Discriminative Chemical Profiles of Shan Tuyet Tea (Camellia sinensis var. Shan) and Sinensis Tea (Camellia sinensis var. sinensis) Collected in Ta Xua, Son La, Vietnam and Their Correlation With Antioxidant Activity

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Abstract

Chè Shan Tuyet (Camellia sinensis var. Shan) is one of the precious tea resources of Vietnam; however, there is little research on its chemical composition. The purpose of this study was to characterize the main quality components, such as free amino acids and catechins, in Camellia sinensis var. Shan and Camellia sinensis var. sinensis collected in the high mountain of Ta Xua, Son La, Vietnam by using an amino acid analyzer and liquid chromatography coupled with tandem mass spectrometry, respectively. Principal component analysis (PCA) discrimination analysis of chemical profiles revealed a clear metabolic difference between the young leaves of Shan Tuyet tea and mature leaves of the same variety and of sinensis tea. The amino acids serine, glutamic acid, arginine, ornithine, and aspartic acid contributed mainly to the discrimination and could be considered biomarkers for Shan Tuyet tea. The levels of caffeine and 7 catechins, catechin, catechin 3-gallate, epicatechin, epicatechin-3-gallate, epigallocatechin 3-gallate, gallocatechin, and gallocatechin 3-gallate, in young leaves of Shan Tuyet tea were significantly higher than in the other types. Notably, the pair correlation among catechins revealed strong coefficients of the epistructures and non-epistructures, which suggested that these compounds can be converted naturally to each other. The strong correlation between epicatechin-3-gallate and catechin 3-gallate with antiradical ABTS activity of Shan Tuyet tea leaves indicates that these 2 catechins are mainly responsible for the antioxidant activity. This is the first report on the bioactive compounds of Shan Tuyet tea, as well as its potential for the production of health supplements.

Keywords

Camellia sinensis, Shan Tuyet, amino acid, catechins, antioxidant activity, discrimination

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Introduction

Tea has been considered for a long time the most popular and widely consumed beverage in the world because of its refreshing taste, attractive aroma, and potential health benefits.¹ Various components such as amino acids, polyphenols, carbohydrates, vitamins, xanthine, and purine alkaloids present in tea help induce various physiological and pharmacological activities.² Tea is processed using the leaves of Camellia sinensis L., a plant now commonly grown in Southeast Asia as well as in central Africa.

There are many varieties of C sinensis, one of the most precious of which is C sinensis var. Shan, grown in Vietnam and used to prepare Shan Tuyet tea.³ C sinensis var. Shan is an ancient tree that can be hundreds of years old and is several meters tall (Figure 1). This variety of C sinensis is often cultivated in the highlands, about 1200 to 2000 m above sea level. In Vietnam, Shan Tuyet tea plants are often found in the northwest and northeast regions such as Son La, Cao Bang, Ha Giang, Lao Cai, and Yen Bai. Although Shan Tuyet tea is a high-value Vietnamese specialty, little research has been done on its chemical composition, as well as on biomarkers that assist tracing of

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the tea products’ provenance. Many tea products come from this variety, but, unfortunately, their quality has not yet been controlled by any scientific manner in terms of both variety and leaf age.

Among tea phytochemicals, polyphenols (in particular, catechins) and amino acids have received immense attention as bioactive compounds and important primary metabolites in biosynthetic pathways of the featured compounds. These compounds were thus considered as quality components for tea quality. On the one hand, the major green tea catechins are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Among them, EGCG accounted for a significant amount of the total catechin content and is regarded as the major antioxidant ingredient in green tea. Tea polyphenols have a wide range of biological effects, including suppression of proliferation and angiogenesis, induction of cell cycle arrest and apoptosis, and alteration of cell signaling. Further roles for tea polyphenols include aiding weight reduction and, as a result, they have been included in weight loss supplements. On the other hand, free amino acids perform individually as active compounds in tea, affecting the taste, aroma, and color quality of the tea. Glutamine (Gln) and glutamic acid (Glu) are free amino acids that give the tea a distinct umami taste, which makes tea savory and brisk. Tryptophan (Trp) and phenylalanine (Phe) contribute to the intensity of the bitter and astringent taste of the tea. Serine (Ser) exhibits aspects of a wine scent. Histidine (His) and leucine (Leu), respectively, act as the antioxidant and branched-chain amino acid balance. The aromatic amino acids, such as phenylalanine, are considered precursors of phenolics, the group of compounds responsible for the antioxidant activity of tea leaves. Therefore, the content and quantity of catechins and free amino acids in different types of tea are important factors in determining the quality of tea.

Our study provides useful information regarding the characterization of catechins and free amino acids present in Shan Tuyet and sinensis variety teas collected at the same location. These data were used to differentiate the chemical profiles of the 2 varieties of C. sinensis, as well as identify biomarkers for traceability. The antioxidant properties of these samples were also determined and correlated with chemical profiles to have insights into the compounds which contribute to this bioactivity.

Results and Discussion

Free Amino Acids Profile

The free amino acid profile is one of the keys determining variables of tea quality. Our analysis characterized 23 amino acids in methanolic extracts of Shan Tuyet and sinensis tea leaves (Table 1S, Supporting Information). The discrimination of free amino acids profile between young and mature leaves of Shan Tuyet and sinensis tea was analyzed by using principal component analysis (PCA), which is the most generally used technique to determine how one sample is differentiated from another. The loading scatter plot of PCA visualized the separation between clusters indicating the discriminative level of samples. Overall, the metabolic discrimination was found greater in terms of leaf age than in terms of plant varieties. Indeed, Figure 2A shows the highly distinguished amino acid profile between young leaves of Shan Tuyet tea and both mature leaves of Shan Tuyet and sinensis tea. Two principal components are illustrated in Figure 2A, contributing 49.5%
to the total variance that perfectly represents the data. The first principal component (Dim-1) attributed 29.5%, while the second (Dim-2) explained 20% of the total. Zooming into the free amino acid profile of the 2 latter sample types, Figure 2B demonstrates weaker discrimination compared to the first PCA plot, with the 2 first components contributing 39.5% to the total variance. However, this plot still showed significant separation between Shan Tuyet and sinensis leaves. The amino acid profile of the 2 types of tea extracts was discriminated as the first-dimensional component interpreted more the Shan Tuyet tea (24.9% of the total variance), while the second component (explaining 14.6% of the total) was correlated with sinensis tea more than the first one.

The structure and quantity of free amino acids not only contribute directly to the flavor and taste of tea, but also affect the quality by their products of transformation and degradation. Because amino acids are precursors of volatile aldehydes and other compounds during tea preparation, they play an important function in the process of forming the aroma of tea. In addition, the building blocks of proteins are amino acids, which are also crucial parts of active peptidases and other bioactive compounds. Therefore, the distinctive amino acid profile could help to explain the responsibility of these primary metabolites to the specific taste, flavor and then bioactivity of Shan Tuyet and sinensis tea. That may also be the reason why Shan Tuyet tea has been considered as a precious natural resource of Vietnam and as a material to process into various specialty tea products. From this starting point, the biosynthetic pathway of bioactive compounds from these tea leaves should be investigated comprehensively to valorize this tea variety.

Figure 1S shows the contribution of different amino acids to the separation of 2 types of tea extracts, in which, serine (Ser), isoleucine (Ile), glutamic acid (Glu), valine (Val), methionine (Met), glycine (Gly), phenylalanine (Phe), tyrosine (Tyr), methionine (Met), arginine (Arg), proline (Pro), cysteine (Cys), and ornithine (Orn) were revealed as the most differential (with their high values estimating the quality of the representation, cos2 > 0.5). Among these compounds, Ser, Glu, Arg, and Orn were found in young leaves of Shan Tuyet in the greatest content (Figure 3). The distinguishing levels of these important amino acids contributed to explaining the different aroma and

Figure 2. Principal component analysis (PCA) scatter plots of 2 first components of amino acids in (A) young leaves of Shan Tuyet tea (triangle), mature leaves of Shan Tuyet tea (circle), and mature leaves of sinensis tea (square) and (B) mature leaves of Shan Tuyet tea (circle) and mature leaves of sinensis tea (square).
Taste characteristics of Shan Tuyet tea and sinensis tea. Indeed, Ser exists in Shan Tuyet young leaves at a level of 5302.9 ± 848.9 µM corresponding to 5.56 ± 0.89 mg/g dry weight (d.w.). This content was the highest for Ser in all the tea varieties and products reported in other research. Similar to Ser, Glu presented in Shan Tuyet young leaves with a concentration of 1247.3 ± 54.4 µM, corresponding to 1.84 ± 0.08 mg/g d.w., which is higher than that in other sinensis teas in previous studies. In contrast, even though Arg was found in the highest concentration in Shan Tuyet young leaves in this study with 86.1 ± 9.6 µM, corresponding to 0.15 ± 0.02 mg/g d.w., this amino acid represented a lower content than in other C. sinensis leaves reported in other studies. Orn, an amino acid that was rarely quantified in tea in other investigations, was detected in young leaves with a content of 0.06 ± 0.02 mg/g d.w. This concentration in Shan Tuyet leaves was significantly higher than that in other types of our samples. Asp was not detected in Shan Tuyet young leaves while existing in mature leaves of the same variety and in the sinensis variety. Therefore, all the above-mentioned reasons let us hypothesize that Ser, Glu, Arg, Orn, and Asp could be biomarkers in the determination of tea quality and contribute to the traceability of Shan Tuyet tea originating from Son La province of Vietnam. This agrees with a previous study which reported Glu, Arg, and Asp as the most discriminating amino acids.

Besides these differential amino acids, histidine (His) is not a variable that contributes much to the discrimination of the whole profile, but was present in Shan Tuyet young leaves at a significantly higher level compared to mature leaves (136.4 ± 28.1 µM corresponding to 0.023 ± 0.005 mg/g d.w.). In contrast, leucine (Leu) was absent from Shan Tuyet young leaves, but present in mature leaves. All amino acids themselves have a distinct scent and are among the most vital components that affect the flavor of the tea. Therefore, the difference in the content of each amino acid could explain the distinguishing flavor and taste of tea leaves analyzed in this study. This is the first time that such kind of characterization has been undertaken for Shan Tuyet tea leaves collected in the high mountains of Vietnam, providing information about the chemical composition of this specialty tea.

**Catechins Profile and its Correlation With Antioxidant Activity**

Besides free amino acids, catechins and caffeine are important indicators of the value of tea leaves because of their strong antioxidant ability and the effect on the central nervous system, respectively. In order to quantify catechins and caffeine in tea extracts, UPLC-MS/MS triple quadrupole with selected reaction monitoring (SRM) scanning for each analyte was considered to be an ideal choice, with high sensitivity and selectivity. In this study, the target solution was calibrated on MS systems to obtain the ion fragments used for quantification. The electrospray ionization was performed in both positive and negative modes. The results showed that 7 catechins had a higher response in the negative mode and so this was used for detection in this study. In contrast, caffeine was more sensitive in positive ionization mode. The order of elution is
demonstrated in Figure 4 with the total ion chromatogram and extract ion chromatogram at the transitions 304.97→124.88, 288.97→108.88, 457.03→168.88, and 441.03→168.88 for catechins (Figure 4) and 195.03→138.03 for caffeine. Among catechins, GC eluted first, followed by C, EGCG, GCG, EC, ECG, and CG. This order is consistent with the analysis mentioned in previous reports.\(^{21}\) The limit of detection (LOD) was determined by the lowest concentration of the peak having a signal-to-noise ratio of 3, and the limit of quantification (LOQ) was defined as 3.3-fold of LOD (Table 1).

The matrix-matched calibration curves (Table 1) were used to quantify 7 catechins and caffeine in young and mature leaves of Shan Tuyet tea, and mature tissues of sinensis tea. In general, the young leaves of Shan Tuyet tea possessed the highest level of catechins and caffeine, especially GC, EC, EGCG, and GCG with 43.4±5.6, 37.7±11.4, 34.1±11.9, and 34.1±11.9 mg/g d.w., respectively. However, mature leaves of Shan Tuyet tea and leaves of sinensis tea did not show any big differences in catechins levels. Among those catechins, (+)-catechin attributed the lowest content in the 3 types of tea extracts and EGCG dominated the catechin profile in most of the samples. These data are relevant to a previous report, which indicated that EGCG was the most abundant catechin in green tea.\(^{22}\) On the other hand, regarding GCG, the content of this compound in young leaves of Shan Tuyet tea was the highest among the 3 types of leaves in this study and especially higher than that in non-processing tea leaves in the previous investigation with 34.1±11.9 mg/g d.w.\(^{21,23}\) Generally, this result indicated that Shan Tuyet tea, especially the young leaves, can deliver more valuable products due to its extremely high content of catechins.

Caffeine, one of the important components of tea leaves, does not contribute to the tea’s taste, but has a big impact on the central nervous system. In this study, the variety of Shan Tuyet was found to produce more caffeine than the sinensis type, while this alkaloid was present in a higher concentration in young leaves of Shan Tuyet tea (122.5±5.6 mg/g d.w.) than in the mature leaves (of 77.8±11.9 mg/g d.w.). Even though the young leaves of the tea species are well known to be the main starting material for the processing of tea products, the variety of Shan Tuyet was found to produce more caffeine than the sinensis type, while this alkaloid was present in a higher concentration in young leaves of Shan Tuyet tea (122.5±5.6 mg/g d.w.) than in the mature leaves (of 77.8±11.9 mg/g d.w.).

![Figure 4. Total ion chromatograms (TIC) and extracted ion chromatograms (EIC) of transitions corresponding to C and EC (288.97→108.88), GC (304.97→124.88), CG, and ECG (441.03→168.88), EGCG, and GCG (457.03→168.88).](image)

| Type | Calibration curve | \(r^2\) | LOD (ppb) | LOQ (ppb) | SLN (n=6) | SLG (n=31) | TX (n=32) |
|------|------------------|--------|----------|-----------|-----------|-----------|-----------|
| C    | \(y = 577.22x + 124.8\) | 0.998  | 5.38     | 17.94     | 7.40±4.62\(^a\) | 1.10±0.73\(^b\) | 0.81±0.76\(^b\) |
| EC   | \(y = 470.76x + 220.19\) | 0.998  | 9.34     | 31.14     | 37.75±11.41\(^a\) | 4.19±3.20\(^b\) | 6.21±4.34\(^c\) |
| GC   | \(y = 826.44x + 54.316\) | 0.998  | 19.04    | 63.45     | 43.44±5.60\(^b\) | 2.25±1.10\(^b\) | 2.14±2.02\(^b\) |
| CG   | \(y = 2892.2x – 3654.2\) | 0.998  | 36.12    | 120.4     | 18.33±3.45\(^c\) | 4.10±2.93\(^b\) | 6.68±3.15\(^c\) |
| ECG  | \(y = 2941x – 3627.6\) | 0.997  | 25.65    | 85.49     | 18.01±3.38\(^b\) | 4.08±2.84\(^b\) | 6.57±3.09\(^c\) |
| EGCG | \(y = 3648.7x – 6606.3\) | 0.997  | 12.66    | 42.21     | 34.11±11.92\(^a\) | 4.56±2.41\(^b\) | 7.00±5.34\(^c\) |
| GCG  | \(y = 3658x – 8444.6\) | 0.996  | 49.49    | 164.97    | 34.06±11.86\(^a\) | 4.74±2.70\(^b\) | 7.22±6.64\(^c\) |
| Caffeine | \(y = 2265.4x + 845.34\) | 0.999  | 1.47     | 4.85      | 122.50±5.60\(^a\) | 77.84±11.86\(^a\) | 28.63±12.26\(^c\) |

\(n\): number of samples.

\(^{a,b,c}\): Different letters showed the significant differences between sample types, according to t-test with \(P\)-value < .05.
The mature leaves are usually used for direct preparation of tea infusions. The significant difference in caffeine level between young and mature leaves of Shan Tuyet variety help to explain the fact that the effect on the central nervous system of non-processed tea infusion (originating from mature leaves) was lower than the processed one (from young leaves). Therefore, mature leaves should be recommended for specific consumers who do not want to be affected by caffeine.

A pairwise correlation analysis was performed to reveal the relationship between catechins profiles (Figure 5, input data in Table 2S, Supporting Information). In this study, a correlation coefficient of above 0.6 was defined as a significant correlation.

The result also accessed the relationship between different pairs of catechin and epicatechin, in which C and EC, CG and ECG, GCG and EGCG, respectively, correlated with a high coefficient: 0.74, 0.95, and 1 (P-value < .001). These significant correlations suggest that catechins and their epimers can be transformed inversely in nature. Several studies revealed the epimerization of catechins in green tea infusions and this reaction was considered the most important conversion in the manufacture of green tea. However, research on the epimerization inside the leaf tissue is limited. In the possible biosynthetic pathways of catechins in tea proposed by Liu et al, there is no direct conversion from the epistucture to the non-epistucture.25

Figure 5. The pair correlation between 7 catechin levels and antioxidant activity in Shan Tuyet tea. On the bottom of the diagonal, the bivariate scatter plots with a fitted line are visualized. On the top of the diagonal, the correlation level between pairs is displayed by correlation coefficients (calculated by Pearson method) and the significance level is denoted as stars. *** P-value < .001, ** P-value < .01, and *P-value < .05.
Table 2. UPLC Parameters: Gradient Table of UPLC-QqQ-MS/MS Analysis.

| Time (min) | Flow (mL/min) | Solvent A (%) | Solvent B (%) |
|-----------|---------------|---------------|---------------|
| 1         | 0.3           | 75            | 25            |
| 2         | 1             | 70            | 30            |
| 3         | 2.50          | 70            | 30            |
| 4         | 2.51          | 75            | 25            |
| 5         | 5.51          | Stop          | 75            | 25            |

Solvent A: 0.01% (v/v) formic acid in water.
Solvent B: Methanol 100%.

Therefore, this is the first time that such a kind of conversion has been proposed. Similarly, the strong correlation between GC and EGCG ($r^2 = 0.95$, $P$-value < .001) and GCG ($r^2 = 0.95$, $P$-value < .001) suggested that there is this conversion in the biosynthetic pathway among all three catechins. These pathways have never been reported in any previous research and need to be confirmed by further investigations.

The correlation between the total phenolic content and antioxidant activity in tea was well studied, but there is limited information about which catechin(s) is/are responsible for this property. In this study, to understand the contribution of catechins’ composition to antioxidant activity, the correlation coefficient between catechin concentrations and antioxidant activity of a number of samples was determined. A catechin showing a strong and significant correlation with bioactivity was deduced to be responsible for this activity. To do that, the antioxidant activity of these extracts was assessed using free radical scavenging activity with ABTS as a free radical model. The inhibition percentage of each extract on ABTS radicals is shown in Table 3S (Supporting Information). Looking at the correlation of catechin profiles and antiradical properties of Shan Tuyet extracts, revealed that the antioxidant activity was correlated significantly most with ECG ($r^2 = 0.74$), followed by CG ($r^2 = 0.68$) and EC ($r^2 = 0.61$). This result was not fully in agreement with previous reports, in which EGCG possessed the highest antioxidant activity for green tea measured by ABTS and DPPH assays. The authors also indicated that high levels of EGCG and EGC and low levels of C and GCG attributed most to the antioxidant activity of green tea. However, those dissimilarities may be due to the different species and the age of tea leaves, as well as the extraction and analysis methods. This result also indicated that Shan Tuyet tea can provide differential values that have never been seen in other varieties of C. sinensis.

Conclusions

In this study, the chemical compositions including free amino acids and catechins of Shan Tuyet and sinensis varieties were determined and the results suggest that C. sinensis var. Shan provides higher values for these markers. There was also clear discrimination in the amino acid profile between Shan Tuyet and sinensis varieties, which contribute to the distinct taste of these tea leaves. The young leaves of Shan Tuyet tea significantly contained a higher concentration of catechins and caffeine, especially EGCG and GCG. A strong correlation between catechins and its epi forms was also confirmed, suggesting their naturally inverted conversion. Additionally, the apparent relationship between individual catechins and antioxidant activity using the ABTS assay showed that the antioxidant activity was dependent on the catechins composition, due to an important correlation coefficient. As such, these findings suggest that the young leaf of Shan Tuyet tea is a valuable source of antioxidants. The results obtained in the present study are expected to provide a further understanding of the bioactive compounds of Shan Tuyet tea, as well as its potential in the production of health supplements.

Experimental

Plant material. Leaves of Camellia sinensis var. Shan (Shan Tuyet tea) were collected from 30 individual plants in Be village, Ta Xua ward, Bac Yen district, Son La province (GPS 21° 15′49.5″N 104°28′28.1″E) with an altitude of 2751 m above sea level. These plants were selected from the population of 200 year-old Shan Tuyet tea trees of Ta Xua and are Vietnam Heritage trees. The leaves were classified into 2 types: 6 young leaf samples (1 bud and 3 first leaves) and 30 mature leaf samples (the 4th to 5th leaves). In the same village, the mature leaves of Camellia sinensis var. sinensis (sinensis tea) were collected from 30 individual trees. All the leaf materials were cleaned to remove dust, dried in a thermostatic oven at 45 °C until unchanged weight, then ground by shaking with zirconium bead at a frequency of 25 times per second for 2 min using a Mixer Mill MM 400 (Retsch, Germany). Samples were stored at −80 °C.

Chemicals. A catechins mix standard including (+)-catechin, (-)-catechin 3-gallate, (-)+-epicatechin, (-)-epicatechin 3-gallate, (-)-epigallocatechin 3-gallate, (-)-gallocatechin, (-)-gallocatechin 3-gallate with 100 μg/mL of each component certified reference material was purchased from Supelco, Merck. The internal standard, 10-camphorsulfonic acid, was provided by Acros Organics, Fisher Scientific.

Oxidized hydrolysate standard, including 23 amino acids (L-cysteic acid, taurine, D, L-methionine sulfioxide, L-methionine sulfone, L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-cystine, L-valine, L-methionine, L-isoleucine, L-tyrosine, L-phenylalanine, L-histidine, L-ornithine, L-lysine, ammonia, L-arginine) with 2.5 mM concentration of each component, all loading buffers and reagents for derivatization were provided by Biochrom, United Kingdom.

Liquid chromatographic solvents, including acetonitrile (ACN), methanol (MeOH), formic acid (FA), and extraction solvent chloroform (CHCl₃) were purchased from Sigma Aldrich, Singapore. Ultra-pure water (UPW) was provided by the Milli-Q integral water purification system (Milli-Q system, Singapore).
Determination of free amino acids level. The infusion was achieved by adding 1 mL of boiling water to 100 mg of finely powdered tea sample before ultrasonic extraction for 15 min at 70 °C. Then, the mixture was extracted for 45 min while shaking and heating using a ThermoMixer® C (Eppendorf, Germany). After centrifugation, the supernatants were filtered through 0.22 μm membranes and loaded into vials. The samples were injected into a Biochrom 30 + Amino acid analyzer physiological system (Biochrom, UK). The instrument parameters were set as follows: flow rate of 0.25 mL.min⁻¹, pressure of 35 bar, reactor temperature of 130 °C, injection volume of 10 μL, and detection wavelength of 440 nm (for proline and leucine) and 570 nm for the remaining amino acids.

Quantification of catechins content. The catechins profile of tea leaves was quantified by using ultra performance liquid chromatography coupled to a tandem mass spectrometer. For sample extraction, 10 mg powered tea leaves was added to 1 mL MeOH 80% in an Eppendorf tube under ultrasonic treatment during 15 min, before being filtered through a 0.22 μm membrane and injected into an ACQUITY UPLC H-Class system (Waters Corporation, US). The mass spectrometry was carried out on a Xevo TQD MS/MS triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. The mixture of catechin standards was infused directly into a triple quadrupole mass spectrometer to optimize the cone voltage (CV) in Q1 and collision cell energy (CE) in Q2 using an automatic tuning process by the IntelliStart tool (Waters Corporation, US). The following parameters of ESI source were respectively set up for negative ion detection mode: desolvation temperature of 350 °C, capillary voltage of 2.4 V, extractor voltage of 3 V, RF lens (hexapole) voltage of 2.5 V, nebulization gas flow rate of 650 L/h, cone gas flow rate of 150 L/h, and CV of 35 V. SRM was performed with auto dwell time in transitions for negative ion detection mode: 304.97→124.88 (CV 50 V, CE 18 eV), 288.97→108.88 (CV 50 V, CE 28 eV), 457.03→168.88 (CV 46 V, CE 16 eV), 441.03→168.88 (CV 50 V, CE 18 eV). For separation, the analytical column C18 BEH (100 mm×2.1 mm i.d.; 1.7 μm, Waters Corporation, US) was kept at constant temperature (40 °C) during chromatographic separation, with an injection volume of 1.5 μL. The samples were stored at 4 °C in a sample manager. The UPLC condition was as in Table 2. The data were acquired and processed using Masslynx software version 4.1 (Waters Corporation, US).

Determination of antioxidant activity. Extracts from tea leaves were prepared by macerating with methanol 80% at room temperature then vortexed for 5 s. The extracts were sonicated for 20 min before being filtered through a 0.22 μL membrane. After being centrifuged at 10000 rpm, the supernatants were transferred into other tubes which subsequently were concentrated by speedvac (Labconco, Fisher Scientific, Austria) until unchanged weight. Sample extracts were also prepared by serial dilutions in ethanol to obtain final concentrations of 500, 250, 125, 62.5, and 31.25 mg/mL. ABTS (2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonate)) stock solution used for this test was prepared by mixing 7 mmol/L ABTS solution with a 2.45 mmol/L potassium persulfate solution in the same ratio by volume. This stock solution was then diluted in distilled water to give an absorbance of approximately 0.7 (wavelength at 734 nm) each use. The prepared sample extracts were mixed with ABTS solution at a ratio of 1:19 to obtain final concentrations of 25, 12.5, 6.25, 3.125, and 1.0625 mg/mL, respectively. The same adjustment to the Trolox standard was performed to obtain a final concentration decreasing from 10 to 0.625 mg/mL. All samples were incubated at room temperature for 10 min and then the absorbance was measured at 734 nm (SpectraMax® iD5 Multi-Mode Microplate Readers, VWR, Germany). The entire process was conducted in a light-induced condition.

Data analysis. Each experiment was carried out in triplicates and statistical analysis was performed in R version 4.0.2. Data of peak areas were processed by Target lynx (Masslynx 4.1, Waters Corporation, US). PCA was constructed to observe the discrimination of the amino acid profile of Shan Tuyet and sinensis teas. Likewise, a boxplot diagram was adopted by tidyverse and ggplot2 R-packages to reveal the concentration of different catechins in 3 types of tea extracts. The correlation between the catechins level and antioxidant activities and the P-value of these comparisons were calculated and illustrated by PerformanceAnalytics R-package.

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Statement of Informed Consent
There are no human subjects in this article and informed consent is not applicable.

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References
1. Kuo KL, Weng MS, Chiang CT, Tsai YJ, Lin-Shiau SY, Lin JK. Comparative studies on the hypolipidemic and growth suppressive effects of oolong, black, pu’erh, and green tea leaves in rats. J Agric Food Chem. 2005;53(2):480-489.
2. Bolling BW, Chen CY. Tea and health: preventive and therapeutic usefulness in the elderly. Curr Opin Clin Nutr Metab Care. 2009;12(1):42-48.
3. Hoang TX, Thu DV, Ha NM, Ha NH, Hue HT, Zhao Y, Liu X, Arkorfal E, Chen X, Li X. Genetic diversity of Shan tea (Camellia sinensis var. Shan) grown in the Northern of Vietnam using ISSR markers. Basic Clin Pharmacol Toxicol. 2020;126:24-25.
4. Cabrera C, Giménez R, López MC. Determination of tea components with antioxidant activity. J Agric Food Chem. 2003;51(15):4427-4435.
5. Liu J, Wen B, Liu X, Yang Y, Li M, Wang X. Molecular and metabolic changes under environmental stresses: the biosynthesis of quality components in preharvest tea shoots. Horticulturae. 2022;8(2):173.
6. Chen L, Zhou ZX. Variations of main quality components of tea genetic resources Camellia sinensis (L.) O. Kuntze preserved in the China national germplasm tea repository. Plant Foods Hum Nutr. 2005;60(1):31-35.
7. Cheng Z, Zhang Z, Han Y, et al. A review on anti-cancer effect of green tea catechins. J Funct Foods. 2020;74:104172.
8. Rothenberg DO, Zhou C, Zhang L. A review on the weight-loss effects of oxidized tea polyphenols. Molecules. 2018;23(5):1176.
9. Mu WM, Zhang T, Jiang B. An overview of biological production of L-theanine. Biotechnol Adv. 2015;33(3-4):335-342.
10. Liu ZW, Wu ZJ, Li H, Wang YX, Zhuang J. L-Theanine content and related gene expression: novel insights into theanine biosynthesis and hydrolysis among different tea plant (Camellia sinensis L.) tissues and cultivars. Front Plant Sci. 2017;8:498-507.
11. Lee LS, Choi JH, Son N, et al. Metabolomic analysis of the effect of shade treatment on the nutritional and sensory qualities of green tea. J Agric Food Chem. 2013;61(2):332-338.
12. Yilmaz C, Ozbekler F, Gökmen V. Investigation of free amino acids, bioactive and neuroactive compounds in different types of tea and effect of black tea processing. JIFTP. 2020b;17:108655.
13. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids. 2009;37(1):1-17.
14. Wang W, Xin H, Wang M, et al. Transcriptomic analysis reveals the molecular mechanisms of drought-stress-induced decreases in Camellia sinensis leaf quality. Front Plant Sci. 2016;30(7):385.
15. Azevedo RSA, Teixeira BS, Sauthier MCDS, Santana MVA, Santos WNLD, Santana DA. Multivariate analysis of the composition of bioactive in tea of the species Camellia sinensis. Food Chem. 2019;273:39-44.
16. Duan Y, Shang X, Liu G, et al. The effects of tea plants-soybean intercropping on the secondary metabolites of tea plants by metabolomics analysis. BMC Plant Biol. 2021;21:482.
17. Liang Y, Lin C, Huang S, Xu Y. Traditional Chinese medicine and intestinal microbiota. Holist Nutr Pract. 2019;33(5):259-265.
18. Li M, Li D, Tai Y, et al. Determination of free amino acids in tea by a novel method of reversed-phase high performance liquid chromatography applying 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate reagent. J Food Sci Technol. 2018;55(10):4276-4286.
19. Li J, Ma J, Li Q, et al. Determination of 35 free amino acids in tea using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Front Nutr. 2021;8:767801.
20. Zhou P, Li Z, Ouyang L, et al. A multi-element stable isotope approach coupled with chemometrics for the determination of Tieguanyin tea geographical origin and harvest season. Anal Methods. 2019;11:346-352.
21. Spáčel Z, Nováková I, Solich P. Comparison of positive and negative ion detection of tea catechins using tandem mass spectrometry and ultra high performance liquid chromatography. Food Chem. 2010;123(2):535-541.
22. Reygaert WC. Green tea catechins: their use in treating and preventing infectious diseases. Biomed Res Int. 2018;2018:9105261.
23. Fernández PL, Pablos F, Martín MJ, González AG. Study of catechin and xanthine tea profiles as geographical tracers. J Agric Food Chem. 2002;50(7):1833-1839.
24. Wang H, Helliwell K. Epimerisation of catechins in green tea infusions. Food Chem. 2000;70(3):337-344.
25. Liu M, Tian H, Wu JH, et al. Erratum: relationship between gene expression and the accumulation of catechin during spring and autumn in tea plants (Camellia sinensis L.), Hortic Res. 2015;2:15023.
26. Dobrinas S, Soceana A, Popescu V, Popovic IC, Jitaria D. Relationship between total phenolic content, antioxidant capacity, Fe and Cu content from tea plant samples at different brewing times. Processes. 2021;9(8):1311.
27. Lee LS, Kim SH, Kim YB, Kim YC. Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity. Molecules. 2014;19(7):9173-9186.
28. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. URL https://www.R-project.org/.
29. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag; 2016, https://ggplot2.tidyverse.org.
30. Peterson BG, Carl P, Boudt K, et al. Performance analytics: Econometric tools for performance and risk analysis. R package version. 2014;1(3).