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Research Article

Metabolomic Profiling Using UPLC–MS/MS Revealed Differential Changes in Metabolites of Three Tea Plant Varieties and Their Potential Antioxidant Activities

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Tea plants, as essential health beverages, are widely recognized for their rich content of antioxidant metabolites. This study utilized metabolomics analysis techniques to compare the metabolic differences between *Camellia tachangensis* F. C. Zhang, *Camellia gymnogyna* Hung T. Chang, and *Camellia sinensis* (L.) O. Kunzte, aiming to reveal their potential antioxidant activities. By applying UPLC–MS/MS technology, we analyzed metabolites in the samples and identified 1056 metabolites. We utilized statistical methods such as orthogonal partial least squares discriminant analysis (OPLS-DA) and principal component analysis to evaluate and compare the variations in the samples. An intersection analysis of metabolites from the three types of tea plants identified 29 major differential metabolites. Functional annotation and pathway analysis of these differential metabolites were conducted, ultimately discussing 24 antioxidant-related metabolites, primarily belonging to flavonoids, phenolic acids, and other categories. Among the three types of tea, *C. tachangensis* has the highest content of procyanidin B4, which is the most abundant substance in this tea. In terms of the impact of this substance on tea, it is known to have antioxidant properties and contributes to the overall antioxidant capacity of tea. In both *C. gymnogyna* and *C. sinensis*, the top 5 antioxidant metabolites include substances such as apigenin-6,8-di-C-glucoside (vicenin-2), epigallocatechin, and apigenin-5-O-glucoside. These substances belong to the flavonoid class and contribute to the antioxidant properties of these teas. Beyond the flavonoids and phenolic acids, other classes of metabolites in tea plants also exhibited significant antioxidant properties. These different metabolites play a key role in the antioxidant function of tea, contributing to their potential health benefits. The metabolites provide crucial insights for exploring and developing high-antioxidant products that occur naturally.

Keywords: antioxidant metabolites; metabolomic; tea

1. Introduction

The origins of tea can be traced back thousands of years, and the Yunnan–Guizhou Plateau is among the significant birthplaces of tea plants [1]. Within this region, several distinct tea plant varieties, including *Camellia tachangensis* F. C. Zhang, *Camellia sinensis* (L.) O. Kunzte, and *Camellia gymnogyna* Hung T. Chang, have been identified. These tea plant varieties hold significant importance in local consumption; however, scientific research regarding their chemical composition [2] and potential health benefits remains relatively limited.

Tea plant varieties have garnered substantial attention in domestic and international tea research communities. Notably, *C. gymnogyna*, *C. tachangensis*, and *C. sinensis*, representing diverse tea plant types [3], have captivated researchers worldwide due to their unique qualities and antioxidant properties. In China, comprehensive studies involving chemical analyses and antioxidant activity assessments have been conducted on the bioactive components of these tea tree varieties. In recent years, high-throughput techniques, such as metabolomic profiling, including metabolomics, have been applied to unveil the metabolic pathways and key metabolites associated with the antioxidant properties [4] of *C. gymnogyna*, *C. tachangensis* tea, and *C. sinensis* [5]. These comprehensive examinations provide fresh angles for delving deeper into the antioxidant mechanisms found in various tea plant varieties. On a global scale, there has been a growing interest in exploring different tea plant varieties. Overseas scholars have employed similar methods to investigate the chemical composition [6] and antioxidant potential [7] of *C. gymnogyna*, *C. tachangensis*, and *C. sinensis*. Metabolomic approaches have been widely adopted in international studies to further uncover the metabolic characteristics [8, 9] and antioxidant mechanisms [10] of these tea plant varieties. These international research efforts not only improve knowledge on the antioxidant properties of tea plants but also provide scientific support for their global standing in the tea market.

Antioxidant metabolites [11] represent a class of biologically active compounds that are crucial for cellular redox balance and protection mechanisms [12]. As knowledge of health and diseases deepens, research and application of antioxidant metabolites have gained increasing attention. Particularly in fields such as food, pharmaceuticals, and health products, antioxidant compounds are essential in the management and alleviation of ailments linked to oxidative stress [13]. Tea plants, as significant sources of health-promoting beverages, are recognized for their abundance of antioxidant metabolites [14]. Among these metabolites, tea plant varieties are known to contain high levels of flavonoids, amino acids, and their derivatives, which are the main components responsible for providing antioxidant properties. Flavonoids exhibit potent free radical scavenging capabilities and antioxidant activities and have been extensively demonstrated to confer protective effects against cardiovascular diseases, cancer, and inflammation [15]. Prior research has indicated that compounds such as catechins and gallic acid have been discovered in tea and exhibit antioxidant characteristics [16, 17]. Amino acids and their derivatives play pivotal roles in maintaining redox balance and regulating antioxidant enzyme activities, thereby possessing antioxidant and anti-inflammatory functions. After evaluating the selection of 30 types of tea, it was found that one of the characteristics of phenolic compounds is that they play a key role in reducing and neutralizing free radicals. Phenolics are a major source of powerful antioxidant properties [18]. The types as well as the amounts of antioxidant metabolites in tea plant varieties are also influenced by a variety of factors, such as cultivation conditions [19], processing techniques [20], and storage methods [21]. Therefore, comprehensive research into the antioxidant metabolites of tea plant varieties necessitates the integrated use of modern analytical techniques and bioinformatics methods to holistically understand their composition, variations, and mechanisms of action.

This study aims to screen the metabolites in the tea plants of three species, *C. tachangensis*, *C. gymnogyna*, and *C. sinensis*, using the ultra performance liquid chromatography (UPLC)–MS/MS metabolomics approach [22]. The differences in antioxidant metabolites among different tea leaf samples will be compared, and the functions and pathways of these metabolites will be annotated and analyzed. In addition, the differences of the levels of various metabolites in three kinds of tea were also studied.

An understanding of the composition and functions of antioxidant metabolites in tea plant varieties will provide a scientific basis for tea plants' antioxidant properties and health-promoting effects. These insights will serve as an important theoretical and practical foundation for further exploration and development of natural products with high antioxidant capacity, thereby providing new avenues for protecting human health [23, 24].

2. Materials and Methods

2.1. Collection of Samples. Samples of three different tea plant varieties were obtained from the Tea Experimental Base of Guizhou University in Guiyang City, Guizhou Province. The selected tea plant varieties for this study were *C. tachangensis*, *C. gymnogyna*, and *C. sinensis*. These tea plants were cultivated under natural light and temperature conditions. Tender leaves of these three tea varieties were chosen for the experiment. A total of nine samples were obtained by collecting three biological replicate samples for each variety of tea plant. The biological samples were subjected to vacuum freeze-drying with a Scientz-100F freeze dryer (Scientz, Zhejiang, China) and subsequently pulverized into powder utilizing an MM 400 Retsch grinder (30 Hz, 1.5 min) (Germany).

2.2. Sample Extraction and Analysis. After vacuum freeze-drying, the biological samples were ground into powder (30 Hz, 1.5 min) using a grinder (MM 400 Retsch grinder, Germany.). Dissolving 100 mg of the powder in 1.2 mL of a 70% methanol extraction solution was carried out for further analysis. The solution was subsequently subjected to

a sequence of vortexing rounds, with the mixture being vortexed every 30 min for 30 s each time, amounting to a total of 6 vortexing cycles. After refrigerating the samples at 4°C overnight, the liquid portion was passed through a microfiltration membrane possessing a pore size of 0.22 μm and then preserved in sample containers after undergoing centrifugation.

2.3. UPLC–MS Analysis. This data acquisition instrument system includes UPLC (SHIMADZU Nexera X2 in Japan). It also includes Tandem mass spectrometry (MS/MS) (Applied Biosystems QTRAP in America).

Liquid phase conditions mainly include chromatographic column [25], Agilent SB-C18 (Agilent, USA) 1.8 μm, 2.1 $*$ 100 mm. The mobile phase is separated into two distinct phases, labeled Phase A and Phase B. In the method described, Phase A is composed of ultrapure water with the addition of 0.1% formic acid, while Phase B consists of acetonitrile with 0.1% formic acid included. The process of elution gradient is structured as follows: initially, at 0.00 min, the composition is 5% B, which then steadily increases to 95% over a period of 9.00 min and is maintained at this level for the subsequent minute. Subsequently, between 10.00 and 11.10 min, the proportion of B is reduced back to 5% and remains constant at this level until the 14-min mark. The parameters for this process include a flow rate of 0.35 mL/min, a column temperature of 40°C, and an injection volume of 4 μL.

The mass spectrum conditions are described by Wang et al. [25]. The LIT and triple quadrupole (QQQ) scans were conducted using a triple quadrupole linear ion trap mass spectrometer (Q TRAP), specifically the AB6500 Q TRAP UPLC/MS/MS system. This system was outfitted with an ESI Turbo ion spray interface. It operated in both positive and negative ion modes, controlled by Analyst 1.6.3 software developed by AB Sciex. The instrument was tuned and mass calibrated in QQQ mode using a 10 μmol/L polyethylene glycol solution and in the LIT mode using a 100 μmol/L polyethylene glycol solution. The device underwent calibration and tuning in the QQQ mode by utilizing a 10 μmol/L polyethylene glycol solution and in the LIT mode with a 100 μmol/L polyethylene glycol solution. The QQQ scan was conducted in multiple reaction monitoring (MRM) mode, with medium collision gas (nitrogen) setting. The DP and CE values for each MRM ion pair were fine-tuned. This was achieved through additional optimization of DP and CE parameters. In each time period, distinct MRM ion pairs were identified that matched the metabolites eluting during that specific timeframe. Substance identification was conducted by utilizing a self-built database and MS/MS spectral information.

In this study, the scientists utilized a cutting-edge method called triple quadrupole mass spectrometry–based MRM to accurately measure the levels of metabolites. In the MRM mode, the target species' precursor ions (parent ions) were initially isolated with a quadrupole, while ions associated with different molecular weight species were rejected, effectively removing potential interferences. Afterward, the precursor ions were subjected to collision-induced dissociation, resulting in the production of a multitude of fragment ions. Then, a three-quadrupole setup was used to filter out the characteristic fragment ion needed to exclude nontarget ion interferences, enhancing the precision and repeatability of quantification.

2.4. Metabolite Analysis. The analysis was conducted by Biomarker Corporation. Using mass spectrometry, the metabolites in the samples were subjected to qualitative analysis. The Biomarker's metabolite database was used as a reference for this analysis. Each unique set of ions for the compounds was identified using a triple quadrupole screening method, with the detector measuring the signal intensity of these ions in counts per second (CPS). The initial mass spectrometry data obtained from the samples underwent processing with the MultiaQuant software for peak analysis and calibration. The peak area of each chromatographic peak reflects the relative content of the corresponding substance.

2.5. Data Analysis. Total ion chromatogram (TIC) of quality control (QC) sample mass spectrometry detection analysis can be viewed in Supporting Figure 1. The unprocessed peak area data were normalized to the overall peak area. Further investigation and analysis followed. The reproducibility of group samples and QC samples was assessed using principal component analysis (PCA) and Spearman's correlation analysis. Compound classification and pathway information were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/). The identified compounds are then classified according to this information. Differential multiples were calculated based on group information, and significant p values were computed using tests for each compound. The ropls R package (R-3.1.1) was employed for orthogonal partial least squares discriminant analysis (OPLS-DA) modelling, and model reliability was validated through 200 permutation tests. Variables in projection (VIP) values were determined through a rigorous process of cross-validation, and a sophisticated algorithm incorporating differential multiples, p values, and VIP scores were employed to identify potential differential metabolites. The screening criteria included FC > 1, p value < 0.05, and VIP > 1. The significant enrichment of different metabolites in KEGG pathways is evaluated using the hypergeometric distribution method.

3. Results

3.1. PCA. In this investigation, PCA was employed to explore metabolic differences among tea plant samples and visualized in a PCA of samples (Figure 1(a)). From the PCA of samples, samples are distributed in three distinct regions. The proportion of variance explained by the first principal component (PC1) and the second principal component (PC2) is 51.57% and 16.90%, respectively. This indicates that these two principal components effectively capture the variation in metabolites across samples. Clustering of tea plant samples in the score plot suggests that different types of

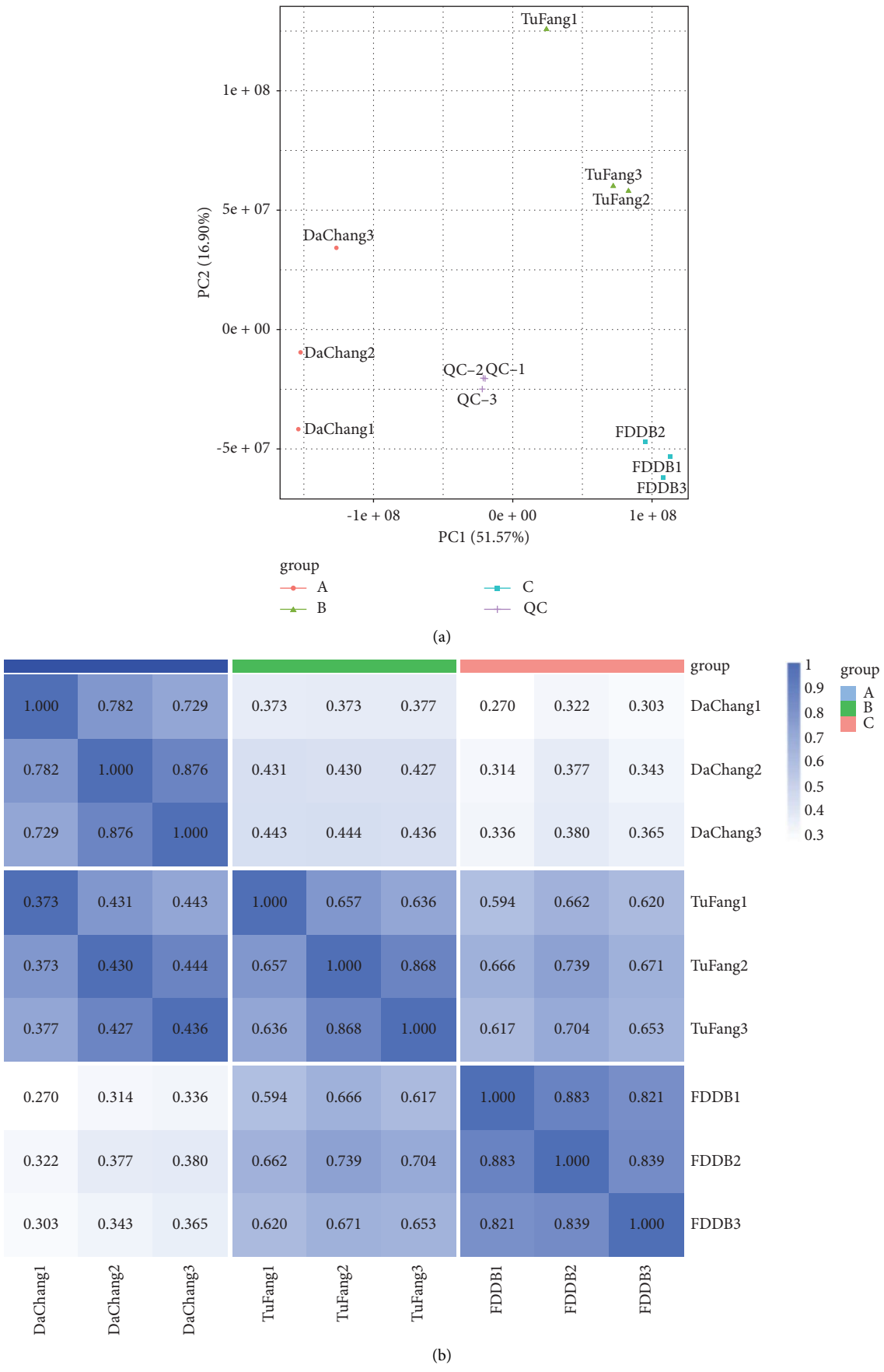


Figure 1: (a) PCA of samples: A represents *C. tachangensis*, B represents *C. gymnogyna*, and C represents *C. sinensis*. (b) Sample correlation plot. The horizontal and vertical axes represent sample names, with color intensity indicating the correlation coefficient (r^2), and groups are labeled.

tea plants exhibit significant metabolic differences at the metabolite level. Moreover, samples of the same tea plant type are relatively close to each other, indicating high internal similarity within the same type of tea plant samples.

Furthermore, the study assessed the correlation of replicate samples by calculating the square of the Spearman rank correlation coefficient (r). A higher r^2 value closer to one indicates a stronger correlation between replicate samples, demonstrating that the experiment exhibits good reproducibility and stability. The analysis of correlation coefficients among replicate samples revealed high similarity in metabolic composition (Figure 1(b)). Combining the PCA score plot and the correlation assessment of replicate samples, it is evident that different types of tea tree samples possess distinct metabolic characteristics, while samples of the same type exhibit relatively similar metabolite compositions. These findings provide crucial insights for further in-depth investigation into the metabolic processes and antioxidant-related pathways of tea plant.

3.2. OPLS-DA. In this research, this data from mass spectrometry was analyzed using OPLS-DA. The comparison of the outcomes generated by OPLS-DA revealed a clear division between the samples originating from the two groups of tea plants, as evidenced by the clear demarcation within the confidence interval. This observation suggests notable variations among the three sample groups, underscoring the presence of significant differences in their chemical profiles (the three figures above are labeled as Figures 2(a), 2(b), and 2(c)).

Models with higher r2y and q2y values are stable and reliable and capable of selecting differential metabolites. An effective model is typically indicated by a q2y value greater than 0.5, while a value exceeding 0.9 suggests an excellent model. Differential metabolites may be chosen according to the importance of VIP significance analysis. Since the original model's r2y and q2y values are relatively low and the permutation test results are unsatisfactory, VIP may not be a selection criterion for identifying discriminatory metabolites. To identify distinct metabolites among the three types of tea plants, OPLS-DA was utilized for comprehensive multivariate statistical analysis. Comparative analysis of OPLS-DA score plots indicated significant separation among the three types of tea group plants, with good predictive ability of the three models (Q2Y values of 0.938, 0.973, and 0.869, respectively). In order to verify the reliability of the OPLS-DA model, a permutation test was performed. The samples were randomly grouped (permutated) for OPLS-DA modelling, and R2Y and Q2Y were calculated based on the permutation groups. This process was repeated 200 times, and the results were plotted as a scatter plot. Through cross-validation, the model is deemed to possess a strong predictive capability (Figures 2(a), 2(b), and 2(c)).

3.3. Metabolite Identification. For this research, the UPLC–MS/MS technology was utilized to investigate the primary and secondary metabolites found within the three distinct cultivars of tea plants. Metabolomic analysis revealed the presence of 1056 metabolites, encompassing a diverse range of compounds such as flavonoids, phenolic acids, catechins, coumarins, tannins, alkaloids, terpenes, organic acids, and lipids.

3.4. Differential Metabolite Screening. The significance of VIP derived from multivariate analyses was used in conjunction with fold change and p values to screen for differential metabolites based on OPLS-DA results. Metabolites satisfying both criteria ($FC > 1$, $p < 0.05$, $VIP > 1$) were considered differential metabolites, thereby preliminary screening for metabolites that exhibit differences between different varieties or tissues. Differences greater than a fold change of 1 between control and experimental samples were considered significant. Differential metabolite screening and analysis were performed for the three types of tea plants, revealing significant differences in metabolites. Among the three categories, a total of 163, 255, and 119 metabolites were examined and analyzed (Figure 3(a)). The metabolites among the three types of tea plants were compared and noticeable intergroup differences were observed. The largest differences were observed between Groups A and B, as well as between Groups C and A. Comparatively, the differences between Groups C and B were smaller and more stable. Among these, in comparison to *C. tachangensis* (A) and *C. gymnogyna* (B), 123 metabolites showed upregulation and 41 metabolites that exhibited downregulation in A. On the other hand, when compared to *C. tachangensis* (A) and *C. sinensis* (C), there are 67 metabolites increased and 189 metabolites decreased in Group C. In addition, C showed 58 upregulated metabolites and 62 downregulated metabolites compared to B (Supporting Figure 2). In total, 29 different metabolites were found in the three groups.

The clustering analysis of the 29 differential metabolites common to the three tea plant samples at the metabolic level revealed distinct clustering patterns in their metabolic profiles (Figure 3(b)). These samples exhibited substantial differences in metabolite abundance, indicating significant variations in metabolic products (Supporting table 1). These disparities could stem from variances in metabolic pathways or differences in enzyme system activities. The metabolite clustering was primarily categorized into flavonoids, phenolic acids, lipids, and other metabolites. Among these differential metabolites, those possessing antioxidant properties (Table 1) included flavonoid metabolites, such as gallocatechin, epigallocatechin, apigenin-5-O-glucoside, apigenin-6,8-di-C-glucoside (vicenin-2), dihydrokaempferol-7-O-glucoside, kaempferol-3-O-(6″-galloyl) glucoside, kaempferol-3,7-O-dirhamnoside (kaempferitrin), and 5,3′, 4′-trihydroxy-7,8-dimethoxyflavone-6-C-[apiosyl-(1-2)]-glucoside. Phenolic acid metabolites included vanillic acid-4-O-glucuronide, 2-O-di-gallic acyl-glucoside-glucoside, 1,6-di-O-caffeoyl-β-D-glucose, (7S,8R)-dehydrodiconiferylalcohol-9′-O-β-D-glucopyranoside, mandelic acid, 1-O-caffeoyl-β-D-glucose, feruloyl syringic acid, and 3,6-di-O-caffeoyl glucose. Other metabolite categories included N-acetylputrescine, 4-hydroxyisoleucine, 1-methyladenine, 10-

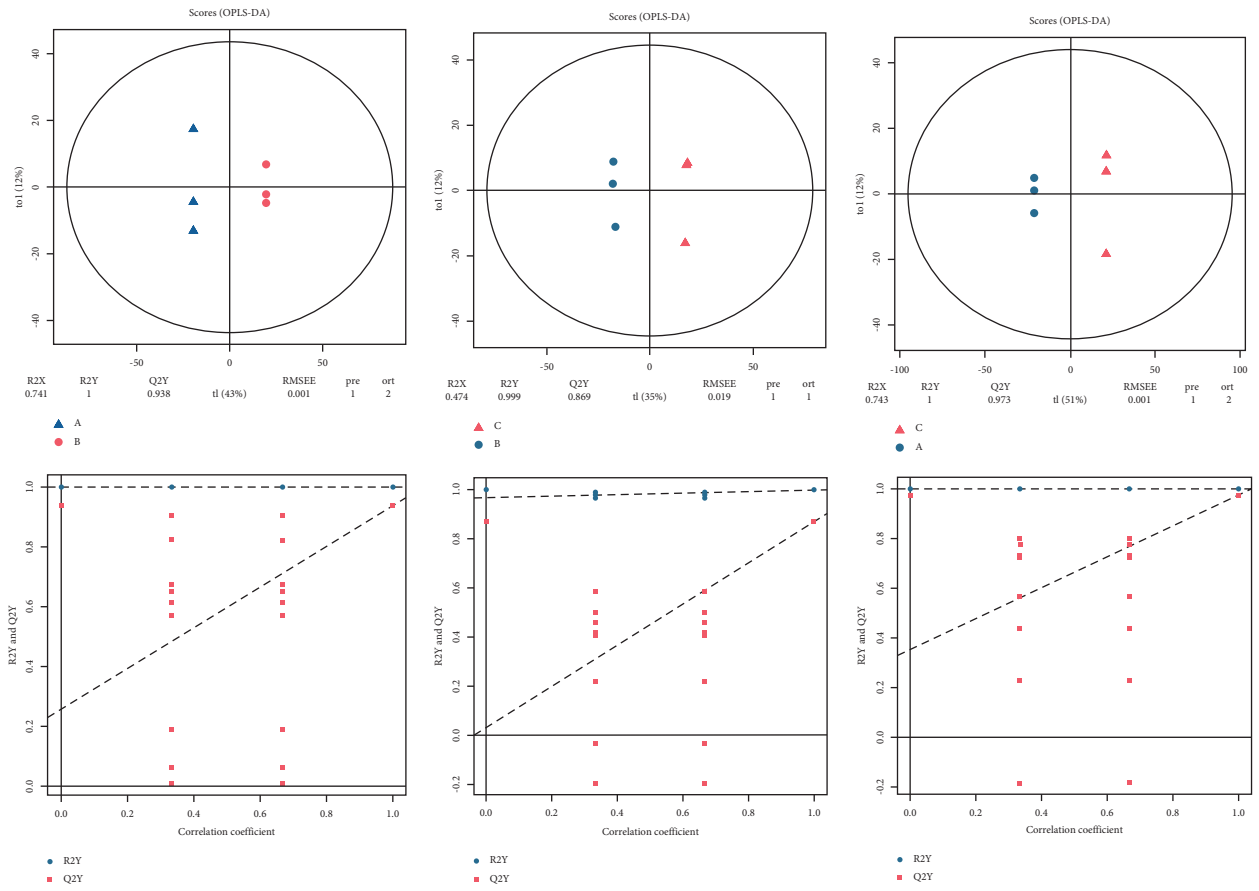


Figure 2: OPLS-DA score plot and OPLS-DA model permutation test plot. The diagram displays the permutation validation plot for the OPLS-DA model. The *x*-axis shows the correlation between the permutation group and the original model group, while the *y*-axis represents the values of R2Y or Q2Y. Blue dots indicate the R2Y values of the permutation models, while red dots represent the Q2Y values. The two dashed lines indicate the regression lines fitted to R2Y and Q2Y. (a) *C. tachangensis* was compared with *C. gymnogyna*. The blue triangle represents *C. tachangensis* and the red circle represents *C. gymnogyna*. (b) *C. sinensis* was compared with *C. gymnogyna*. The red triangle represents *C. sinensis* and the blue circle represents *C. gymnogyna*. (c) *C. sinensis* was compared with *C. tachangensis*. The red triangle represents *C. sinensis* and the blue circle represents *C. tachangensis*.

heptadecenoic acid, ricinoleic acid, LysoPC 17:1, LysoPC 18:4, and procyanidin B4. The antioxidant-related metabolites among these differential metabolites exhibited significant differences in the samples, indicating that the three tea tree varieties possess distinct antioxidant capabilities.

3.5. Functional Annotation and Pathway Analysis of Antioxidant Metabolites. This study analyzed pathway enrichment of different metabolites using the KEGG database. Within the context of the metabolomics analysis, several metabolites with antioxidant properties were identified and subsequently subjected to functional annotation and pathway analysis. The results revealed the presence of 20 pathways associated with the differential metabolites, among which 12 pathways exhibited significant enrichment in antioxidant metabolites (Figure 4). Among the enriched pathways, flavonoid biosynthesis and flavonoids are widely recognized for their antioxidant properties; these compounds potentially operate by scavenging free radicals and employing other antioxidant mechanisms. The metabolism of arginine and proline is also important, as these amino acids play roles in intracellular redox reactions, maintaining oxidation–reduction balance and contributing to antioxidant functions. In alanine, aspartate, and glutamate metabolism [26], amino acids such as aspartate, alanine, and glutamate provide substrates to produce antioxidants and help sustain cellular redox equilibrium. For glycine, serine, and threonine metabolism, the amino acids are engaged in various antioxidant pathways, including the synthesis of antioxidant enzymes and the regulation of intracellular redox balance. The pathway enrichment analysis underscores the potential significance of these pathways and metabolites in orchestrating antioxidant responses. This study provides insight into the molecular pathways involved in the antioxidant action of the studied tea. The pathway of amino acid synthesis is critical to the process of protein production as it provides the building blocks needed for cell

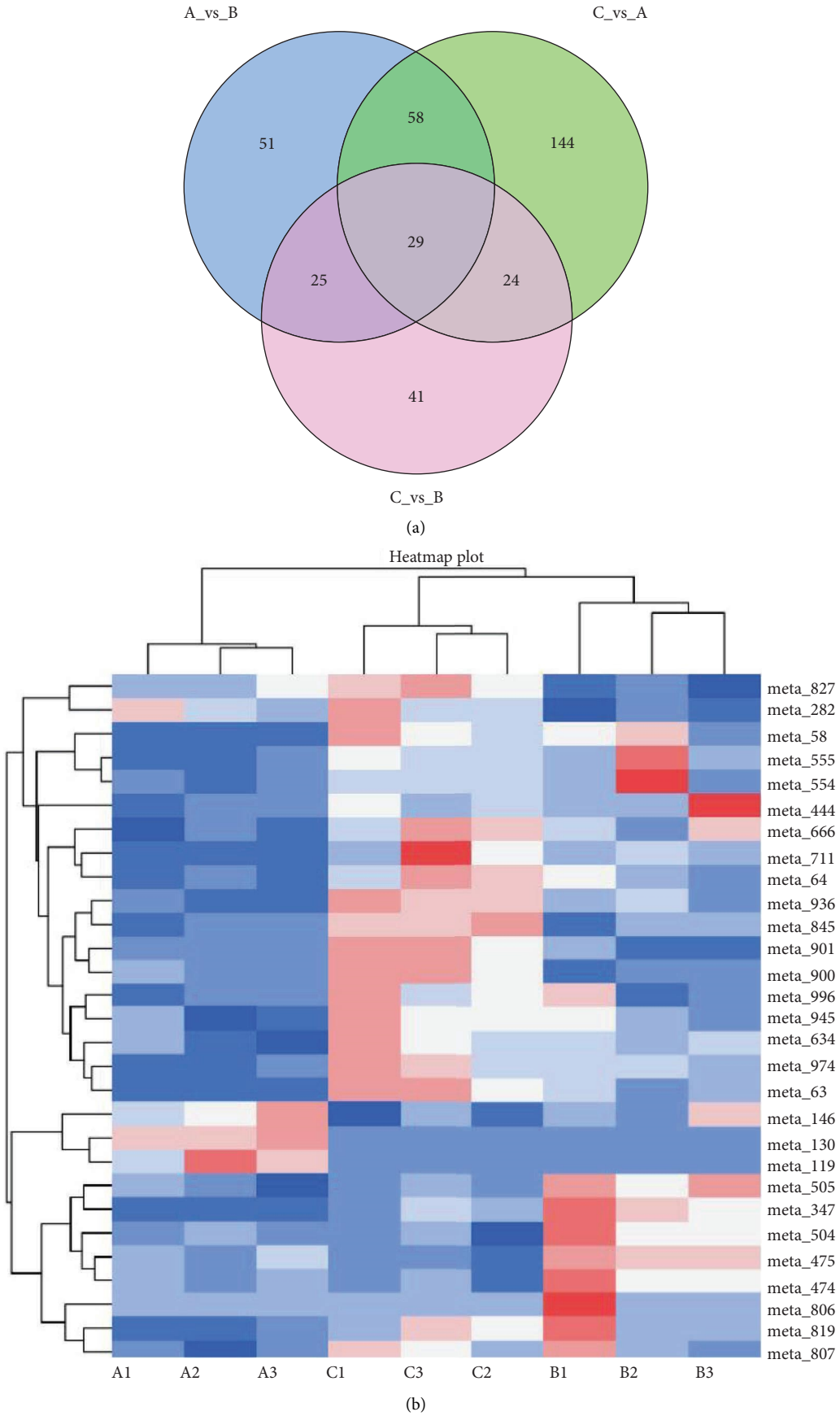


Figure 3: (a) Metabolites in Wayne diagram: A is *C. tachangensis*, B is *C. gymnogyna*, and C is *C. sinensis*. Each circle represents a contrast group, and the numbers within the overlapping circles representing the total differential metabolite data across the control subjects. The color bar above distinguishes the different groups. (b) Heatmap plot.

Table 1: Table of differential metabolite statistics.

ID	Rt (min)	Name	Formula	CAS	Class I	KEGG pathway
meta_58	0.72	N-Acetylputrescine	$C_6H_{14}N_2O$	—	Alkaloids	K00330
meta_63	1.47	3-Guanidinopropionic acid	$C_4H_9N_3O_2$	—	Organic acids	—
meta_119	0.83	4-Hydroxyisoleucine	$C_6H_{13}NO_3$	—	Amino acids and derivatives	—
meta_130	1.03	1-Methyladenine	$C_6H_7N_5$	5142-22-3	Nucleotides and derivatives	—
meta_146	2.91	Mandelic acid	$C_8H_8O_3$	90-64-2	Phenolic acids	—
meta_282	3.14	Caffeine	$C_8H_{10}N_4O_2$	58-08-2	Alkaloids	—
meta_375	11.47	10-Heptadecenoic acid	$C_{17}H_{32}O_2$	126761-43-1	Lipids	—
meta_347	0.82	1-beta-D-Arabinofuranosylurac	$C_9H_{12}N_2O_6$	—	Nucleotides and derivatives	—
meta_444	9.19	Ricinoleic acid	$C_{18}H_{34}O_3$	141-22-0	Lipids	—
meta_474	2.34	Gallocatechin	$C_{15}H_{14}O_7$	1617-55-6	Flavonoids	Ko0941
meta_505	2.32	5-(2-Hydroxyethyl)-2-O-glucosylphenol	$C_{14}H_{20}O_8$	—	Phenolic acids	—
meta_475	2.53	Epigallocatechin	$C_{15}H_{14}O_7$	141-22-0	Flavonoids	Ko0941
meta_554	2.45	Vanillic Acid-4-O-Glucuronide	$C_{15}H_{18}O_9$	18191-12-3	Phenolic acids	—
meta_504	2.30	Cimidahurinine	$C_{14}H_{20}O_8$	—	Phenolic acids	—
meta_711	4.36	Dihydrokaempferol-7-O-glucoside	$C_{21}H_{22}O_{11}$		Flavonoids	—
meta_555	2.45	1-O-Caffeoyl-β-D-glucose	$C_{15}H_{18}O_9$	—	Phenolic acids	—
meta_624	3.43	Feruloyl syringic acid	$C_{19}H_{18}O_8$	—	Phenolic acids	—
meta_666	3.75	Apigenin-5-O-glucoside	$C_{21}H_{20}O_{10}$	—	Flavonoids	—
meta_806	3.89	3,6-Di-O-caffeoyl glucose	$C_{24}H_{24}O_{12}$	—	Phenolic acids	—
meta_807	3.83	1,6-Di-O-caffeoyl-β-D-glucose	$C_{24}H_{24}O_{12}$	—	Phenolic acids	—
meta_819	8.50	LysoPC 17:1	$C_{25}H_{50}NO_7P$	—	Lipids	—
meta_827	7.40	LysoPC 18:4	$C_{26}H_{46}NO_7P$	—	Lipids	—
meta_845	4.79	(7S,8R)-Dehydrodiconiferylalcohol-9′-O-β-D-glucopyranoside	$C_{26}H_{32}O_{11}$	—	Phenolic acids	—
meta_900	3.78	Procyanidin B4	$C_{30}H_{26}O_{12}$	29106-51-2	Tannins	—
meta_901	3.77	Kaempferol-3,7-O-dirhamnoside (kaempferitrin)	$C_{27}H_{30}O_{14}$	—	Flavonoids	—
meta_936	3.18	Apigenin-6,8-di-C-glucoside (vicenin-2)	$C_{27}H_{30}O_{15}$	23666-13-9	Flavonoids	—
meta_945	4.23	Kaempferol-3-O-(6″-galloyl) glucoside	$C_{28}H_{24}O_{15}$	—	Flavonoids	—
meta_974	3.88	5,3′,4′-Trihydroxy-7,8-dimethoxyflavone-6-C-[apiosyl-(1-2)]-glucoside	$C_{28}H_{32}O_{16}$	—	Flavonoids	—
meta_996	2.56	2-O-Di-gallic acyl-glucoside-glucoside	$C_{26}H_{30}O_{19}$	—	Phenolic acids	—

Note: Rt (min) stands for retention time.

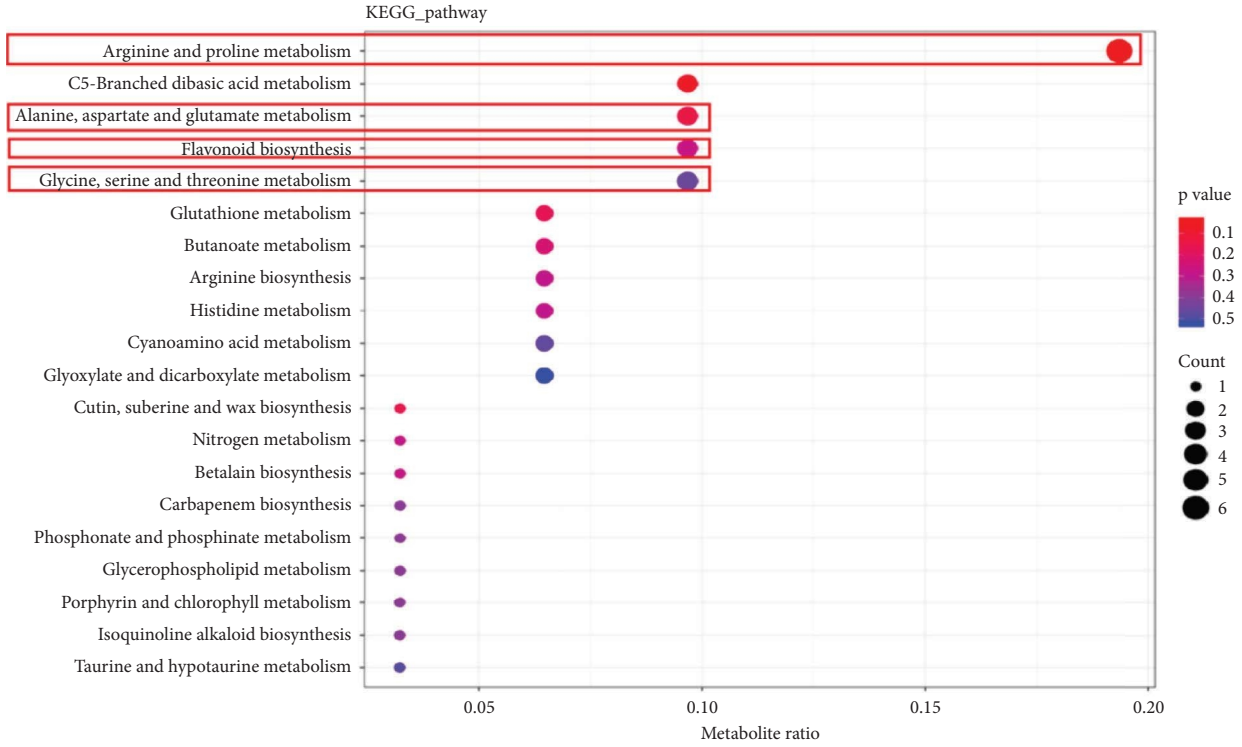


Figure 4: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of diverse metabolites. The *x*-axis indicates the ratio of the number of differentially expressed metabolites in each of the pathways to the total number of metabolites annotated in that pathway. The *y*-axis displays the names of the pathways. The dots' color depth is directly linked to the logarithm of the *p* value, where darker shades of red signify a higher level of enrichment significance. The dots' sizes are determined by the quantity of distinct metabolites that are enriched in each pathway.

growth and development. During the enrichment analysis of the substances mentioned above in KEGG pathways, it was found that 4 substances were enriched in the four annotated pathways. Among them, epigallocatechin and gallocatechin were found to be abundant in the flavonoid biosynthesis. N-Acetylputrescine was enriched in the Ko00330 arginine and proline metabolism pathway.

4. Discussion

For this research project, a metabolomics approach utilizing UPLC–MS/MS technology was utilized to examine variations in metabolites across three distinct varieties of tea plants to uncover their possible antioxidant properties. The primary and secondary metabolites of the three tea plant species were analyzed, revealing a grand total of 1056 metabolites that were successfully identified. Based on the screening criteria of FC > 1, $p < 0.05$, and VIP > 1, differential metabolites were carefully chosen for further analysis. A comparison of the differential metabolites among the three types of tea plants revealed 29 common differential metabolites (Figure 3(a)). The discussion mainly focuses on 24 common antioxidant metabolites. Procyanidin B4 had the highest content in *C. tachangensis*, which was also the highest among the three types of tea plants. Epigallocatechin had the highest content in *C. gymnogyna*, which was also the highest among the three types of tea plants. The most highly abundant antioxidant metabolite in Chinese acacia is a flavonoid called apigenin. N-Acetylputrescine is enriched in KEGG pathways and had the highest content in *C. sinensis*. Gallocatechin and epigallocatechin had the highest content in *C. gymnogyna* (Table 1). Metabolomics analysis revealed differential antioxidant metabolites among the three tea plant varieties. Analysis of antioxidant metabolites in tea plant samples identified a wide variety of substances, primarily involving flavonoids, phenolic acids, and other categories [27, 28]. These metabolites likely play a significant role in combating oxidative stress in tea plants, providing a robust scientific foundation for their health-promoting properties.

4.1. Antioxidant Properties of Flavonoid Metabolites. Within the flavonoid metabolites, several compounds that exhibit significant antioxidant potential were identified [29], including apigenin-5-O-glucoside, gallocatechin, epigall ocatechin, dihydrokaempferol-7-O-glucoside, , apigenin-6,8-di-C-glucoside (vicenin-2), kaempferol-3,7-O-dirham noside (kaempferitrin), kaempferol-3-O-(6″-galloyl)gluco-side, and 5,3′,4′-trihydroxy-7,8-dimethoxyflavone-6-C-[apiosyl-(1-2)]-glucoside. Extensive research [15, 30] confirms the exceptional antioxidant capabilities of these flavonoid compounds; as a result, these compounds are essential bioactive components in tea plants. Studies suggest [31] that flavonoids exert antioxidant effects through various mechanisms, including free radical scavenging, inhibition of

oxidative enzyme activities, and reduction of oxidative stress reactions. Apigenin, a type of flavonoid compound, has 84also been found to exist in tea [32]. Apigenin-6,8-di-C-glucoside (vicenin-2) and apigenin-5-O-glucoside are classified under the umbrella of apigenin compounds. Apigenin is most abundant in *C. sinensis*, followed by *C. gymnogyna*, and is least abundant in *C. tachangensis*. Apigenin exerts an antioxidant effect by scavenging free radicals [33], and the variation in the apigenin content might influence the antioxidant potential of tea. In three types of tea plants, studies have shown that in the young tea buds of *Camellia sinensis*, the most abundant metabolites of flavan-3-ol derivatives are catechins [17]. Kaempferol-3,7-O-dirhamnoside (kaempferitrin), dihydrokaempferol-7-O-glucoside, and kaempferol-3-O-(6″-galloyl) glucoside are all derivatives of kaempferol and are most abundant in *Camellia tachangensis*. Catechins are polyphenols derived from tea, including gallocatechin and epigallocatechin [34, 35]. These two substances are enriched in the flavonoid biosynthesis pathway. Gallocatechin and epigallocatechin have the highest content in *Camellia tachangensis*, followed by *C. sinensis*, and have been confirmed to exist in tea and enhance antioxidant capacity [18, 36]. Phenolic compounds such as gallocatechin and epigallocatechin may contribute to the observed antioxidant activity in tea samples [37]. One way in which catechins exhibit antioxidant properties is by neutralizing free radicals within cells, minimizing cellular harm caused by oxidative stress, as well as elucidating the antibacterial properties within cells and cell membranes [38]. The total content of flavonoid compounds is most abundant in *C. sinensis*, followed by *C. gymnogyna*, and least in *C. tachangensis*. Flavonoid metabolites such as apigenin, catechins, and kaempferol may be associated with the antioxidant activity of *C. sinensis*, *C. gymnogyna*, and *C. tachangensis*.

4.2. Antioxidant Properties of Phenolic Acid Metabolites. Phenolic acid metabolites exhibit substantial antioxidant properties in tea plant samples. They play a vital role in preserving redox equilibrium and shielding cells from oxidative harm in natural settings. These phenolic acid metabolites likely function through various pathways in the antioxidant mechanism, including redox state regulation and enhancement of antioxidant enzyme activities. The antioxidant properties of these phenolic acid metabolites have been substantiated in related research, and the current study further underscores their significance in tea plants. Xiao et al. have found that *C. sinensis* contains hydroxycaffeic acid and vanillic acid [39]. 1,6-Di-O-caffeoyl-*β*-D-glucose and 1-O-caffeoyl-*β*-D-glucose are compounds derived from the combination of caffeic acid through a process known as esterification. Caffeic acid derivatives have been identified in tea [40], and caffeic acid is a naturally occurring derivative of cinnamic acid [41]. Prior research has demonstrated that caffeic acid can enhance the function of specific soluble peroxidases and antioxidant enzymes in mung bean plants, showcasing its antioxidant properties [42]. Vanillic acid can scavenge free radicals, reduce lipid peroxidation levels, and enhance antioxidant status [43, 44]. In experiments, vanillic acid-4-O-glucuronide has been detected in urine after a single intake of red tea [45]. Feruloyl syringic acid has been found to exist in black tea and belongs to the fatty acid profile of this tea [46]. Past research findings regarding Cimidahurinine have unveiled its potent ability to suppress melanin synthesis and scavenge free radicals effectively [47]. 1,6-Di-O-caffeoyl-*β*-D-glucose has the highest concentration of phenolic acids among the three teas, with the highest levels found in *C. sinensis*, followed by Cimidahurinine which has the highest content in *C. gymnogyna*. *C. sinensis* has been found to contain the highest amount of phenolic metabolites, with *C. gymnogyna* following closely behind, and *C. tachangensis* exhibiting the lowest levels in comparison. *C. sinensis* may have higher phenolic synthase activity, resulting in higher levels of phenolic compounds such as catechins and theaflavins.

4.3. Antioxidant Properties of Other Metabolite Categories. Caffeine is also an important metabolite in tea and is a key characteristic metabolite for tea quality [48]. High levels of caffeine provide the foundation for tea flavor [48]. Among the trio of tea varieties, *C. tachangensis*, *C. gymnogyna*, and *C. sinensis*, the caffeine content is relatively high and ranks among the top in terms of shared differential metabolites. The highest caffeine content is found in *C. gymnogyna*, followed by *C. sinensis*, and finally, *C. tachangensis*. The amount of caffeine present could affect the taste and flavor of the tea.

Beyond flavonoids and phenolic acids, other metabolite classes in tea plants also demonstrate significant antioxidant properties. Procyanidin B4 is a pivotal antioxidant component in tea plants [49]. Multiple studies indicate that procyanidin B4 typically possesses potent antioxidant abilities, and its cell-protective effects have been verified. Procyanidin B4 has the highest content in *C. tachangensis*, which may influence the antioxidant activity of this tea species. Comparative studies [50] suggest that procyanidin B4 might mitigate oxidative stress-induced cellular damage by inhibiting inflammatory responses, protecting cells from inflammation-induced oxidative stress. The unique chemical structure of procyanidin B4 might be linked to its antioxidant properties, providing novel insights for further exploration of its antioxidative mechanisms. The antioxidant characteristics of the original anthocyanin B4 could enrich the categories and types of antioxidant metabolites in tea plants, potentially enhancing their health-promoting functions [48].

In addition to procyanidin B4, the metabolite N-acetylputrescine is an effective antioxidant, neutralizing free radicals and alleviating oxidative stress-induced cellular damage. By utilizing extracts from lemongrass, ginger, and moringa leaves, it was discovered that an instant tea contains ricinoleic acid. The analgesic and anti-inflammatory effects of ricinoleic acid have been demonstrated [51, 52]. 4-Hydroxyisoleucine has been discovered in fenugreek and displays the capacity to suppress the generation of reactive oxygen species [53]. The existence of 1-methyladenine in tea

has been confirmed and it is a specific methylation precursor [54].

4.4. Relationship Between Antioxidant Properties and Molecular Structures. Regarding the structure–activity relationship, the molecular structures of these metabolites may be correlated with their antioxidant properties. Compounds that contain phenolic or hydroxyl groups typically exhibit good antioxidative properties [55], as they can donate hydrogen atoms or electrons and neutralize free radicals. For instance, the phenolic and hydroquinone structures of phenol and catechol contribute to their antioxidant properties. In terms of physiological effects, the concentration and mode of action of these metabolites in physiological environments could influence their antioxidative properties [56]. Nicotinamide (a derivative of niacin) participates in various redox reactions within cells and functions as a coenzyme in energy metabolism, which may impact cellular antioxidative capability [57]. Moreover, these metabolites might interact or synergize with other antioxidants or intracellular antioxidant enzymes, enhancing their overall antioxidative effects [58]. Histamine and salicylic acid might interact with other antioxidants or intracellular antioxidant enzymes, strengthening their antioxidative capability [59].

In conclusion, the antioxidative properties of these metabolites could be influenced by various factors, including concentration [60], physiological environment, and target sites. Moreover, complex biochemical processes within cells and tissues might affect the antioxidative properties of metabolites [61]. Therefore, a deeper level of experimentation and research is needed to reveal the specific antioxidative mechanisms [62] and effects of these metabolites due to their antioxidative properties.

5. Conclusions

In summary, tea plant samples contain a diverse array of antioxidative metabolites encompassing flavonoids, phenolic acids, and other compound classes. These metabolites operate through distinct molecular mechanisms to exert antioxidative activity, shielding cells from oxidative harm. Through metabolomics analysis of tea plant samples, a range of compounds with significant biological functions and potential antioxidative activities have been identified. Among the antioxidative metabolites, the total number detected is 29. In *C. tachangensis*, the highest content is procyanidin B4, and the content of procyanidin B4 in *C.* tachangensis is the highest among these three types of tea. In *C. gymnogyna*, the highest content is epigallocatechin. Epigallocatechin also has the highest content in *C. gymnogyna* among these three types of tea. In *C. sinensis*, the highest content of antioxidative metabolites is celery flavonoids. *C. gymnogyna* and *C. sinensis* are rich in antioxidative metabolites, with the top 5 compounds being apigenin-5-O-glucoside, epigallocatechin, and apigenin-6,8-di-C-glucoside (vicenin-2). These metabolites, predominantly flavonoids, play a crucial role in combating oxidative stress and promoting overall health.

This research lays a crucial scientific groundwork for investigating the antioxidant properties of tea plants and creating innovative functional products based on this knowledge. Determining the antioxidative metabolites in tea, as a potentially health-promoting beverage [63], helps reveal their antioxidative mechanisms, paving the way for the development of applications to prevent diseases related to oxidative stress [64]. This study also provides new perspectives and directions for the utilization and development potential of tea plants [65].

Nevertheless, further experimental validation and in-depth research are needed to fully confirm the antioxidative properties of these metabolites. In addition, clarifying how the concentrations and modes of action of these metabolites in physiological contexts affect their antioxidative capacity is crucial for future research. Moreover, the interactions and synergistic effects among these metabolites may significantly influence their overall antioxidative effectiveness, warranting further exploration. Through more comprehensive research, a more profound comprehension of the mechanisms of the antioxidative metabolites in tea plants can be achieved, providing robust support for the development of tea plant products with excellent antioxidant properties [66] and opening new horizons for the application and development potential of tea plants.

Data Availability Statement

The authors have verified that the data underpinning the conclusions of this research are accessible in the article and accompanying supporting information.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: T.S.; methodology: Y.Y. and Y.L.; data analysis and supervision: L.L., X.W., and Q.L.; experimental work: X.W. and X.M.; data collection: H.D., and Q.Z.; article writing: L.L., X.W., and Q.L.; supervision: X.Z., Z.C., and Z.Z. All authors have read and agreed to the publishing version of the manuscript. L.L., X.W., and Q.L. contributed equally to this work.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Supporting Information includes two figures and a table. Supporting Informations are available in the Journal of Food Biochemistry online and consists of data provided by the author. Supporting Figure 1: Total ion chromatogram (TIC) of quality control (QC) sample mass spectrometry detection analysis. Supporting Figure 2: Volcano plot of differential metabolites. Supporting Table 1: Table of differential metabolite statistics.

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