authenticated by associate professor Lin Ma from Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (IMM). A voucher specimen (S-2441) was deposited at the Herbarium of IMM.

4.3. Extraction and isolation

4.3.1. Isolation of I

Dried pu-erh tea (10.0 kg) was extracted three times with 20 L boiling water for 50 min. Then the solvent was evaporated and the resulting residue (3.4 kg) was suspended in H2O and extracted with EtOAc for 3 times, yielding a concentrated extract (1.4 kg). The EtOAc fraction (1.4 kg) was subjected to CC (silica gel; CHCl3–MeOH, 1:0–0.1) to obtain fractions A1–A6. Fr. A2 (127 kg) was submitted to repeated CC (silica gel, CHCl3–MeOH, 9:1–0.1) to afford 4 fractions, Fr. B1–B4. Fr. B2 (32 g) was further purified using CC (RP-C18 silica gel, MeOH–H2O, 95:1–0.1) to obtain fractions C1–C7. Fr. C3 (4.7 g) was separated by the RP-MLPC using an ODS column, and further purified by preparative HPLC to obtain 4 (85 mg), 5 (27 mg) and 6 (49 mg). Fr. C4 (3.2 g) was subjected to an NKA resin column (EtOH–H2O, 0:1–1:0), and further purified by Sephadex LH-20 CC and preparative HPLC to afford 7 (47 mg). Fr. C5 (2.1 g) and Fr. C6 (1.4 g) were subjected to Sephadex LH-20 CC (MeOH–H2O, 0:1–1:0) and further purified by preparative HPLC to obtain 8 (14 mg), 9 (46 mg), 10 (36 mg), 11 (21 mg), 12 (53 mg) and 17 (25 mg). Fr. B3 (22 g) was separated on a RP-C18 silica gel column eluting with MeOH–H2O (5:95–1:0) to afford 7 fractions, Fr. D1–D7. Fr. D3 (11 g) and Fr. D7 (4.8 g) were subjected to repeated CC purifications (NKA resin, EtOH–H2O 0:1–1:0; Sephadex LH-20, MeOH–H2O 0:1–1:0) and further purified by preparative HPLC to obtain 15 (12 mg), 16 (8 mg), 1 (19 mg) and 2 (6 mg). Using analogous separation and purification procedures as for Fr. B4 (13 g), Fr. B6 (18 g) afforded 3 (5 mg), 13 (23 mg) and 14 (50 mg).

4.3.2. Identification of I

White amorphous powder, [α]D20^20 66.8 (c 0.025, MeOH); UV (MeOH) λmax (log ε) 208 (3.02), 314 (3.51) nm; IR (KBr) νmax 3276, 1709, 1623, 1604, 1514 cm⁻¹; 1H NMR (DMSO-d6, 500 MHz), and 13C NMR (DMSO-d6, 125 MHz), see Table 1; positive ion mode HR-ESI-MS m/z 437.1242 [M+H]^+ (Calcd. for C24H21O8 437.1231).

4.4. In vitro hypoglycemic and hypolipidemic activities

4.4.1. Assessment of α-glucosidase inhibitory activity

Rat small intestinal brush border membrane vesicles were prepared and a suspension of this material in 0.1 mol/L phosphate buffer (pH 6.0) was used as the small intestinal α-glucosidases of maltase, sucrase, isomaltase and trehalase. The enzyme suspension was diluted to hydrolyze sucrose to produce D-glucose in the following reaction. Reaction was performed in a 96-well plate. The substrate (sucrose: 100 mg/dL), test compounds and the enzyme in 0.1 mol/L phosphate buffer (pH 6.0, 0.2 mL) were incubated together at 37 °C. After 30 min of incubation, the plate was immediately heated to 80–85 °C for 3 min to stop the reaction, then cooled. Glucose concentration was

### Table 3

| Compd. | Sucrase Inhibition (%) | IC50 (μmol/L) | Maltase Inhibition (%) | IC50 (μmol/L) | Lipase Inhibition (%) | IC50 (μmol/L) |
|--------|------------------------|----------------|------------------------|----------------|------------------------|----------------|
| 1      | 45.4                   | –              | 71.8                   | 49.7           | 42.6                   | –              |
| 2      | 71.3                   | 62.1           | –                      | –              | 41.6                   | –              |
| 3      | 64.5                   | 107.1          | –                      | –              | 36.3                   | –              |
| 4      | 21.5                   | 74.6           | 16.1                   | –              | 64.9                   | 39.7           |
| 5      | 90.4                   | 74.6           | 92.0                   | 199.9          | 92.1                   | 20.4           |
| 6      | 39.2                   | –              | 48.5                   | –              | 70.4                   | 32.2           |
| 7      | 31.5                   | –              | 25.5                   | –              | 44.9                   | –              |
| 8      | 61.9                   | 104.6          | 84.7                   | 27.3           | 91.8                   | 16.0           |
| 9      | 83.5                   | 84.2           | 91.4                   | 63.1           | 97.0                   | 43.9           |
| 10     | 79.2                   | 71.9           | 89.7                   | 27.5           | 96.4                   | 13.6           |
| 11     | 88.1                   | 59.4           | 91.8                   | 15.8           | 95.9                   | 19.8           |
| 12     | 41.2                   | –              | 67.6                   | 61.8           | 55.8                   | –              |
| 13     | 84.6                   | 71.3           | –                      | –              | 89.5                   | 51.7           |
| 14     | 93.8                   | 78.1           | 78.5                   | –              | 56.7                   | –              |
| 15     | 92.7                   | 32.5           | 98.7                   | 1.3            | 93.8                   | 13.3           |
| 16     | 77.5                   | 78.7           | –                      | –              | 75.8                   | 62.6           |
| 17     | 41.9                   | –              | 68.7                   | 60.3           | 47.5                   | –              |

Acarbose (IC50 value for sucrose: 0.97 μmol/L, for maltase: 0.13 μmol/L) and Orlistat (IC50 value for lipase: 0.012 μmol/L) were used as positive control.

– Not applicable.

*Inhibition at the concentration of 10⁻⁵ mol/L.
determined by the glucose oxidase method. The assay was performed in triplicates with five different concentrations around the IC₅₀ values. The IC₅₀ values were calculated from the dose–response curves, thus being obtained in the experiments.

4.4.2. Assessment of lipase inhibitory activity
An enzyme buffer was prepared by the addition of 40 μL (5 mg/mL) of a solution of porcine pancreatic lipase in Tris buffer (pH 7.1) 20 μL of compounds or orlistat at the test concentration was mixed with the enzyme buffer, and incubated for 10 min at 37 °C. Then, 5 μL of the substrate solution (10 mg/mL triglyceride) was added and the enzymatic reaction was allowed to proceed for 60 min at 37 °C. Pancreatic lipase activity was determined by measuring the hydrolysis of triglyceride to glycerol, which was monitored at 500 nm using a plate reader. The inhibition of lipase activity was observed by IC₅₀ values. The IC₅₀ values were calculated from the dose–response curves.

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Appendix A. Supplementary material
Supplementary data associated with this paper can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2016.12.007.

References
1. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. *Phytotherapy Research* 1995;2:137–89.
2. Lu HP, Gu JP, Lin Z, Guo L, Tan JF, Peng QH, et al. Advance in the study on the chemical composition and biological activity of Pu-erh Tea. *J Tea Sci* 2007;27:8–18.
3. Cao ZH, Gu DH, Lin QY, Xu ZQ, Huang QC, Rao H, et al. Effect of pu-erh tea on body fat and lipid profiles in rats with diet-induced obesity. *Phytotechnology* 2011;25:234–8.
4. Wang D, Luo X, Zhong Y, Yang W, Xu MJ, Liu Y, et al. Pu-erh black tea extract supplementation attenuates the oxidative DNA damage and oxidative stress in Sprague–Dawley rats with renal dysfunction induced by subchronic 3-methyl-2-quinoxalino benzenevinylketo-1,4-dioxide exposure. *Food Chem Toxicol* 2012;50:147–54.
5. Tu SH, Chen MY, Chen LC, Mao YT, Ho CH, Lee WJ, et al. Pu-erh tea extract attenuates nicotine-induced foam cell formation in primary cultured monocytes: an in vitro mechanistic study. *J Agric Food Chem* 2016;64:3186–95.
6. Wang TD, Lin HY, Kuo DH, Tsai SJ, Shieh JC, Wu JC, et al. Pu-erh tea attenuates hyperlipogenesis and induces hepatoma cells growth arrest through activating AMP-activated protein kinase (AMPK) in human HepG2 cells. *J Agric Food Chem* 2009;57:5257–64.
7. Du WH, Peng SM, Liu ZH, Shi L, Tan LF, Zou XQ. Hypoglycemic effect of the water extract of Pu-erh tea. *J Agric Food Chem* 2012;60:1026–32.
8. Lv HP, Zhu Y, Tan JF, Guo L, Dai WD, Lin Z. Bioactive compounds from Pu-erh tea with therapy for hyperlipidaemia. *J Fun Food* 2015;19:194–203.
9. Wu XD, Cheng JT, He J, Zhang XJ, Dong LB, Dong LB, et al. Benzophenone glycosides and epicatechin derivatives from *Malania oleifera*. *Fitoterapia* 2012;83:1068–71.
10. Liu F, Li FS, Peng ZM, Yang YN, Jiang JS, Li L, et al. Neurprotective naphthalene and flavan derivatives from *Polygonum cuspidatum*. *Phytochemistry* 2015;110:150–9.
11. Zhou ZH, Yang CR. Chemical constituents of crude green tea, the material of Pu-er tea in Yunnan. *Acta Bot Yunnanica* 2000;22:343–50.
12. Lin YP, Chen TY, Tseng HW, Lee MH, Chen ST. Neural cell protective compounds isolated from *Phoenix hanceana var. formosana*. *Phytochemistry* 2009;70:1173–81.
13. Huang B, Huang ZY, Wu LB, Li W, Jiang B, Li SH. Chemical constituents from *Cassia agnes* Brenan. *Nat Prod Res Dev* 2012;24:437–9.
14. Ivanov SA, Nomura K, Malfanov IL, Sklyar IV, Ptitsyn LR. Isolation of a novel catechin from Bergenia rhizomes that has pronounced lipase-inhibiting and antioxidative properties. *Fitoterapia* 2011;82:212–8.
15. Wan SB, Chan TH. Enantioselective synthesis of afzelechin and epiafzelechin. *Tetrahedron* 2004;60:8207–11.
16. Hashimoto F, Nonaka GI, Nishioka I. Tannins and related compounds LVI isolation of four new acylated flavan-3-ols from Oolong Tea. *Chem Pharm Bull* 1987;35:611–6.
17. Petereit F, Kolodziej H, Nahrstedt A. Flavan-3-ols and proanthocyanidins from *Cistus incanus*. *Phytochemistry* 1991;30:981–5.
18. Saijo R. Isolation and chemical structures of two new catechins from fresh tea leaf. *Agric Biol Chem* 1982;46:1969–70.
19. Slade D, Ferreira D, Marais JP. Circular dichroism, a powerful tool for the assessment of absolute configuration of flavonoids. *Phytochemistry* 2005;66:2177–215.
20. Zeng XB, Qiu Q, Jiang CG, Jing YT, Qiu QF, He XJ. Antioxidant flavanes from *Livistona chinensis*. *Fitoterapia* 2011:82:609–14.