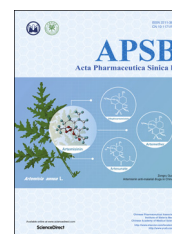




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ORIGINAL ARTICLE

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. *ginnala* are significantly higher than those from *Camellia* tea; teas from *I. serra*, *Pistacia chinensis* and *A. tataricum* subsp. *ginnala* have remarkable antioxidant activities similar to the activities from green tea (44.23 μ g/mL). Purine alkaloids (caffeine, theobromine

Abbreviations: AABA, α -aminobutyric acid; AccQ, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; AMQ, 6-aminoquinoline; AQC, 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; EA, essential amino acid; EDTA, ethylene diamine tetraacetic acid; FAAs, free amino acids; F-C, Folin-Ciocalteu; GABA, γ -aminobutyric acid; GAE, gallic acid equivalents; HCA, hierarchical cluster analysis; HEA, half-essential amino acid; NEA, non-essential amino acid; PCA, principal component analysis; RSD, relative standard deviation; Thea, theanine; UHPLC, ultra-high performance liquid chromatography

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and theophylline) were not detected in non-*Camellia* teas. The investigation suggest 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 alternatives 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^{1–3}. 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^{4,5}. GABA is an important inhibitory neurotransmitter in the mammalian central nervous system and is known to exhibit antihypertensive effects⁶. Teas rich in GABA can decrease blood pressure in rats⁷. Amino acids also participate in the biosynthesis of polyphenols and alkaloids^{8,9}. 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 cancers¹⁰. However, purine alkaloids (such as caffeine, theobromine and theophylline), as additional key components of tea and coffee, may have negative impacts on our wellness when the intake is high¹¹.

A variety of folk teas originated from plants other than *Camellia* have been used by various indigenous and minority groups in China for centuries. Their potential nutritional and medical value in preventing chronic diseases has not been widely recognized or compared with common green teas until recent years. Folk teas are also a part of the traditional Chinese tea culture and are widely consumed as beverages and medicines in folk for disease prevention and treatment^{12,13}. Recently, our team performed a systematic collection and some chemical and pharmacological investigations of these traditional teas and named them as non-*Camellia* teas (or in Chinese: Bie-yang-cha)^{14–16}. Our

previous studies demonstrated that many non-*Camellia* teas contain abundant polyphenols, show striking antioxidant effects *in vitro*, and have many other health benefits^{17–20}.

Due to the biological significance of the amino acids, polyphenols and alkaloids contained in green tea, for the first time we systematically investigated these important types of constituents and the antioxidant activity of extracts of plants for non-*Camellia* teas, in order to provide information on the health properties and promote the development of non-*Camellia* teas as functional beverages. In this study, 33 non-*Camellia* tea samples were collected based on these criteria: those teas (i) are not originated from *Camellia* plants; (ii) are popularly used for at least three hundred years in some minorities' areas or indigenous groups in China; and (iii) are presently consumed.

Several well-established biochemical methods were used in our study. The ultra-high performance liquid chromatography (UHPLC) method was used to determine the amino acid content. It is a rapid, modern, and effective method which has been used to detect amino acids in cheese and green tea^{21,22}. Precolumn derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidylcarbamate (AccQ) is a widely used method for the derivatization of amino acids^{21,23,24}, but the use of UHPLC with precolumn derivatization with AccQ to detect 20 amino acids has not been 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

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