

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Tea silkworm droppings as an enriched source of tea flavonoids

Tzu-Yun Chou^a, Meei-Ju Yang^b, Shih-Kung Tseng^b, Shoei-Sheng Lee^{a,*}, Chia-Chuan Chang^{a,*}^a School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan, ROC^b Tea Research and Extension Station, Taoyuan, Taiwan, ROC

ARTICLE INFO

Article history:

Received 1 September 2016

Received in revised form

8 November 2016

Accepted 9 November 2016

Available online 25 January 2017

Keywords:

1,7-dimethyl xanthine

Andraca theae

droppings

flavonoids

metabolites

ABSTRACT

Andraca droppings is the waste excreted from the tea biter *Andraca theae*. Its chemical constituents and potential medical use, unlike those of the traditional Chinese medicine silkworm droppings, have not been reported yet. To explore new nutraceuticals, the chemical constituents of this substance were investigated. Since the bioactive ingredients are generally present in the EtOAc-soluble fraction, this fraction, obtained from the ethanolic extract of the dried *Andraca* droppings by liquid–liquid partitioning, was separated by chromatographic methods, including Sephadex LH-20, centrifugal partition chromatography, and RP-18 columns, to produce 14 compounds (1–14). They were characterized as 1,7-dimethyl xanthine (1), three benzoic acids (2, 3, and 5), and 10 flavonoids (4, 6–14). The amount of compounds 6, 7, 10, 13, and 14 in the droppings were 1.7–15.5-fold compared to those of tea leaves. In addition, 1,7-dimethyl xanthine (1) was found present only in the *Andraca* droppings but absent in tea leaves. Therefore, except for compound 1, which might be transformed from caffeine by microflora in the insect, the compounds were believed not to be absorbed by the worm gut and excreted directly. The present study suggests the *Andraca* droppings are an enriched source of the bioactive flavonoids from tea leaves and are potential as a useful nutraceutical.

Copyright © 2017, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Silkworm feculae (also called *Can-Sha* in Chinese) is the droppings of silkworm (*Bombyx mori* L.; Bombycidae). In traditional Chinese medicine, it is used to expel wind, harmonize stomach, disperse dampness, and transform turbidity. It has been reported to promote wound healing, hematopoiesis by bone

marrow, liver protection, antiulcer, antitumor, antidiabetes, and antihyperlipidemia [1]. The mulberry leaf, the fodder of silkworm, is also a traditional Chinese medicine.

Andraca theae is a common tea pest. The larvae of *A. theae* flock and bite the leaves of tea shrub (*Camellia sinensis*) and only the leaf vein remains after they grow up and disperse. The epidemic of *A. theae* is seriously harmful to the production and quality of tea leaves. Aside from its destruction, its

* Corresponding authors. School of Pharmacy, College of Medicine, National Taiwan University, Taipei 10050, Taiwan, ROC.

E-mail addresses: shoeilee@ntu.edu.tw (S.-S. Lee), chiachang@ntu.edu.tw (C.-C. Chang).

<http://dx.doi.org/10.1016/j.jfda.2016.11.011>

1021-9498/Copyright © 2017, Food and Drug Administration, Taiwan. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

droppings, which might possess similar bioactivities to *C. sinensis* leaf, are considered as a potential material for medical use.

About 160 compounds have been isolated from leaves and other parts of *C. sinensis*. The compounds are classified into triterpenoids, flavonoids, catechins, aromatic glycosides, and others [2–9]. However, no chemical studies on *Andraca* droppings have been conducted. For the reasons mentioned above, the present study aimed to isolate its chemical constituents by chromatographic methods, and deduce the relationship of the chemical constituents between *C. sinensis* leaf and *Andraca* droppings.

2. Experimental

2.1. General

Instruments to obtain the physical data for the compounds were as follows. Circular dichroism: Jasco J-710 Spectropolarimeter (Tokyo, Japan); optical rotation: Jasco DIP-370 Digital Polarimeter; nuclear magnetic resonance (NMR): a Bruker DPX-200 (200 MHz), AV-400 (400 MHz), or AVIII-600 (600 MHz) NMR (Bruker Daltonics, Billerica, MA, USA) with a dual CryoProbe, J in Hz, δ in ppm calibrated by δ_{H} 3.30/ δ_{C} 49.0 for CD_3OD , δ_{H} 2.49/ δ_{C} 39.5 for $\text{DMSO}-d_6$, or δ_{H} 7.19 for $\text{C}_5\text{D}_5\text{N}$, the 2D NMR spectra acquired by standard pulse sequences; ESI-MS: an Esquire 2000 Ion Trap Mass Spectrometer (Bruker Daltonics); thin-layer chromatography: Silica gel 60 F_{254} aluminum sheets (0.25 mm; Merck KGaA, Darmstadt, Germany); column chromatography: Sephadex LH-20 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) and Lobar, Lichrospher RP-18 (size B; 40–63 μm , 310 $\times$ 25 mm; Merck); centrifugal partition chromatography (CPC): Model L.L.B-M (230 mL; Sanki Engineering Limited, Tokyo, Japan); high-performance liquid chromatography (HPLC): a Hitachi HPLC (Tokyo, Japan) equipped with a D-7000 interface, L-7100 pump, and an L-7400 UV-VIS or an L-7455 diode array detector; HPLC columns: Phenomenex Prodigy ODS (3) 100A columns (5 μm ; 250 $\times$ 4.6 mm for analysis or 250 $\times$ 10 mm for semi-preparation; Torrance, CA, USA).

2.2. Material

Dried *Andraca* droppings (Figure 1) (380 g) were provided by Tea Research and Extension Station, Council of Agriculture, Executive Yuan, Taoyuan, Taiwan, R.O.C., on November 10, 2014.

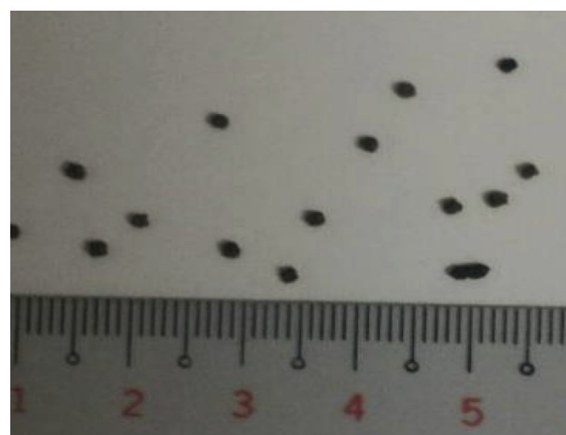
2.3. Extraction and isolation

The *Andraca* droppings (380 g) were extracted by 95% EtOH (3 $\times$ 2 L) and concentrated under reduced pressure at 45°C to give the EtOH extract (40 g). The suspension of the EtOH extract (25 g) in H_2O (200 mL) was partitioned in sequence against CHCl_3 , EtOAc, and *n*-BuOH, each 3 $\times$ 200 mL, to give three corresponding fractions (1.9 g, 2.4 g, and 2.4 g, respectively), and H_2O soluble (17.9 g) and insoluble (358.2 mg) fractions (see Figure 1).

Most of the EtOAc-soluble fraction (2.1 g) were fractionated by a Sephadex LH-20 column (4 cm outer diameter $\times$ 8 cm) into



A



B

Figure 1 – *Andraca* droppings. (A) General appearance in piles; (B) individual appearance.

16 fractions (fraction E1–16). Fraction E4 (149.9 mg) was separated by a Lobar RP-18 column (size B; MeOH– H_2O 3:7; 2 mL/min) to give compound 1 (6.2 mg). Fraction E5 (127.7 mg) was separated by the same column (MeOH– H_2O 9:11; 2.0 mL/min) to give nine fractions, of which fraction 6 (5.8 mg) was compound 4, fractions 2 (25.2 mg) and 3 (25.0 mg) yielded compounds 2 (5.0 mg; t_{R} 9.5 minutes) and 3 (3.4 mg; t_{R} 10.2 minutes), respectively, upon separation on the semi-preparative HPLC column, eluted individually by MeOH– H_2O (9:11) and (11:9) with a flow rate of 2.0 mL/min and detection at UV 254 nm. Recrystallization of an aliquot of fraction E6 (35 mg out of 266.7 mg) from MeOH yielded compound 5 (7.8 mg). Separation of fraction E8 (116.8 mg) on a CPC, using the lower and upper layers of CHCl_3 –MeOH– H_2O –*n*-BuOH (10:10:6:1) as mobile and stationary phases, respectively, with a flow rate of 1.2 mL/min and rotation speed 800 rpm, to give three fractions (fraction E8-1–3), then the delivery system was reversed to give fraction E8-4. Separation of fraction E8-2 (82.0 mg) by the semipreparative HPLC column (MeOH– H_2O 28:72; 2.0 mL/min; UV 254 nm) to give compounds 6 (13.1 mg; t_{R} 8.5 min) and 7 (26.1 mg; t_{R} 14.2 min). Separation of fraction E9 (127.7 mg) on the Lobar RP-18 column (MeOH– H_2O 35:65; 2 mL/min) to give

seven fractions, of which fractions 3 and 4 were compounds 6 (2.3 mg) and 7 (17.7 mg), respectively, and fraction 6 (15.2 mg) gave compounds 8 (2.8 mg; t_R 8.7 min) and 9 (3.2 mg; t_R 10.4 min) upon separation over the semipreparative HPLC column (MeOH–H₂O 11:9; 2.0 mL/min; UV 254 nm). Separation of fraction E10 (175.8 mg) by the Lobar column (MeOH–H₂O 2:8, 2 mL/min) to give compounds 10 (22.0 mg) and 11 (17.6 mg), respectively. Separation of fraction E13 (176.5 mg) by the Lobar column (MeOH–H₂O 33:67, 2 mL/min) gave compound 12 (17.1 mg). Fractionation of fraction E14 (548.1 mg) by a Sephadex LH-20 column (2.0 outer diameter $\times$ 29 cm; MeOH–H₂O 6:4), followed by the Lobar column (23%–28% MeOH/H₂O, 2 mL/min) to give five fractions, of which fraction 4 was compound 13 (46.6 mg) and fraction 5 gave compound 14 (11.8 mg; t_R 9.7 min) upon separation by the semipreparative HPLC column (MeOH–H₂O 35:65, 2.0 mL/min; UV 254 nm).

3. Results

A total of 14 compounds (1–14) (Figure 2) were isolated from the EtOAc-soluble fractions of the dried *Andraca* droppings by chromatographic methods, including Sephadex LH-20, CPC, and RP-18 column chromatography. Ten of them were flavonoids, including seven flavanol derivatives (compounds 6, 7, 10–14) and three flavonol 3-O-glycosides (compounds 4, 8, 9).

The ¹H NMR spectrum of compound 1 showed three singlets at δ 7.79 (H-8), 3.95 (7-Me), and 3.31 (1-Me) and thus compound 1 was identified as 1,7-dimethyl xanthine (paraxanthine) [10]. This suggestion was confirmed by further comparison with the ¹³C NMR data [11].

Compounds 6 and 7 were identified as catechin and l-epicatechin [12–14], respectively. Their ¹H NMR spectra showed the distinct signals of H-2 at δ 4.55 (d, J = 7.5 Hz) and 4.80 (br. s), respectively. The CD spectra of both compounds are similar in shape and showed respective a positive Cotton

effect around 240 nm and a negative CE around 280 nm, supporting 2R- configuration [14,15]. However, the optical property of compound 6 with $[\alpha]_D$ value close to 0 was not consistent with that of d-catechin present in tea leaf. This optical property indicates compound 6 to be a mixture of d- and l- catechins, with d-from enantiomeric excess as verified from CD data.

Similarly, compounds 10 and 11 were identified as gallo-catechin and l-epigallocatechin, respectively. Their ¹H NMR spectra are similar to those of compounds 6 and 7 except that the ABX system in the B ring of compounds 6 and 7 ($\delta_{H-2'}$ 6.82, d, J = 1.8 Hz; $\delta_{H-5'}$ 6.75, d, J = 8.0 Hz; $\delta_{H-6'}$ 6.69, dd, J = 8.0, 1.8 Hz) was replaced by a two-proton singlet (δ ~6.40). As with compound 6, the optical property of compound 10 with $[\alpha]_{26}^D$ +0.5 (c 1.0, MeOH) was not consistent with that of d-gallocatechin present in tea leaf, indicating compound 10 to be a mixture with d-from enantiomeric excess.

Compounds 12 and 13 were identified as l-3''-O-methyl-epigallocatechin gallate and l-epigallocatechin gallate [16–18], respectively. The ¹H NMR spectrum of compound 13 was similar to that of compound 11 except for showing the much more downfield shifted H-3 (δ 5.52 vs. 4.16) and an additional two-proton singlet ($\delta_{H-2''/6''}$ 6.94). The ¹H NMR spectrum of compound 12 was similar to that of compound 13 except for showing an additional O-methyl singlet (δ 3.80) and replacement of a two-proton singlet for H-2''/6'' by an AB system (δ 7.01 and 7.05, J = 1.9 Hz). Compound 14 was identified as l-epicatechin gallate [19]. Its ¹H NMR spectrum was similar to that of compound 7 except for showing the much more downfield shifted H-3 (δ 5.52 vs. 4.15) and an additional two-proton singlet ($\delta_{H-2''/6''}$ 6.94) (see Figure 2).

Compounds 2, 3, and 5 are simple benzoic acid derivatives and were identified as 3-O-methylgallic acid [20], 4-hydroxybenzoic acid [21], and gallic acid [22], respectively, by comparison of their ¹H and ¹³C NMR data with those reported.

Compounds 4, 8, and 9 were 3-O-glycosyl flavonols as exemplified by their ¹H NMR spectra, showing an AX system

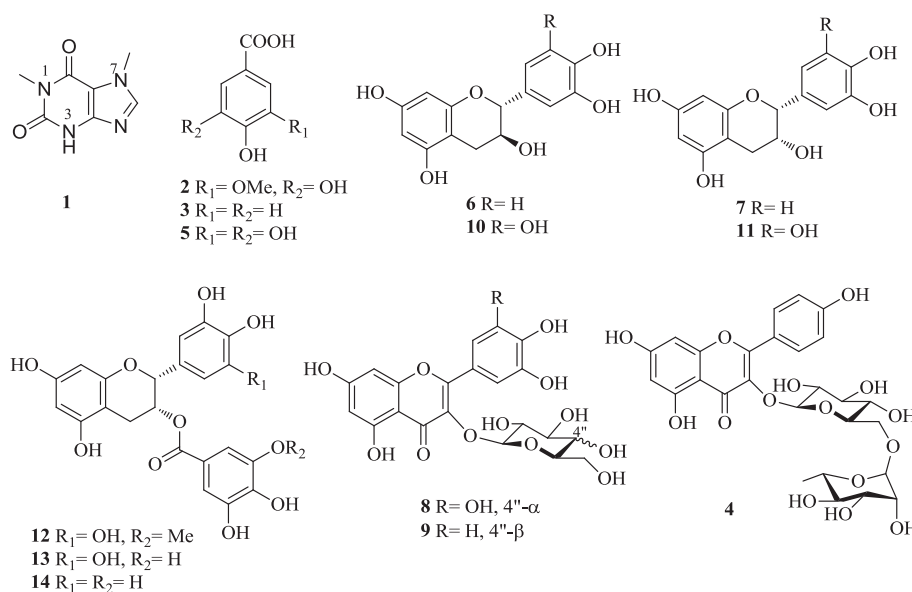


Figure 2 – Structures of compounds (1–14) from the *Andraca* droppings.

for H-6 (δ ~6.20) and H-8 (δ ~6.40), an AA'XX' (4) or A₂ (8) or AMX (9) system for protons in ring B, and characteristic signals for the anomeric protons, e.g. δ 5.12 (d, J = 7.4 Hz) for Glc H-1 and δ 4.51 (d, J = 1.1 Hz) for Rha H-1 in compound 4. They were identified as kaempferol 3-O-rutinoside (compound 4) [23], isomyricitrin (compound 8) [23], and quercetin 3-O- β -D-galactopyranoside (compound 9) [13] by comparison of their physical data (^1H and ^{13}C NMR, $[\alpha]_D$) data with those reported.

Among these isolated compounds, compound 12 is the most abundant (2.22%, w/w) while the content of the unusual 3-desmethylated caffeine (compound 1), having not been reported from tea leaf (see Table 1), is about 0.30%.

Comparison of the content of the corresponding compounds isolated from *Andraca* droppings in the present study to our unpublished data and the data from those reported in the literature [4–6, 24–26] indicated that most of them are obviously more abundant, e.g. up to 15.5 folds for catechin (compound 6), except the content of one compound (11, 1.0-fold) was identical (Table 1). From the aforementioned data we speculated that the *Andraca* cannot digest these flavonoids efficiently, leading to the concentration of these compounds in the digestion processes.

These flavonoids possess various bioactivities, such as antioxidative, anti-inflammatory and anticancer activities [2–9]. The most abundant compound, 13 (EGCG; 2.22%), is reported to be the most effective cancer chemopreventive polyphenol in green tea [27]. The second and third abundant epicatechin (compound 7; 1.24%) and gallic acid (compound 5; 0.37%) also possess anticancer activities [28]. The methylated EGCG (compound 12) exhibits antiallergic functions by inhibition of mast cell activation and suppression of leukotriene and interleukin-2 secretion, and suppression of TNF- α and MIP1- α production, and its potency is higher than EGCG (13) [29]. In addition, 1,7-dimethyl xanthine

(paraxanthine; 1), the only compound not found in the fodder, *C. sinensis* leaf, is a nonselective antagonist for phosphodiesterase [30] and adenosine receptor [31]. Paraxanthine, the 3-N-demethylated metabolite of caffeine in humans via cytochrome P-450 oxidation [32], might be produced by microbial transformation in the gut of *Andraca* worm.

This study indicates that *Andraca* droppings are a much better source of the bioactive ingredients than tea leaf. The findings also indicated that AD should possess at least similar bioactivity to tea leaf and has great potential to be developed as a useful nutraceutical.

4. Discussion

Table 1 lists the percentage and the relative content of the isolated compounds in the fodder, *C. sinensis* leaf, and *Andraca* droppings. This comparison provided important hint about how these compounds were digested by the gut microflora. All the flavanol derivatives (compounds 6, 7, 10–14) were enriched in *Andraca* droppings, indicating them not readily metabolized by insect gut and microflora, where 3-N-demethylation on caffeine was undertaken efficiently to give compound 1. The optical properties of catechin (compound 6) and gallic acid (compound 10) revealed that some biotransformations occurred during the digestion process, including oxidation at C-3 hydroxy group, followed by C-2 epimerization and reduction at the formed C-3 ketone. Through these, the *l*-form product will be produced from the natural *d*-form. From the relative content of flavanol glycosides, β -glucosidase seems abundant but β -galactosidase absent in the gut system since the content of compound 8 is much lesser but that of compound 9 much more (42-fold) in *Andraca* droppings than in tea leaf. In addition, mono-O-

Table 1 – Estimated amount (g) of compounds 1–14 in 100 g of the corresponding extracts^a from *Camellia sinensis* leaf and *Andraca theae*.

Compound	Droppings (A)	<i>C. sinensis</i> (B) ^b	Relative content (A/B)
Flavanol derivatives			
Catechin (6)	0.62	0.04	15.5
Epicatechin (7)	1.24	0.12	10.3
Gallocatechin (10)	1.05	0.12	8.8
Epigallocatechin (11)	0.84	0.82	1.0
Epigallocatechin 3''-O-methyl-gallate (12)	0.81	NE	—
Epigallocatechin gallate (13)	2.22	1.30	1.7
Epicatechin gallate (14)	0.56	0.24	2.3
Flavanol glycosides			
Kaempferol 3-O-rutinoside (4)	0.28	NE	—
Isomyricitrin (8)	0.11	NE	—
Quercetin 3-O- β -D-galactopyranoside (9)	0.84	NE	—
Xanthine			
1,7-dimethyl xanthine (1)	0.30	—	—
Benzoic acids			
gallic acid (5)	0.37	NE	—
3-O-methylgallic acid (2)	0.24	NE	—
4-hydroxybenzoic acid (3)	0.16	NE	—

NE = not estimated.

^a Extracts were soluble in ethanol and the concentrate of the ethyl acetate-soluble part from *C. sinensis* leaf and *A. theae* droppings, respectively.

^b The data for compounds 6–7 and 10–14 were provided by Tea Research and Extension Station.

methylation on the phenolic –OH in the galloyl moiety seems to be a general metabolic pathway by the gut system as verified in compounds 2 and 12.

This study demonstrates that *Andraca* droppings might become a new nutraceutical similar to the droppings of silkworm. A lot of substandard tea, so-called vice tea, is discarded during tea preparation but is good fodder for the insect *Andraca theae*. Accompanied with the present finding of the potential use of *Andraca* droppings, sufficient utilization of vice tea to nurture this distinct insect might create a new agriculture-industry joined business and should be beneficial to human welfare and economic development.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by the grant 103AS-14.3.3-TS-T1 from Council of Agriculture, Taiwan, Republic of China.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jfda.2016.11.011>.

REFERENCES

- [1] Shi MG, Li JL, Pu YZ. Research situation of silkworm excrement. *Tradit Chin Drug Res Clin Pharm* 2013;4:1–6.
- [2] Yoshikawa M, Sugimoto S, Nakamura S, Matsuda H. Medicinal flowers. XXII. Structures of chakasaponins V and VI, chakanoside I, and chakaflavonoside A from flower buds of Chinese tea plant (*Camellia sinensis*). *Chem Pharm Bull* 2008;56:1297–303.
- [3] Yoshikawa M, Sugimoto S, Kato Y, Nakamura S, Wang T, Yamashita C, Matsuda H. Acylated oleanane-type triterpene saponins with acceleration of gastrointestinal transit and inhibitory effect on pancreatic lipase from flower buds of Chinese tea plant (*Camellia sinensis*). *Chem Biodivers* 2009;6:903–15.
- [4] Saito ST, Welzel A, Suyenaga ES, Bueno F. A method for fast determination of epigallocatechin gallate (EGCG), epicatechin (EC), catechin (C) and caffeine (CAF) in green tea using HPLC. *Food Sci Technol (Campinas)* 2006;26:394–400.
- [5] Yoshikawa M, Morikawa T, Yamamoto K, Kato Y, Nagatomo A, Matsuda K. Floratheasaponins A–C, acylated oleanane-type triterpene oligoglycosides with antihyperlipidemic activities from flowers of the tea plant (*Camellia sinensis*). *J Nat Prod* 2005;68:1360–5.
- [6] Morikawa T, Nakamura S, Kato Y, Muraoka O, Matsuda H, Yoshikawa M. Bioactive saponins and glycosides. XXVIII. New triterpene saponins, foliatheasaponins I, II, III, IV, and V, from Tencha (the leaves of *Camellia sinensis*). *Chem Pharm Bull* 2007;55:293–8.
- [7] Morikawa T, Li N, Nagatomo A, Matsuda H, Li X, Yoshikawa M. Triterpene saponins with gastroprotective effects from tea seed (the seeds of *Camellia sinensis*). *J Nat Prod* 2006;69:185–90.
- [8] Varughese T, Manir MM, Rahaman M, Kim JK, Lee BG, Moon SS. Tea triterpenoidal saponins from the roots of *Camellia sinensis* have inhibitory effects against alcohol dehydrogenase. *Planta Med* 2011;77:2029–36.
- [9] Kobayashi K, Teruya T, Suenaga K, Matsui Y, Masuda K, Kigoshi H. Isotheasaponins B1–B3 from *Camellia sinensis* var. *sinensis* tea leaves. *Phytochemistry* 2006;67:1385–9.
- [10] Fujii T, Saito T, Tamura K. Purines. XLIX. Synthesis and proton nuclear magnetic resonance study of 3,7-dialkylxanthines and 1,3,7-trialkyl-xanthines. *Chem Pharm Bull* 1991;39:2855–62.
- [11] Osterman RM, McKittrick BA, Chan TM. Regiochemical assignment of methylated purines and pyrimidines by selective INEPT. *Tetrahedron Lett* 1992;33:4867–70.
- [12] Bilia AR, Morelli I, Hamburger M, Hostettmann K. Flavans and A-type proanthocyanidins from *Prunus prostrata*. *Phytochemistry* 1996;43:887–92.
- [13] Balde AM, Pieters LA, Gergely A, Kolodziej H, Claeys M, Vlietinck AJ. A-Type proanthocyanidins from stem-bark of *Pavetta owariensis*. *Phytochemistry* 1991;30:337–42.
- [14] Kiatgrajai P, Wellons JD, Gollob L, White JD. Kinetics of epimerization of (+)-catechin and its rearrangement to catechinic acid. *J Org Chem* 1982;47:2910–2.
- [15] Rinaldo D, Batista JM, Rodrigues J, Benfatti AC, Rodrigues CM, Dos Santos LC, Furlan M, Vilegas W. Determination of catechin diastereomers from the leaves of *Byrsonima* species using chiral HPLC-PAD-CD. *Chirality* 2010;22:726–33.
- [16] Aihara Y, Yoshida A, Furuta T, Wakimoto T, Akizawa T, Konishi M, Kan T. Regioselective synthesis of methylated epigallocatechin gallate via nitrobenzenesulfonyl (Ns) protecting group. *Bioorg Med Chem Lett* 2009;19:4171–4.
- [17] Melikuziev FA, Nishanbaev SZ, Turgunov KK, Bobakulov K. Phenolic compounds from roots of *Rhodiola litvinovii*. *Chem Nat Compd* 2013;49:349–50.
- [18] Masuda T, Someya T, Fujimoto A. Phenolic inhibitors of chemical and enzymatic oxidation in the leaves of *Myrica rubra*. *Biosci Biotechnol Biochem* 2010;74:212–5.
- [19] Ohmori K, Yano T, Suzuki K. General synthesis of epi-series catechins and their 3-gallates: reverse polarity strategy. *Org Biomol Chem* 2010;8:2693–6.
- [20] Liao CR, Kuo YH, Ho YL, Wang CY, Yang CS, Lin CW, Chang YS. Studies on cytotoxic constituents from the leaves of *Elaeagnus oldhamii* Maxim. in non-small cell lung cancer A549 cells. *Molecules* 2014;19:9515–34.
- [21] Chan CC, Chen YW, Su CS, Lin HP, Lee CF. Green catalysts derived from agricultural and industrial waste products: the preparation of phenols from CsOH and aryl iodides using CuO on mesoporous silica. *Eur J Org Chem* 2011;2011:7288–93.
- [22] Zhang HM, Wang CF, Shen SM, Wang GL, Liu P, Liu ZM, Wang YY, Du SS, Liu ZL, Deng ZW. Antioxidant phenolic compounds from Pu-erh Tea. *Molecules* 2012;17:14037–45.
- [23] Luyen BTT, Tai BH, Thao NP, Eun KJ, Cha JY, Xin MJ, Lee YM, Kim YH. Anti-inflammatory components of *Euphorbia humifusa* Willd. *Bioorg Med Chem Lett* 2014;24:1895–900.
- [24] Zuo Y, Chen H, Deng Y. Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector. *Talanta* 2002;57:307–16.
- [25] Manir MM, Kim JK, Lee BG, Moon SS. Tea catechins and flavonoids from the leaves of *Camellia sinensis* inhibit yeast alcohol dehydrogenase. *Bioorg Med Chem* 2012;20:2376–81.

- [26] Zhou ZH, Zhang YJ, Xu M, Yang CR. Puerins A and B, two new 8-C substituted flavan-3-ols from Pu-er tea. *J Agric Food Chem* 2005;53:8614–7.
- [27] Du GJ, Zhang Z, Wen XD, Yu C, Calway T, Yuan CS, Wang CZ. Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients* 2012;4:1679–91.
- [28] Hara Y. Green tea: health benefits and applications. Tokyo: CRC Press; 2001.
- [29] Maeda-Yamamoto M, Ema K, Monobe M, Tokuda Y, Tachibana H. Epicatechin-3-O-(3''-O-methyl)-gallate content in various tea cultivars (*Camellia sinensis* L.) and its *in vitro* inhibitory effect on histamine release. *J Agric Food Chem* 2012;60:2165–70.
- [30] Essayan DM. Cyclic nucleotide phosphodiesterases. *J Allergy Clin Immunol* 2001;108:671–80.
- [31] Daly JW, Jacobson KA, Ukena D. Adenosine receptors: development of selective agonists and antagonists. *Prog Clin Biol Res* 1987;230:41–63.
- [32] Guerreiro S, Toulorge D, Hirsch E, Marien M, Sokoloff P, Michel PP. Paraxanthine, the primary metabolite of caffeine, provides protection against dopaminergic cell death via stimulation of ryanodine receptor channels. *Mol Pharmacol* 2008;74:980–9.