Investigation of free amino acid, total phenolics, antioxidant activity and purine alkaloids to assess the health properties of non-Camellia tea

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Abstract To find novel functional beverages from folk teas, 33 species of frequently used non-Camellia tea (plants other than Camellia) were collected and compared with Camellia tea (green tea, pu-erh tea and black tea) for the first time. Data are reported here on the quantities of 20 free amino acids (FAAs) and three purine alkaloids (measured by UHPLC), total polyphenols (measured by Folin-Ciocalteu assay), and antioxidant activity (DPPH). The total amounts of FAAs in non-Camellia tea (0.62 – 18.99 mg/g) are generally less than that of Camellia tea (16.55 – 24.99 mg/g). However, for certain FAAs, the quantities were much higher in some non-Camellia teas, such as γ-aminobutyric acid in teas from Ampelopsis grossedentata, Isodon serra and Hibiscus sabdariffa. Interestingly, theanine was detected in tea from Potentilla fruticosa (1.16 – 0.81 mg/g). Furthermore, the content of polyphenols in teas from A. grossedentata, Acer tataricum subsp. gninla are significantly higher than those from Camellia tea; teas from I. serra, Pistacia chinensis and A. tataricum subsp. gninla have remarkable antioxidant activities similar to the activities from green tea (44.23 μg/mL). Purine alkaloids (caffeine, theobromine...
and theophylline) were not detected in non-Camellia teas. The investigation suggests some non-Camellia teas may be great functional natural products with potential for prevention of chronic diseases and aging, by providing with abundant polyphenols, antioxidants and specific FAAs.

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1. Introduction

Our expected lifespan continues increasing, but many of us will lead a higher percentage of our lives in poor health conditions due to aging and increasing threats by many chronic diseases such as hypertension, hyperlipidemia, diabetes, chronic inflammation, and other stressors. As healthcare costs increase, preventive approaches (e.g., supplemental diets, functional foods or drinks) are gaining in popularity. These approaches represent inexpensive and readily applicable approaches to reduce the incidence of chronic diseases. Such preventive health products have gained a great deal of attention from both the scientific community and the general public. Additionally, exploration of the natural and sustainable resources for healthcare and supplementary nutrition has become crucial for the future of developing countries and for those with large populations such as China.

It is well-known that green teas prepared from leaves of Camellia plants have many important physiological properties and health benefits. Drinking green teas may reduce the risk of many diseases, such as cancers and cardiovascular diseases, as teas have a variety of biological activities including anti-tumor, anti-oxidation, and anti-obesity. Previous studies have demonstrated that amino acids, polyphenols and purine alkaloids are important nutritional and active components in green teas. Examples include the nutritional roles for essential amino acids and the pharmacological effects of theanine and γ-aminobutyric acid (GABA). Theanine is a major amino acid uniquely found in green tea which can decrease norepinephrine and serotonin levels in the brain, lower blood pressure and produce neuroprotective and cognitive-enhancing actions. GABA is an important inhibitory neurotransmitter in the mammalian central nervous system and is known to exhibit antihypertensive effects. Teas rich in GABA can decrease blood pressure in rats. Amino acids also participate in the biosynthesis of polyphenols and alkaloids. The antioxidant and free radical-scavenging abilities of polyphenols in green tea may play an important role in the prevention of cardiovascular disease, chronic gastritis and some cancers. However, purine alkaloids (such as caffeine, theobromine and theophylline), as well as the antioxidant activity, were previously reported. UHPLC was also applied to determine the purine alkaloid content. Lastly, the phenolic content and antioxidant activity of these tea products were determined using the Folin-Ciocalteu (F-C) assay and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method, respectively. The F-C assay and DPPH methods provide convenient, rapid and simple estimation of the total phenols content and antioxidant activity, respectively.

In the present paper, the content of the 20 free amino acids (FAAs), polyphenols, three purine alkaloids (caffeine, theobromine and theophylline), as well as the antioxidant activity, were investigated in 33 non-Camellia teas. The 20 investigated FAAs were: 9 essential amino acids (EA), threonine (Thr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), tryptophan (Trp) and histidine (His); 6 conditionally essential amino acids (HEA): arginine (Arg), cysteine (Cys), glycine (Gly), glutamine (Glu), proline (Pro) and tyrosine (Tyr); 3 dispensable or non-essential amino acids (NEA): alanine (Ala), aspartic acid (Asp), serine (Ser), and 2 activated amino acids (GABA and Thea).

2. Materials and methods

2.1. Collection of tea samples

The 33 non-Camellia teas (119 accessions) were collected in China from 2008 through 2013, the 9 Camellia teas (3 green teas, 3 pu-erh teas and 3 black teas, respectively) were purchased from...
| Tea No. | Sample No. | Origin | Family | Chinese name | Source provinces or regions | Collection time |
|--------|------------|--------|--------|--------------|-----------------------------|-----------------|
| 1      | BYC 1–4   | Engelhardia roxburghiana Lindl. | Juglandaceae | Luo-han-cha | Lingyun and Jinxiu, Guangxi | 2011–2012 |
| 2      | BYC 5–7   | Elsholtzia bodinieri Vaniot | Lamiaceae | Feng-wei-cha | Yunnan | 2009–2010 |
| 3      | BYC 8–13  | Mallotus peltatus (Geiseler) Mull.Arg. | Euphorbiaceae | Zhe-gu-cha | Hainan | 2009–2012 |
| 4      | BYC 14–16 | Chinonanthus nitens Oliv. | Calycanthaceae | Xiang-feng-cha | Zhejiang | 2011–2012 |
| 5      | BYC 17–19 | Hibiscus sabdariffa L. | Malvaceae | Mei-gui-qie-cha | Guangxi | 2012–2013 |
| 6      | BYC 20–22 | Rubus chingii var. suavissimus (S.K.Lee) | Rosaceae | Tian-cha | Medicinal plant garden and Jinxiu, Guangxi | 2010–2013 |
| 7      | BYC 23–25 | Fagopyrum tataricum (L.) Gaertn. | Polygonaceae | Ku-qiao-cha | Shanxi | 2011–2012 |
| 8      | BYC 26–28 | Thamnolia vermicularis (Sw.) Ach. | Thamnoliaceae | Tai-bai-cha | Shaanxi | 2008–2009 |
| 9      | BYC 29–34 | Apocynum venetum L. | Apocynaceae | Luo-bo-ma-cha | Cangzhou, Hebei; Fenglingdu and Pinglu, Shanxi; Liaoning; Alatai, Xinjiang | 2009–2011 |
| 10     | BYC 35–39 | Ampelopsis grossedentata (Hand.-Mazz.) W.T.Wang | Vitaceae | Teng-cha | Hunan; Guizhou; Shaowu, Fujian | 2010–2011 |
| 11     | BYC 40–45 | Ligustrum robustum (Roxb.) | Oleaceae | Xiao-ye-ku-ding-cha | Yuqing, Jinxian and Yuqing, Guangxi; Zhaotong, Yunnan; Hainan | 2008–2009 |
| 12     | BYC 46–48 | Adinandra nitida Merr. ex H.L.Li | Pentaphylacaceae | Shi-ya-cha | Jinxian and Shentangshan, Guangxi | 2011–2012 |
| 13     | BYC 49–52 | Ilex latifolia Thunb. | Aquifoliaceae | Da-ye-ku-ding-cha | Emei, Sichuan; Zhejiang; Guangxi; Wuzhishan, Hainan; Sichuan; Guizhou; Hangzhou, Zhejiang | 2008–2010 |
| 14     | BYC 53–56 | Litsea coreana var. lanuginosa (Migo) Yang & Huang | Lauraceae | Lao-yin-cha | 2008–2011 |
| 15     | BYC 57–59 | Sarcandra glabra (Thunb.) Nakai | Chloranthaceae | Jiu-jie-cha | Jiangxi | 2011–2012 |
| 16     | BYC 60–62 | Cassia obtusifolia (L.) H.S.Irwin & Barneby | Leguminosae | Jue-ming-zi-cha | Linxia | 2010 |
| 17     | BYC 63–65 | Isodon serra (Maxim.) Kudô | Lamiaceae | Xi-huang-cao-cha | Shaoguan, Guangdong | 2011–2012 |
| 18     | BYC 66–68 | Forsythia suspensa (Thunb.) Vahl | Oleaceae | Lian-qiao-ye-cha | Shandong | 2012 |
| 19     | BYC 69–71 | Chrysanthemum indicum L. | Compositae | Ye-ju-hua-cha | Zhejiang | 2011–2012 |
| 20     | BYC 72–74 | Dendranthema morifolium (Ramat.) Tzvelev | Compositae | Ju-hua-cha | Huangshan, Anhui | 2011–2012 |
| 21     | BYC 75–80 | Potentilla fruticosa L.* | Rosaceae | Yao-wang-cha | Shaanxi; Jilin | 2008–2012 |
| 22     | BYC 81–83 | Malus hupelensis (Pamp.) Rehder | Rosaceae | Hua-hong-cha | Xiangyang, Hubei | 2012–2013 |
| 23     | BYC 84–86 | Gynostemma pentaphyllum (Thunb.) Makino | Compositae | Jiao-gu-lan-cha | Jinxiu, Guangxi | 2012–2013 |
| 24     | BYC 87–89 | Cratoxylum cochinense (Lour.) Blume | Hypericaceae | Huang-niu-cha | Xinyi, Guangdong | 2012–2013 |
| 25     | BYC 90–93 | Lycium barbarum L. | Solanaceae | Gou-qi-ye-cha | Yinchuan, Linxia | 2011–2013 |
| 26     | BYC 94–96 | Scoparia dulcis L. | Plantaginaceae | Si-shi-cha | Fujian | 2012–2013 |
| 27     | BYC 97–99 | Cyclocauro paliurus (Batalin) Iljinsk. | Juglandaceae | Qin-qian-ju-cha | Suining, Hainan | 2010–2011 |
| 28     | BYC 100–102 | Acer tataricum subsp. gimala (Maxim.) Wesm. | Sapindaceae | Ku-jin-cha | Jilin; Liaoning | 2011–2013 |
local retailer shops (Table 1). Except for the teas from Hibiscus sabdariffa, Chrysanthemum indicum, Dendranthema morifolium and Coreopsis tinctoria are derived from flowers, Fagopyrum tataricum and Cassia obtusifolia are from seeds, the plant part of all other teas are from leaves. All samples were kept sealed and stored in dry and cool places before testing.

The plant species of the tea samples were authenticated by Dr. Peigen Xiao and all species were validated taxonomically as described on the web site www.theplantlist.org. The voucher specimens were deposited in Xiao’s laboratory at the Institute of Medicinal Plant Development of the Chinese Academy of Medical Sciences in Beijing, China.

2.2. Chemicals and standards

AccQ-Tag™ Ultra UPLC™ amino acid analysis derivatization kits were purchased from Waters (Milford, MA, USA). The 20 FAAs, caffeine, theobromine, theophylline and acid gallic standard were purchased from Winherb Medical Technology Co., Ltd. (Shanghai, China). Mixed amino acid standard stock solutions of 2.5 mmol/L were prepared in 0.1 mol/L HCl and stored at 4°C. Diluted solutions were freshly prepared weekly.

HPLC-grade acetonitrile was purchased from Honeywell Burdick & Jackson (Morristown, NJ, USA). HPLC-grade methanol was from Fisher Scientific (Atlanta, GA, USA) and hydrochloric acid, formic acid, and ortho-phosphoric acid were from CNW Technologies GmbH (Düsseldorf, Germany). Sodium acetate and triethylamine were purchased from Guangfu Technology Development Co. (Tianjin, China). Sodium azide, α-aminobutyric acid (AABA, internal standard), 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and Folin-Ciocalteu phenol reagent were from Sigma-Aldrich (St. Louis, MO, USA). Sodium carbonate was purchased from Beijing Chemical Works (Beijing, China). Trolax solution (10 mmol/L) was purchased from Beyotime Biotechnology (Haimen, Jiangsu, China). All reagents used were of analytical grade. All solutions were made with Milli-Q water (Millipore, Bedford, MA). Eluent A concentrate for gradient elution contained sodium acetate (1.4 mol/L), sodium azide (1.5 mmol/L), disodium EDTA (2.6 mmol/L) and tri-ethyl amine (170 mmol/L) in water, and was titrated to pH 4.95 with phosphoric acid.

2.3. Preparation of tea samples

Tea samples were prepared as follows: 250 mg (for amino acid and antioxidant analysis) and 150 mg (for total phenolics and purine alkaloids analysis) of teas were extracted with 10 mL of distilled water at 80°C for 30 min respectively. After the tea water extract was cooled to room temperature, the volume was brought back to 10 mL with water. The sample solutions were filtered through a 0.22 µm nylon membranes purchased from Jinteng experiment equipment Co., Ltd. (Tianjin, China).

2.4. Analysis of total polyphenols

The total phenolic content of the tea samples was determined according to a procedure described by Ainsworth and Gillespie25 with slight modifications. One hundred microliters of each filtered sample solution was mixed with 200 µL of 10% (v/v) F-C phenol reagent and 800 µL of 700 mmol/L sodium carbonate solution in a 2-mL microtube for 2 h before spectrometric analysis. Finally, 200 µL of the sample, standard or blank was transferred to clear 96-well microplates, and the absorbances read at 765 nm using the Quant MQX200 microplate reader from Biotek Instruments, Inc. (Winooski, VT, USA). Gallic acid was used as standards for quantification, and the results were expressed as percent gallic acid equivalents (GAE).

2.5. Antioxidant assay

The antioxidant activities of the tea samples were evaluated by DPPH method as previously reported, with some modifications51,52. Briefly, 7 µL of the diluted sample or Trolax

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**Table 1 (continued)**

| Tea No. | Sample No. | Origin | Family | Chinese name | Source provinces or regions | Collection time |
|---------|------------|--------|--------|--------------|----------------------------|-----------------|
| 29      | BYC 103–105| Pistacia chinesis | Anacardiaceae | Huang-li-ya-cha | Loudi, Hunan | 2013 |
| 30      | BYC 106–108| Coreopsis tinctoria | Compositae | Kun-lun-xue-ju-cha | Xinjiang | 2012–2013 |
| 31      | BYC 109–111| Orthosiphon aristatus | Lamiaceae | Shen-cha | Yunnan | 2011–2012 |
| 32      | BYC 112–116| Scutellaria baicalensis | Lamiaceae | Huang-qin-cha | Hebei; Beijing; Chengde, Hebei; Zhuozi and Yakeshi, Neimenggu | 2008–2012 |
| 33      | BYC 117–119| Lithocarpus litsea (Hance) Chun | Fagaceae | Duo-sui-ke-cha | Mashan, Guangxi | 2012 |
| 34      | BYC 120–122| Camellia sinensis (L.) Kuntze | Theaceae | Green tea | Emei, Sichuan | 2010–2013 |
| 35      | BYC 123–125| Camellia sinensis (L.) Kuntze | Theaceae | Black tea | Fujian | 2010–2013 |
| 36      | BYC 126–128| Camellia sinensis (L.) Kuntze | Theaceae | Pu-erh tea | Yunnan | 2010–2013 |

Most Latin names and families of original plants were identified in TPL (www.theplantlist.org), except the plants signed with ** which were identified according to Flora of China (2000).
(six different concentrations) or ethanol was added to 193 μL of 0.2 mmol/L DPPH (freshly prepared prior to assay), then left at ambient temperature in the dark for 30 min. The absorbance was measured at 517 nm. Trolox and ethanol were used as the reference and control, respectively. The DPPH radical scavenging activity was calculated using the following formula:

\[
\text{DPPH Radical Scavenging Activity (\%)} = \left(\frac{A_0 - A_f}{A_0}\right) \times 100
\]

where \(A_f\) is the absorbance of the test sample, and \(A_0\) is the absorbance of the control. Results are expressed as EC\(_{50}\) and values are referred to the concentration of the tea infusions required for the 50% of the antioxidant activity (g/mL).

2.6. Derivatization reaction of free amino acids

Precolumn 6-aminoquininyl-N-hydroxysuccinimidyl carbamate (AQC) derivatization of FAAs was accomplished using a Waters AccQ·Tag™ reagent kit. Amino acid standards or tea samples (10 μL) were derivatized directly by mixing with 70 μL AccQ·Tag™ borate buffer. After adding 20 μL derivatizing reagent (10 mmol/L AccQ·Tag™ reagent), the mixtures were immediately vortexed, left to rest for 1 min at room temperature and finally heated for 10 min at 55 °C to complete the derivatization. Derivatized sample solutions were then subjected to chromatographic analysis. The concentration level of each analyte was approximately as follows: 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200 and 400 pmol/μL.

2.7. UHPLC instrument and chromatographic conditions

The analysis of FAAs and alkaloids was carried out by using the Dionex Ultimate 3000 UHPLC system equipped with a HPG3400 RS Pump, SRD-3400 degasser, WPS-3000T RS Autosampler, TCC-3000RS Column Compartment, DAD-3000RS Diode Array Detector and Chromelion chromatography software package (6.8 version).

UHPLC separation of AQC-derivatized amino acids was performed with a Waters Acquity UPLC™ column (BEH C18, 100 mm x 2.1 mm, 1.7 μm). The mobile phase A was Eluent A concentrate diluted 1:10 with ultrapure water while B was 60% (v/v) acetonitrile. The temperature of the column oven was set at 37 °C. The elution program is described in Table 2. After the program, the initial conditions were regenerated within 0.5 min and maintained for another 4.5 min, resulting in a net separation time of 11.5 min and a 16 min total cycle time. The injection volume was 2 μL, and UV detection of AQC amino acid derivatives was performed at 254 nm. Data were archived using Chromelion chromatography software package. All of the operations and the data acquisition were controlled by a Dionex ChemStation.

The caffeine, theobromine and theophylline content of the tea samples were determined according to our previously reported procedure29. Briefly, UHPLC was performed with Dionex Acclaim PA2 column (150 mm x 2.1 mm, 2.2 μm). The mobile phase A was acetonitrile, while B was ultrapure water. The flow rate was set to 0.5 mL/min, and the temperature of column oven was set at 30 °C. Elution was accomplished with a linear gradient of phase A from 5% to 15% in 10 min. The injection volume was 10 μL, and UV detection of these alkaloids was performed at 272 nm.

2.8. UHPLC method validation

The precision, repeatability, stability, detection and quantification limits, linearity ranges and recovery of the proposed method were validated following the guideline of the International Conference on Harmonisation (ICH)30. To evaluate the precision of the method, a standard solution was injected six times successively. To get the repeatability of the method, six replicate analyses of a standard solution were performed to determine both the retention time and the peak area of the amino acid standards. The stability was assessed by injecting a standard solution six times within 0, 2, 4, 6, 8, 16, and 24 h, respectively. Detection and quantification limits (LOD and LOQ) were calculated at the respective signal-to-noise ratios of 3 and 10. Linear calibration curves were calculated over a concentration range of 0.001–1600 pmol per 2 μL injection volume. The recovery was assessed by the experiments in which the known amounts of AMQ (6-aminoquinoline)–amino acid standards (0.01, 0.50 and 1 mmol/L) were added to the tea samples. Each standard was analyzed in triplicate, and the peak area was plotted against the corresponding concentrations.

| Time (min) | Flow rate (mL/min) | Eluent A | Eluent B |
|------------|--------------------|---------|---------|
| 0          | 0.40               | 88.0    | 12.0    |
| 1          | 0.20               | 96.0    | 4.0     |
| 2          | 0.20               | 93.0    | 7.0     |
| 3          | 0.15               | 90.0    | 10.0    |
| 3.5        | 0.30               | 83.0    | 17.0    |
| 4          | 0.30               | 82.0    | 18.0    |
| 4.5        | 0.50               | 78.0    | 22.0    |
| 6          | 0.20               | 76.0    | 24.0    |
| 7          | 0.20               | 76.0    | 24.0    |
| 8          | 0.50               | 65.0    | 35.0    |
| 10         | 0.50               | 65.0    | 35.0    |
| 10.1       | 0.50               | 0.0     | 100.0   |
| 11         | 0.50               | 0.0     | 100.0   |

Eluent A: contained sodium acetate (140 mmol/L), sodium azide (0.15 mmol/L), disodium EDTA (0.26 mmol/L) and tri-ethyl amine (17 mmol/L), in water, and was titrated to pH 4.93 with phosphoric acid.

Eluent B: 60% (v/v) acetonitrile.
2.9. Statistical analysis

Mean values of each sample were obtained from at least three replications. The hierarchical cluster analysis was conducted by R 3.1.0 with package Pheatmap 0.7.7 (Free Software Foundation). The Python 2.7.8 software (Python Software Foundation) was used for principal component analysis (PCA). Pearson correlation was performed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Validation of FAA analysis method

The gradient elution program of the UHPLC method was optimized, and the final analytical method achieved a high resolution allowing the identification of target compounds in a short time (Fig. 1A). All analyzed AQC derivatives exhibited appropriate linearity ($R^2 \geq 0.9950$) within the applied calibration range (10–400 pmol per 2 μL injection volume) at 254 nm.

For most of the amino acids, the instrumentation precision of relative standard deviation (RSD) of amino acid standards varied <3%, whereas the repeatability (RSD) was <5% for all analytes. The stability of amino acid standards (RSD) varied <4%. Limits of detection and quantification (LOD and LOQ) were calculated at the respective signal-to-noise ratios of 3 and 10, with on-column amounts of AQC derivatives ranging between 0.05–0.10 pmol and 0.10–0.50 pmol (each per 2 μL injection volume), respectively. The recovery rate was calculated by comparing the obtained amounts with those added, and values ranged between 80% and 105%. Therefore, the overall UHPLC analytical procedure was fast, accurate and suitable for the quantitative analysis of a large number of samples.

3.2. Quantitative analysis of selected FAAs in various non-Camellia teas

The new analytical method was subsequently applied to simultaneously determine 20 FAAs levels of 119 samples from 33 non-Camellia teas representing a variety of types. Green tea (34), black tea (35) and pu-erh tea (36) from Camellia leaves were also analyzed at the same time for comparison. The individual contents of the 20 FAAs in these samples are listed in Table S1.

The results showed that most non-Camellia teas had relatively moderate levels of the 20 FAAs (Table S2). The total amino acid content in green tea (34) (24.99 mg/g) was the highest followed by pu-erh tea (36), black tea (35), teas from Lycium barbarum (25), H. sabdariffa (5), Gynostemma pentaphyllum (23) and Scutellaria.
*baicalensis* (32), with contents ranging from 10.41 to 18.69 mg/g. Teas from *C. tinctoria* (30), *Pistacia chinensis* (29), *Forsythia suspensa* (18), *Litsea coreana* var. *lanuginose* (14), *Adinandra nitida* (12), *Acer tataricum* subsp. *ginnala* (28), *D. morifolium* (20), *Rubus chinensis* var. *suavissimus* (6), *C. indicum* (19), and *Scoparia dulcis* (26) were at intermediate levels ranging from 5.24 to 9.70 mg/g. The other teas had lower concentrations ranging from 0.62 to 4.67 mg/g (Fig. 2A). Moreover, essential amino acid (EAAs) concentrations were high in black tea (35, 18.29 mg/g). Pu-erh tea (36), teas from *H. sabdariffa* (5), *L. barbarum* (25), *P. chinensis* (29), *A. nitida* (12) and green tea (34) with intermediate levels (4.62–8.17 mg/g). Other teas had lower levels (less than 4.50 mg/g) (Fig. 2B). HEAs are considered conditionally essential in the human diet, meaning their synthesis can be limited under special pathophysiological conditions. The content of HEAs was highest in tea from *L. barbarum* (25, 6.24 mg/g), and

**Figure 2** Content of amino acids in non-*Camellia* tea. (A) Total (total amino acid); (B) EA (essential amino acid, including His, Thr, Val, Met, Lys, Ile, Leu, Phe, Trp); (C) HEA (half-essential amino acid, including Glu, Gly, Arg, Pro, Cys, Tyr); (D) NEA (non-essential amino acid including Asp, Ser, Ala); (E) GABA; (F) Thea. I, II, III, IV: corresponding to the 4 classes in Fig. 4, V: three *Camellia* tea (green tea, black tea and pu-erh tea).
followed by green tea (34, 5.74 mg/g), teas from C. tinctoria (30, 4.86 mg/g) and S. baicalensis (32, 4.70 mg/g). Teas from G. pentaphyllum (23, 3.03 mg/g), P. chinensis (29, 2.91 mg/g), Orthosiphon aristatus (31, 2.88 mg/g), C. indicum (19, 2.54 mg/g), R. chinii var. suavissimus (6, 2.31 mg/g). Pu-erh tea (36, 2.13 mg/g) contained relatively large amounts of HEA ranging from 2.13 to 3.03 mg/g, while other teas had less than 1.60 mg/g (Fig. 2C). The three NEAs, which are dispensable in humans and can be synthesized in the body, existed at relatively high amounts in teas from L. barbarum (25, 3.08 mg/g), G. pentaphyllum (23, 3.12 mg/g), H. sabdariffa (5, 3.14 mg/g) and green tea (34, 2.85 mg/g), and less than 1.5 mg/g in the other teas (Fig. 2D).

Although the total content of amino acids in each non-Camellia tea is lower than the content in the three common Camellia teas, the contents of some specific amino acids are significantly higher in non-Camellia teas than in green tea. In terms of the essential amino acids, the respective Thr contents of each of the non-Camellia teas were: P. chinensis (29, 3.85 mg/g), L. barbarum (25, 2.67 mg/g), S. dulcis (26, 3.66 mg/g), S. baicalensis (32, 2.68 mg/g), L. coreana var. lanuginose (14, 2.57 mg/g) and D. morifolium (20, 2.75 mg/g). The respective Val contents were L. barbarum (25, 0.60 mg/g), S. baicalensis (32, 0.76 mg/g), R. chinii var. suavissimus (6, 0.35 mg/g), and A. nitida (12, 0.52 mg/g). Met contents were F. suspensa (18, 2.09 mg/g). The Lys content in F. suspensa was (18, 0.51 mg/g). The Phe contents respectively were L. barbarum (25, 1.33 mg/g), A. nitida (12, 3.03 mg/g) and G. pentaphyllum (23, 1.65 mg/g), significantly higher than in green tea (34, 1.95 mg/g, 0.17 mg/g, 0.56 mg/g, 0.29 mg/g, 0.53 mg/g, respectively). In terms of NEA, the content of Asp in teas from H. sabdariffa (5, 2.61 mg/g) and G. pentaphyllum (23, 2.75 mg/g) were significantly higher than in green tea (34, 1.24 mg/g).

In terms of HEA, Gly showed low contents in all the samples but tea from H. sabdariffa showed the highest content (0.21 mg/g). The content of Arg in tea from P. chinensis (29, 1.93 mg/g) was relatively higher than that in three Camellia teas. The content of Pro in teas from R. chinii var. suavissimus (6, 2.03 mg/g), C. indicum (19, 1.40 mg/g), L. barbarum (25, 3.91 mg/g), C. tinctoria (30, 4.41 mg/g), Orthosiphon aristatus (31, 2.48 mg/g) and S. baicalensis (32, 3.17 mg/g), were significantly higher than in three Camellia teas which ranged from 0.05 to 0.31 mg/g. Except for nine teas (including 4–8, 15, 16, 22, 33), Cys content in most non-Camellia teas also showed higher concentration than in the three Camellia teas.

Of note, GABA was detected in all teas except tea from Sarcandra glabra (15, Fig. 2E). Thea was detected in approximately half of the teas (Fig. 2F). The GABA content in teas from Ampelopsis grossedentata (10), Isodon serra (17) and H. sabdariffa (5) was the highest (all above 1.02 mg/g), much higher than that in green tea (34, 0.28 mg/g). For Thea, green tea (34) was found to have significantly higher content (10.61 mg/g) than the others. Pu-erh tea (36) was the second highest at approximately 4.59 mg/l. The only other tea with a Thea content above 1.0 mg/g was tea from Potentilla fruticosa (21, 1.16 mg/g). All others had less than 0.5 mg/g. We are the first group reporting these results, and further studies on more tea samples remain to be performed.

3.3. Principal component analysis (PCA) of teas

The potential utility of employing PCA using a combination of the key parameters and the contents of the 20 FAAs as a means of classifying the teas was explored. The PCA provided three eigenvalues, which were analyzed to obtain three factors (Z1–Z3, Table S3). The proportion of each eigenvalue was computed, and the cumulative proportion of the three factors was found to total 90.61%. The three factors were retained for further analyses because their cumulative proportions were higher than 90%, which is considered adequate for the estimation of FAAs patterns. The eigenvectors of the patterns of the 20 FAAs used to obtain the three factors are summarized in Table S4. The plot of the PCA scores, as shown in Fig. 3A, was readily divided into two relative clusters, which indicates that the content and distribution of the 20 FAAs are highly varied in the three Camellia teas and 33 non-Camellia teas. The plots of the PCA loadings were utilized to identify the differential FAAs for the discrimination of groups. Gly and Arg were largely influenced by the first factor (PC1), whereas His was dominant by the second factor (PC2). Try, Cys and Arg were mainly influenced by the third factor (PC3, Fig. 3B).

A similar PCA was performed to analyze 33 non-Camellia teas based on content of the 20 FAAs. The cumulative proportion of the three factors was found to total 82.51% (Table S5), and the eigenvectors of the patterns of the 20 FAAs used to obtain the three factors are summarized in Table S6. The plot of the PCA scores, as shown in Fig. 3C, was readily divided into four relative clusters, indicating that the content and distribution of the 20 FAAs is highly varied in the different non-Camellia teas. The plots of the PCA loadings were utilized to identify the differential FAAs for the discrimination of groups. Gly, Arg and Try are largely influenced by the first factor (PC1), whereas His and Thr are dominant by the second factor (PC2). Try and Arg are mainly influenced by the third factor (PC3, Fig. 3D).

3.4. Cluster analysis of non-Camellia tea based on FAA level

Hierarchical cluster analysis (HCA) was used to confirm the results of the PCA analysis. The content of the 20 FAAs obtained for the 33 non-Camellia teas was standardized into the Euclidean equation to obtain the Euclidean distances between the samples. The cluster analysis provided a dendrogram, and Ward’s minimum-variance cluster analysis (WMVCA) produced two large sub-clusters, which were denoted A and B (Fig. 4).

Cluster B was further divided into two sub-clusters, which were denoted II and III. Sub-cluster II, which is comprised of 3, 5, 14, 20, 26 and 29, was defined by relatively high contents of Gly and Thr. Sub-cluster III also consisted of six non-Camellia teas, including 6, 19, 25, 30, 31 and 32; this sub-clusters contained a high amount of Pro, Thr and Glu. Cluster I included 19 non-Camellia teas have relatively low content of the total amino acid (<4.0 mg/g) except 10, 18, and 28 (4.26, 6.77 and 5.91 mg/g, respectively). Among these teas, 10 and 17 with high content of GABA could be considered together for a separate class. Cluster IV included two non-Camellia teas (12 and 23) which exhibited relatively high contents of Phe, Asp and Glu.

HCA was also conducted to accurately describe the content characters among the 20 FAAs (Fig. 4). Thr was the FAA with the highest average content of (1.42 mg/g), followed by Pro (0.69 mg/g). GABA, Phe, Glu and Asp were at intermediate levels in all 20 FAAs ranging from 0.27 to 0.40 mg/g, while the other FAAs had a lower content ranging from 0.09 to 0.25 mg/g.
Figure 3  Principal component analysis of amino acids in teas. (A) Scores plot of 36 teas; (B) loading plot of 36 teas; (C) scores plot of 33 non-
Camellia teas; (D) loading plot of 33 non-Camellia teas. I, II, III, IV: corresponding to the 4 classes in Fig. 4.

Figure 4  Hierarchical cluster dendritic diagram of 33 non-Camellia teas. Cluster (A) is divided into I and IV, cluster (B) is divided into II and III,
respectively.
3.5. Total phenolic content of non-Camellia tea

Fig. 5A shows the total phenolic content of the non-Camellia tea samples (data presented in Table S7). Most of the samples had high polyphenol content. Tea from A. grossedentata (10) had the highest total phenolic content (177.25 mg GAE/g) among all of the non-Camellia teas. It had over 2 times higher phenolic content than green tea (34, 80.07 mg GAE/g), black tea (35, 39.77 mg GAE/g) and pu-erh tea (36, 67.82 mg GAE/g). Teas from A. tataricum subsp. ginnala (28), Mallotus oblongifolius (3) and P. fruticosa (21) all had a high phenolic content over 100.00 mg GAE/g. The total phenolic content of teas from I. serru (17, 97.84 mg GAE/g) and P. chinensis (29, 96.86 mg GAE/g) was relatively lower but still higher than that of green tea. Furthermore, teas from C. tinctura (30, 81.92 mg GAE/g) and Engelhardtia roxburghiana (1, 82.62 mg GAE/g) had a similar level of polyphenolics as green tea. The polyphenol content of some samples of teas from Ligustrum robustum (11), A. nitida (12), I. serra (17, 97.84 mg GAE/g), P. chinensis (29, 96.86 mg GAE/g) was relatively lower than that of green tea. Furthermore, teas from C. tinctura (30, 81.92 mg GAE/g) and Engelhardtia roxburghiana (1, 82.62 mg GAE/g) had a similar level of polyphenolics as green tea. The polyphenol content of some samples of teas from Ligustrum robustum (11), A. nitida (12), I. serra (17, 97.84 mg GAE/g), P. chinensis (29, 96.86 mg GAE/g) was relatively lower than that of green tea.

Figure 5 Total phenolic content, DPPH EC\textsubscript{50} of non-Camellia tea and their relationship. (A) Total phenolic content of non-Camellia tea; (B) antioxidative activity of non-Camellia tea (mean ± SD); (C) Pearson correlation between total phenolic content and antioxidative activity of non-Camellia tea. I, II, III, IV: corresponding to the 4 classes in Fig. 4, V: 3 Camellia teas (green tea, black tea and pu-erh tea). EC\textsubscript{50} was too high to be detected in this study conditions.

Figure 6 Chromatograms of three purine alkaloids in standard solution and different tea samples. (A) Three purine alkaloid standard solution: 1, theobromine (0.76 μmol/L); 2, theophylline (0.78 μmol/L); 3, caffeine (1.6 μmol/L); (B) green tea; (C) Ampelopsis grossedentata; (D) Mallotus oblongifolius.
**Ilex latifolia** (13) and **L. coreana** var. **lanuginose** (14) were much higher than comparable values from green tea, potentially due to different collection times or/and areas. The polyphenol content of teas from **R. chinensis** var. **suavissimus** (6), **F. suspensa** (18), **Malus hupehensis** (22), **Cratoxylum cochinichense** (24), **S. baicalensis** (32) and **Lithocarpus litseyfolius** (33) was slightly lower than that of green tea. The other teas had much lower polyphenol content than found in green tea. Tea from **C. obtusifolia** (16) had the lowest polyphenol content (6.27 mg GAE/g).

### 3.6. Antioxidant potential of non-Camellia tea

As shown in Fig. 5B (and Table S7), there were remarkable differences in the DPPH-scavenging capacity among non-Camellia teas. The EC50 values varied from 38.41 μg/mL to 724.13 μg/mL. According to their antioxidant power, 33 non-Camellia teas can be divided in three groups: (a) strong (EC50 < 100 μg/mL); (b) intermediate (EC50, 100–500 μg/mL); (c) weak (EC50 > 500 μg/mL). The EC50 values of teas from **I. serra** (17, 38.41 μg/mL), **P. chinensis** (29, 41.83 μg/mL), and **A. tataricum** subsp. **ginnala** (28, 48.27 μg/mL) were significantly lower than those of the remaining non-Camellia teas and two Camellia teas, black tea (35, 176.23 μg/mL), pu-erh tea (36, 108.10 μg/mL), and had no significant difference with the green tea (34, 44.23 μg/mL) and the Trolox (17.67 μg/mL). In addition, correlation analysis showed that the DPPH-scavenging capacity of the non-Camellia teas was significantly correlated with their phenolic content (r = −0.5552; P < 0.01) (Fig. 5C).

### 3.7. Caffeine, theobromine, and theophylline in non-Camellia tea

The presence of caffeine, theobromine, and theophylline in the non-Camellia tea samples was determined by comparison to alkaloid standard chromatograms. The results show that these alkaloids were not present in any of the non-Camellia tea samples. In contrast, in the three common Camellia teas, caffeine and theobromine were detected, but theophylline was not detected (Fig. 6). Therefore, non-Camellia tea may be a more suitable beverage in some cases than Camellia tea due to the very low amounts of addictive and potentially harmful purine alkaloids.

### 4. Conclusions

A variety of teas from non-Camellia plant are popularly consumed in many regions of China for centuries. In this study, we report for the first time, that some non-Camellia teas contain higher amount of some specific amino acids (such as Arg, Pro, Cys, Thea and GABA), polyphenols (particularly, teas from **A. grossedentata**, **A. tataricum** subsp. **ginnala**, **M. oblongifolius** and **P. fruticosa**, **I. serra** and **P. chinensis**) than found in Camellia tea. Some non-Camellia teas also have remarkable antioxidant activities (particularly, teas from **I. serra**, **P. chinensis**, **A. tataricum** subsp. **ginnala**, and **M. oblongifolius**). They do not contain addictive substances such as caffeine, theobromine and theophylline. Furthermore, a UHPLC method with precolumn derivatization with AccQ to detect 20 amino acids in teas has been established. This method compares favorably against the HPLC or UHPLC methods in which less than 20 amino acids are quantified.

In a summary, the discovery that some non-Camellia teas contain abundant amino acids and polyphenols, which have significant antioxidant activities, but lack caffeine, demonstrating that some of these teas have potential to prevent many chronic diseases. Additionally, non-Camellia teas have been frequently used for centuries throughout many regions of China, indicating that they are quite safe to use. Furthermore, since the non-Camellia teas are derived from various families of plants, the unique types of polyphenols and corresponding bioactivities are highly diverse and can be their characteristic features different from common Camellia tea. Some bioactivity profiles of several non-Camellia teas have been recently characterized by several preliminary chemical and pharmacological studies31,32. Therefore, our study suggests that non-Camellia teas might be more valuable as a functional beverage than Camellia tea, and could be a beneficial supplement or alternative beverage to Camellia tea. Exploration of the non-Camellia teas should provide a variety of beverage choices for health benefits.

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2015.11.003.

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