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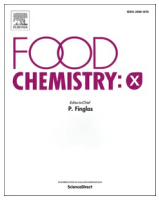

Correlation analysis of secondary metabolites and disease resistance activity of different varieties of Congou black tea based on LC-MS/MS and TCMSP

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ABSTRACT

To investigate the correlation between the difference of secondary metabolites and the disease-resistance activity of different varieties of Congou black tea. Among a total of 657 secondary metabolites identified, 183 metabolites had anti-disease activity, 113 were key active ingredients in traditional Chinese medicine (TCM), 73.22% had multiple anti-disease activities, and all were mainly flavonoids and phenolic acids. The main enriched metabolic pathways were phenylpropanoid biosynthesis, biosynthesis of secondary metabolites, flavonoid biosynthesis, and metabolic pathways. Flavonoid and phenolic acid secondary metabolites were more correlated with anti-disease activity and key active TCM ingredients. Conclusion: The types of JGY and Q601 Congou black tea of the relative contents show large differences in secondary metabolites. Flavonoid and phenolic acid secondary metabolites were identified as the primary factors contributing to the variation in secondary metabolites among different varieties of Congou black tea. These compounds also exhibited a stronger correlation with disease resistance activity.

1. Introduction

The secondary metabolites in tea are small molecule compounds formed from primary metabolites within the tea leaves and catalyzed by enzymes (Wan & Xia, 2015). Characteristic metabolites in tea such as catechins, caffeine, and flavonoids not only relate to the color, taste, and aroma of tea but also have anti-disease properties such as hypoglycaemic (Dogan, Pelvan, Aksu, & Akalin, 2019), antioxidant (Lim, Kim, & Sang, 2021; Xu et al., 2020), antimutagenic (Sun, Zhang, Zhang, Lai, & Sun, 2020), and gastrointestinal-motility activities (Bond & Derbyshire, 2020; Chen et al., 2022), which are closely related to human health.

The secondary metabolites of Congou black tea are formed by processes such as withering and fermentation and are important factors in the qualities of black tea. Among them, theaflavins are an important secondary metabolite of black tea, resulting from the oxidation and condensation of catechin-like substances, which account for 2%–6% of the dry weight of black tea (Bhagwat, Beecher, Haytowitz, Holden, & Balentine, 2015) and are considered characteristic components of black tea. Studies have shown that theaflavin, theaflavin-3′-O-gallate, theaflavin-3-O-gallate, and theaflavin-3,3′-O-digallate have antiviral (Ge, Yang, Hou, Gan, & Geng, 2021; Ohgitani, Shin-Ya, & Ichitani, 2020), anticancer (Banerjee, Kanwar, & Maiti, 2021; Itoh, Toda, & Wakita, 2021), and antitumor (Tsiani, 2021) activities and can protect against liver and kidney damage (El-Mekkawy, Al-Kahtani, Shati, et al., 2020; Zhan et al., 2021) and enhance immunity (Chowdhury, 2020). In addition, polyphenols in black tea have been reported to have antioxidant, anticancer, lipid homeostasis (Yan et al., 2020; Truong & Jeong, 2022), antidiabetic (Chen, Sun, et al., 2022), intestinal flora promotion, and obesity suppression (Liu, Chen, Zhang, & Ni, 2022) health effects. Gas chromatography-tandem mass spectrometry (GC–MS/MS) has been used for the separation and qualitative and quantitative analysis of tea's

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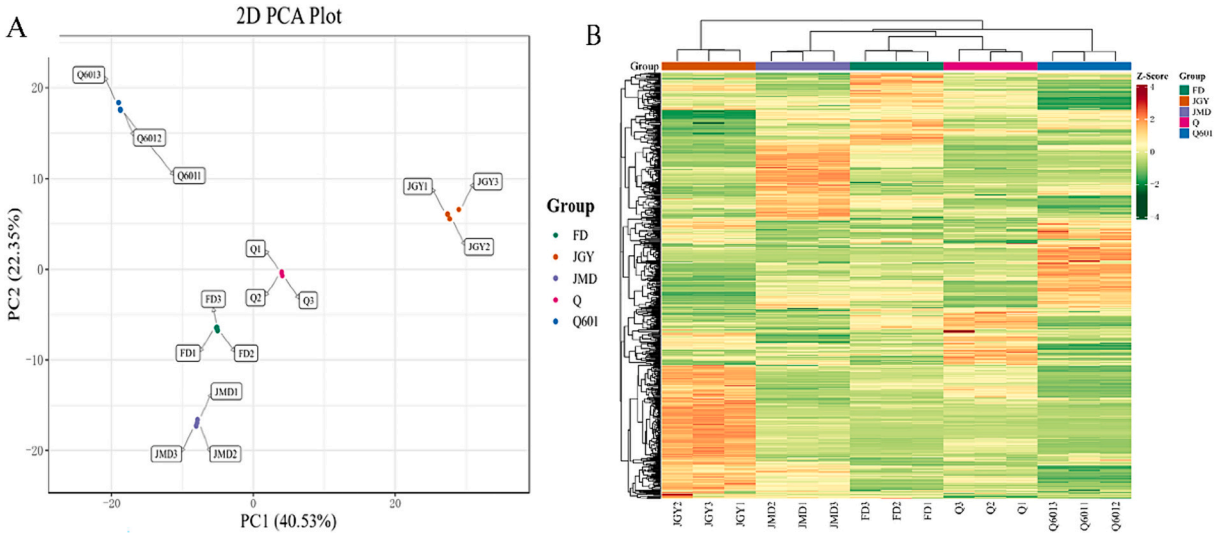

Fig. 1. Principal component analysis(A) and clustering heat map of secondary metabolites(B) of different varieties of Congou black-tea.

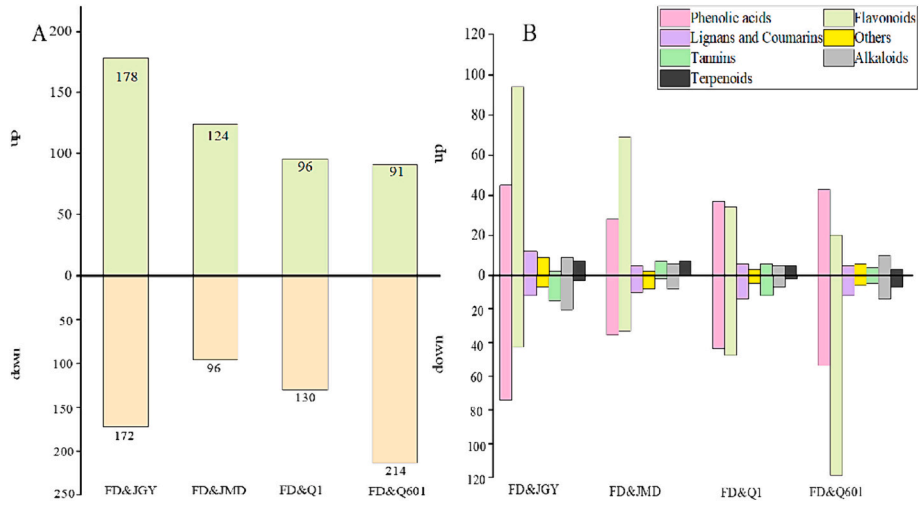

Fig. 2. Differential metabolite statistics of secondary metabolites of different varieties Congou black-tea.
Note: A is for different groups, B is for different types of substances.

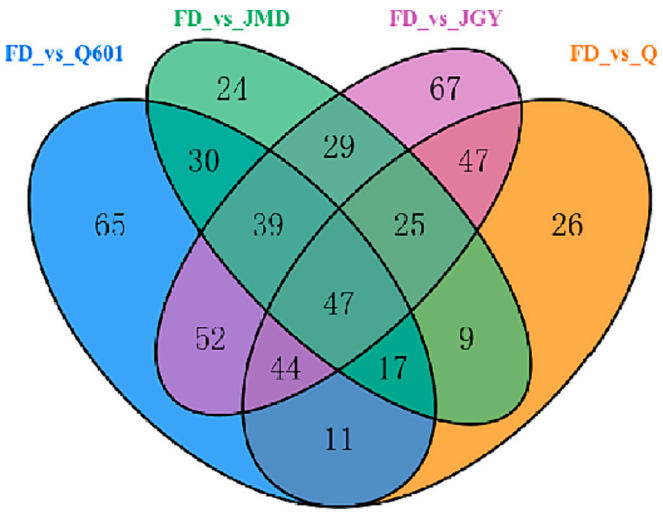

Fig. 3. Venn diagram of the differential metabolites of different varieties of Congou black-tea.

active ingredients owing to its excellent chromatographic separation, high sensitivity, and high resolution (Sun et al., 2024; Hua et al., 2022; Zeng et al., 2022). This has led to the discovery of secondary metabolites such as flavonoids, phenolic acids, and alkaloids in tea (Zhang et al., 2021; Wang, 2021; Liu et al., 2023). However, apart from theaflavins, which have been widely studied, there is still a lack of comprehensive comparisons of the secondary metabolites in different varieties of Congou black tea. Also, it is still largely unclear whether other substances contribute to the manifestation of black tea's anti-disease activity, and the secondary metabolites and their health effects have not been systematically analyzed.

The Traditional Chinese Herbal Medicine Systems Pharmacology Platform (TCMSP) database is a systematic pharmacology platform for herbal medicines that integrates information on drug chemical composition; absorption, distribution, metabolism, and excretion (ADME) properties, and drug similarities. It is used to study the network of disease–drug interactions, including active-ingredient identification, drug similarity (DL), oral bioavailability (OB), drug-target screening, and the visualization of compound-target-disease networks. It provides a new platform for the systematic study of the mechanism of action of herbal medicines (Liu, Tang, & Wang, 2020; Shi, Huang, & Cheng, 2022; Wang,

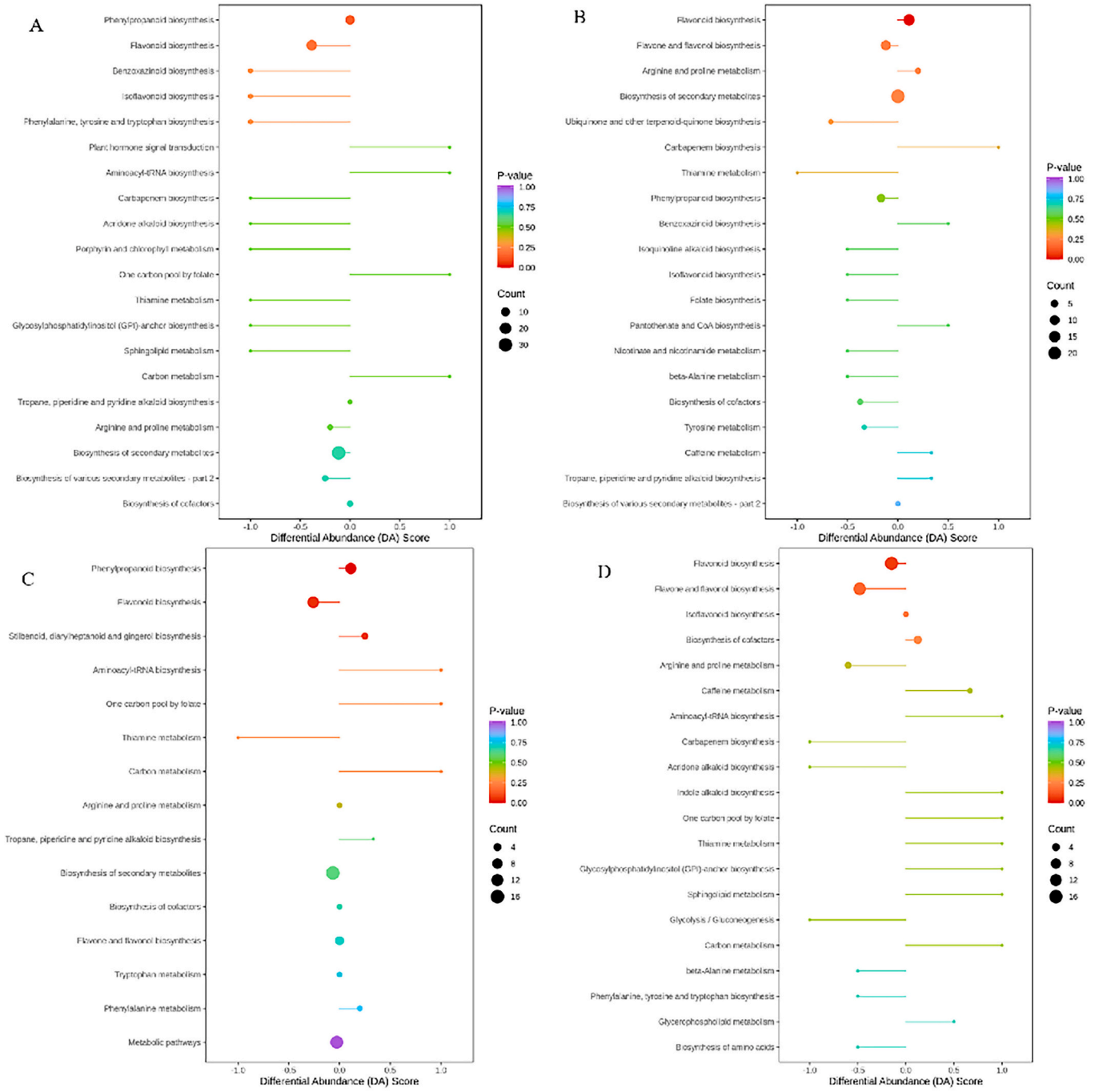

Fig. 4. Diagram of key metabolic pathways in different varieties of Congou black-tea.
Note: The horizontal coordinate is the Rich factor corresponding to the metabolic pathway, the vertical coordinate is the name of the metabolic pathway, the color of the dots is *P*-value(the more red indicates the more significant enrichment), and the size of the dots is the number of differentiated metabolites enriched (the larger indicates the more number). A: FD vs JGY; B: FD vs JMD; C: FD vs Q; D: FD vs Q601.

Cai, & You, 2022). The health benefits of black tea have attracted much attention, and evaluating the anti-disease activity of black tea's secondary metabolites using the TCMSP database can deepen our understanding of such effects.

Fuding Dabai (FD), Golden Guanyin (JGY), Golden Mudan (JMD), QianMei 601 (Q601), and Qiancha 1 (Q) are representative varieties of black tea, with large areas of cultivation in Guizhou, China (Pan et al., 2021). JGY and JMD are oolong tea varieties that have been widely introduced in Guizhou in recent years because of their outstanding floral aroma (Qiao, Guo, Yang, & Chen, 2019). Apart from floral characteristics, however, there is still a lack of data about their secondary metabolites. Q601 and Q are red and green varieties cultivated in Guizhou (Yang, Chen, Guo, Qiao, & Chen, 2019). FD, meanwhile, is the earliest variety introduced in Guizhou; it has the largest planting area, and it is used for both red and green tea production. Research on secondary metabolites in black teas from different tea plant species is still rare. Some studies have found that secondary metabolites vary greatly between different varieties of the same plant, depending on habitat, agronomic practices, and processing (Li et al., 2022). Therefore, examining differences in the secondary metabolites of different varieties Congou black-tea under consistent habitats and processing practices can help us grasp the characteristics of secondary metabolites in different

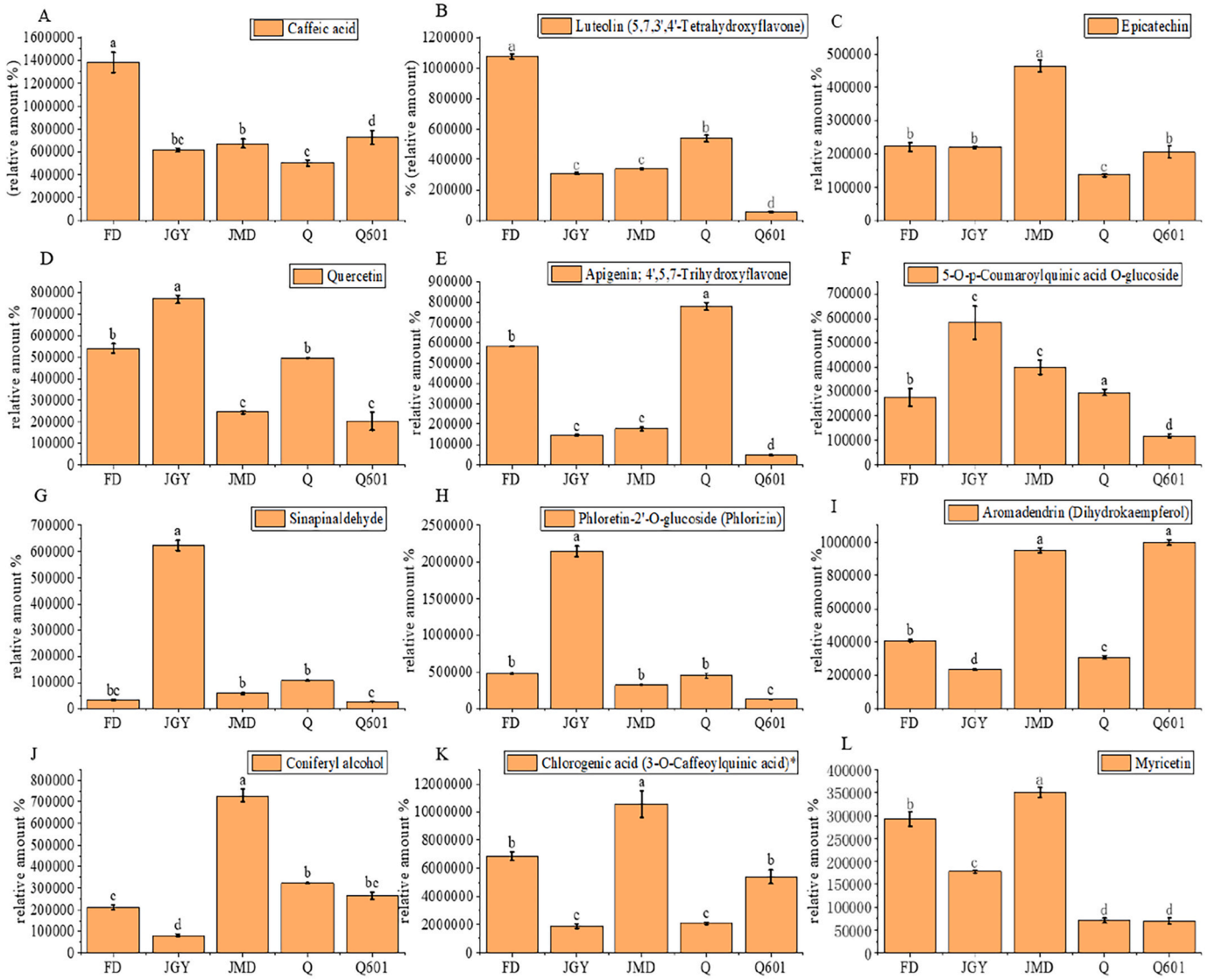

Fig. 5. Relative content of key metabolites in different varieties of Congou black-tea.
Note: A, B, C, D, E, F, G, H, I, J, K,and L, correspond, respectively, to caffeic acid, luteolin (5,7,3′,4′-tetrahydroxyflavone), epicatechin, quercetin, Apigenin: 4′,5,7-trihydroxyflavone, 5-O-P-coumaroylquinic acid, sinapinaldehyde, phloretin-2'-O-glucoside (phlorizin), aromadendrin (dihydrokaempferol), chlorogenic acid, chlorogenic acid (3-O-caffe quinic acid), and myricetin.

black teas and provide a theoretical basis for evaluating the quality of black tea products. Accordingly, this study used GC–MS/MS metabolomics and the TCMSP database to investigate the difference of secondary metabolites of different varieties of Congou black tea (FD, JGY, JMD, Q601, and Q) and the correlation of disease resistance activity. This can provide a reference for the secondary metabolites of Congou black-tea and their related health value.

2. Materials and methods

2.1. Experimental materials

This study focused on the FD, JGY, JMD, Q, and Q601 tea varieties, produced by the Guizhou Meitan Shengxing Tea Co. (Guizhou, China). One bud and one leaf of fresh leaves, free of diseased leaves, red leaves, and debris, were harvested on June 20–25, 2021, and made into Congou black tea through processes such as withering, kneading, fermentation, and after dehydration, take samples, put them in liquid nitrogen, and store them in the −80 °C refrigerator for later use. The tea samples were labeled as FD, JGY, JMD, Q, and Q601, and 500 g of each variety was taken and evenly divided into three portions for testing and analysis.

2.2. Experimental reagents

The following reagents were used: chromatographic purity acetonitrile (ACN) and methanol (MeOH) purchased from Merck (Darmstadt, Germany), and formic acid purchased from Sigma-Aldrich (Shanghai, China). All of the standards were purchased from MedChemExpress (MCE, China). The stock solutions of standards were prepared at a concentration of 2 mg/mL in MeOH. All stock solutions were stored at −20 °C.

2.3. Sample preparation and extraction

Biological samples were freeze-dried using a vacuum freeze dryer (Scientz-100F). Freeze-dried samples were crushed using a mixer mill (MM 400, Retsch) with a zirconia bead for 1.5 min at 30 Hz; then, 50 mg of lyophilized powder was dissolved with 1.2 mL 70% methanol solution. Thirty seconds of vortexing was performed every 30 min, six times in total. Following centrifugation at 12,000 rpm for 3 min, the extracts

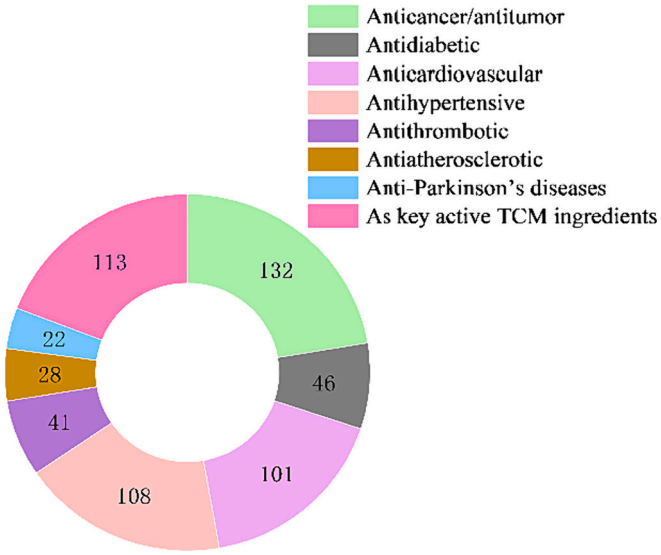

Fig. 6. Antidisease activity of secondary metabolites.

were filtrated (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China, http://www.anpel.com.cn) before UPLC-MS/MS analysis.

2.4. UPLC conditions and ESI-Q TRAP-MS/MS

Detection of secondary metabolites in the UPLC conditions and ESI-Q TRAP-MS/MS were referenced by Wu et al. (2022).

2.5. Evaluation of antidisease activity

The TCMSP database was used to query the anti-disease activity of the identified secondary metabolites of different black-tea varieties (Ru et al., 2014) and determine whether the secondary metabolites were key active traditional Chinese medicine (TCM) ingredients when OB $\geq$ 5% and DL $\geq$ 0.14.

2.6. Data processing

Based on a self-built database and a public database of metabolite information provided by Wuhan Meiwei Metabolic Biotechnology Co., LTD, the substances were characterized based on secondary spectrum information. The substances were scanned by multiple response monitoring mode(MRM) using five parameters of substance detection,

Table 1
Specific differential metabolites and their antidisease activity.

Group	Species	Unique differential metabolites	Up/down	Disease resistance activity	
FD Vs JGY	Flavonoids	Isorhamnetin	Up	anticancer、anticardiovascular、antidiabetic、antihypertensive	The key active TCM substances
		3′-Methoxy-3,4′,5,7-Tetrahydroxyflavone			
		Nepetin-7-O-glucoside(Nepitrin)			
		Rhamnetin-3-O-Glucoside			
		Diosmetin-7-O-Neohesperidoside (Neodiosmin)	Up	anticancer、antidiabetic、anticardiovascular、antihypertensive	
		Quercetin-3-O-(2″-*O*-acetyl)glucuronide			
		Quercetin-7-O-(6″-malonyl)glucoside	Up	Anticancer、	
		Apigenin-7-O-rutinoside (Isorhoifolin)	Down	anticancer、anticardiovascular、antihypertensive、anti-Parkinson's diseases	
	Phenolic acids	3,5-Digalloylshikimic acid	Down	anticancer、anticardiovascular	
		1-O-Galloyl-3-O-p-Coumaroyl-β-D-glucose			
		1,4-Di-O-Galloyl-D-glucose			
		1,6-Di-O-galloyl-β-D-glucose			
		Protocatechuic Acid Methyl Ester	Up	Anticancer、antihypertensive、antithrombotic	
		1-O-p-Hydroxycinnamoyl-3-O-caffeoylglycerol			
	Others	3,4'-Dihydroxy-5-methoxystilbene	Up	Anticancer、anticardiovascular、antihypertensive、anti-Parkinson's diseases	
		E-3,4,5′-Trihydroxy-3′-glucosylstilbene			
		6-Demethoxycapillarisin	Down	Anticancer、anticardiovascular、antihypertensive	The key active TCM substances
	Alkaloids	*N*-Benzylmethylene Isomethylamine	Down	Anticancer、anticardiovascular、antihypertensive、anti-Parkinson's diseases	
FD Vs Q601	Flavonoids	Epiafzelechin	Up	Anticancer	The key active TCM substances
		Kaempferol-3-O-sophoroside			
		Hesperetin	Down	Anticancer、anticardiovascular、antihypertensive	
	Phenolic acids	Dehydrodiconiferyl alcohol	Up	Anticancer、antidiabetic、antihypertensive、anticardiovascular、antithrombotic	The key active TCM substances
		p-Coumaric acid	Down	Anticancer、anticardiovascular、antihypertensive	
FD Vs JMD	Flavonoids	Kaempferol-3-O-rutinoside(Nicotiflorin)	Up	Anticancer	The key active TCM substances
		Kaempferol-3-O-rhamnoside (Afzelin) (Kaempferin)	Down	Anticancer、anticardiovascular、antihypertensive、antithrombotic	
	Phenolic acids	Ferulic acid methyl ester	Down	Anticancer、Antihypertensive、anticardiovascular	
		2,6-Dimethoxybenzoic acid			
	Others	Resveratrol	Up	Anticancer、antidiabetic、anticardiovascular、antihypertensive、antiatherosclerotic	
FD Vs Q	Flavonoids	Kaempferol-3-O-rutinoside(Nicotiflorin)	Up	Anticancer	The key active TCM substances
		Kaempferol-3-O-rhamnoside (Afzelin) (Kaempferin)	Down	Anticancer、anticardiovascular、antihypertensive、antithrombotic	The key active TCM substances
		Kaempferol-7-O-rhamnoside	Down	Anticancer、antihypertensive	The key active TCM substances

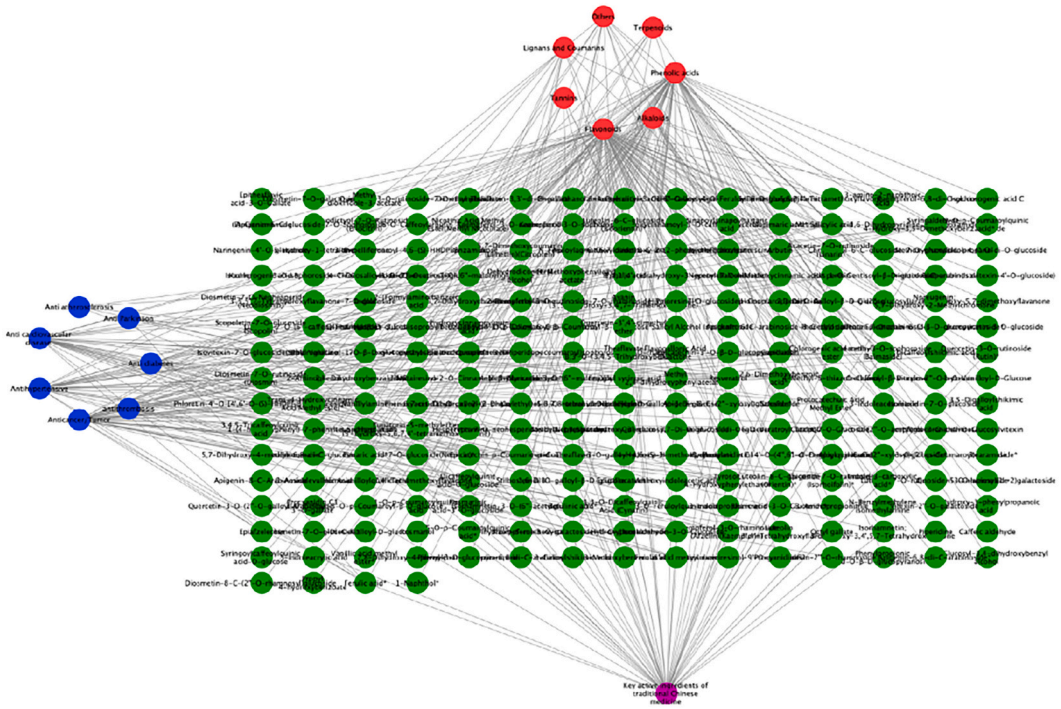

Fig. 7. Network diagram of correlations between differential metabolites and the disease-resistance activity of different varieties Congou black-tea (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).
Note: Green represents differential metabolites, red represents substance types, blue represents the seven disease-fighting activities, and purple represents key active herbal ingredients.

blanking voltage (DP)DP, impact energy (CE), retention time (RT), parent ion molecular weight (Q1), and characteristic fragment ion molecular weight (Q3)-to detect the relative content of substances in different samples and obtain the qualitative and quantitative data of the substances. The metabolite mass spectra of different samples were obtained, and the peak areas of the mass spectral peaks of all substances were integrated, in which the mass spectra of the same metabolites in different samples were corrected for peak integration. For FD, JGY, JMD, Q, and Q601, two comparisons were performed with FD as the control. These were recorded as FD vs JGY, FD vs JMD, FD vs Q, and FD vs Q601. Multivariate statistical analysis was used to perform principal component analysis (PCA) and orthogonal bias analysis (OBA) on the four groups of test samples. PCA, orthogonal partial least-squares discriminant analysis (OPLS-DA), and cluster analysis were used to initially investigate the metabolic characteristics of the secondary metabolites. Differential metabolites between groups were screened based on variable importance in the projection (VIP) values $\geq$1, fold change values (FC) ≥ 2 or ≤ 0.5, and $p < 0.05$ for *t*-tests obtained from the OPLS-DA. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used to analyze the metabolic pathways of the corresponding differential metabolites. A network diagram of the correlations between differential metabolites and disease-resistance activity was created using Cytoscape 3.7.0 (developed by the Open Source Initiative in the United States). Graphs were produced using Origin Pro 2021 (Origin Lab Inc., USA).

3. Results and analysis

3.1. Different varieties of Congou black tea secondary metabolite identification, PCA, and clustering

A total of 657 secondary metabolites were identified in seven categories in Congou black-tea identified by UPLC-MS/MS detection technology; including 223 phenolic acids, 252 flavonoids, 39 lignans and coumarins, 27 tannins, 63 alkaloids, 20 terpenoids, and 33 others.

To clarify the differences in secondary metabolites in Congou black tea (FD, JGY, JMD, Q, and Q601), a PCA model was constructed using the relative contents of the identified secondary metabolites as variables (Fig. 1-A). The contribution of PC1 was 40.53% and that of PC2 was 22.35%. The total contribution of the two principal components was 62.88%, which could basically reflect the main characteristic information of the tea samples. The blacktea samples were clustered together internally, and the separation trend between different varieties was obvious, with FD, Q, and JMD being closer together and having relatively small differences in their secondary metabolites. Meanwhile, JGY and Q601 were further apart, showing more obvious differences in their secondary metabolites. Clustering heat maps (HCA) can effectively reflect information about variation in secondary metabolites within and between different varieties of Congou black-tea groups. The HCA in Fig. 1-B shows that JGY was clustered into a single large group, indicating that the secondary metabolites in JGY varied the most compared with other varieties.

3.2. Different varieties of Congou black tea differential metabolite screening

These four groups were used for comparative analysis, based on a PLS-DA model with a VIP value >1, upregulated metabolite fold change $\geq$2, and downregulated metabolite fold change $\leq$0.5. The relative variable analysis results of *t*-tests were used to screen out significantly different metabolites. As shown in Fig. 2, a total of 350 differential metabolites were screened by FD vs JGY, among which 178 (50.85%) were upregulated (mainly flavonoids), and 172 were downregulated (mainly phenolic acids), followed by flavonoids and alkaloids. A total of 220 differential metabolites were screened by FD vs JMD, among which 124 (56.36%) were upregulated, all of which were dominated by phenolic acids and flavonoids; a higher number of flavonoids were upregulated, and 96 differential metabolites were downregulated. A

total of 226 differential metabolites were screened in FD vs Q, of which 96 (42.47%) were upregulated and 130 were downregulated, all dominated by differential changes in phenolic acids and flavonoids. A total of 305 differential metabolites were screened for FD vs Q601, of which 91 (29.83%) were upregulated. Phenolic acid and flavonoid differential changes dominated, and 214 (70.16%) differential metabolites were downregulated. This was the highest downregulation number compared with the other three groups. This was mainly attributed to the higher downregulation of flavonoids, indicating that the relative content of flavonoid metabolites is lower in Q601 than in other varieties. This is consistent with the results of Fang et al. (2024) And Su et al. (2024) indicating that flavonoids are important secondary metabolites in black tea.

As shown in Fig. 3, 182 specific differential metabolites had been screened, the FD vs JGY and FD vs Q601groups had significantly more than the FD vs JMD and FD vs Q groups, and the specific differential metabolites of each group were successively 67, 65, 24, 26. That is to say, JGY and Q601 show larger differences in secondary metabolites than others.

3.3. The key metabolic pathways of different varieties of Congou black tea

Based on the results for differential metabolites, the top 20 most-enriched metabolic pathways were annotated using the KEGG database (Sun, Wang, Xie, & Yue, 2016), as shown in Fig. 4. Based on the number of annotated differential metabolites and their ratio to the total number of annotated differential metabolites, the differential metabolite pathways in the FD vs JGY, FD vs JMD, FD vs Q, and FD vs Q601 groups were mainly phenylpropanoid biosynthesis, biosynthesis of secondary metabolites, flavonoid biosynthesis, and metabolic pathways, respectively. Among them, the flavonoid pathway is the main metabolic pathway of Congou black tea, which is consistent with the results of Yue, Wang, Peng, Li, and Yang (2023).

Phenylpropanoid biosynthesis and flavonoid biosynthesis in FD vs JGY tended to be downregulated, with a *p*-value close to 0 and a significant enrichment of differential metabolites. Annotated differential metabolites involved in more than three metabolic pathways were considered key differential metabolites. There were four key differential metabolites in FD vs JGY, namely, the flavonoids luteolin (5,7,3′,4′-tetrahydroxyflavone) and epicatechin, and the phenolic acids caffeic acid, and coniferyl alcohol. Among the flavonoids, luteolin (5,7,3′,4′-tetrahydroxyflavone), a common differential metabolite in all four groups, was downregulated and was present at higher levels in FD (Fig. 5-B). Caffeic acid, a common differential metabolite in all four groups, was downregulated in all four groups and was present at relatively high levels in FD (Fig. 5-A).

The *p*-value of the biosynthesis of secondary metabolites, flavone, and flavonol biosynthesis, and the flavonoid biosynthesis of FD vs JMD is smaller, with significant enrichment of differential metabolites. Flavone and flavonol biosynthesis tended to be downregulated, while flavonoid biosynthesis tended to be upregulated. There were four key differential metabolites in FD vs JMD, namely, the flavonoid luteolin (5,7,3′,4′-tetrahydroxyflavone), quercetin, 4′,5,7-trihydroxyflavone, and the phenolic acid caffeic acid. Among them, quercetin, a differential metabolite shared among all four groups, was upregulated and was present at relatively high levels in JGY. Meanwhile, 4′,5,7-trihydroxyflavone is a key active ingredient in TCM, and it was present at relatively high levels in Q and FD (Fig. 5-E).

FD vs Q had the lowest *p*-value for phenylpropanoid biosynthesis and flavonoid biosynthesis, with significant enrichment of differential metabolites. There were six key differential metabolites in FD vs Q, namely, the flavonoids luteolin (5,7,3′,4′-tetrahydroxyflavone) and myricetin; the phenolic acids caffeic acid, 5-O-p-coumaroylquinic acid, and sinapinaldehyde and chlorogenic acid (3-O-caffeoylquinic acid); and chlorogenic acid (3-O-caffeoylquinic acid). Among them, the phenolic acid 5-O-p-coumaroylquinic acid was a unique differential metabolite in the FD vs Q group, upregulated and present at relative highs in JGY (Fig. 5-F). Sinapinaldehyde had the highest relative content in JGY, slightly higher than FD in Q (Fig. 5-G).

Flavonoid biosynthesis and flavone and flavonol biosynthesis of FD vs Q601 tended to be downregulated, with the smallest *p*-value and significant enrichment of differential metabolites. There were five bonded differential metabolites, including the flavonoids luteolin (5,7,3′,4′-tetrahydroxyflavone), phloretin-2'-O-glucoside (phlorizin), aromadendrin (dihydrokaempferol), and quercetin. Among them, the flavonoid phloretin-2'-O-glucoside (phlorizin) was present at relatively high levels in JGY (Fig. 5-H). The flavonoid aromadendrin (dihydrokaempferol) had high relative content in Q601 and JMD (Fig. 5-I).

3.4. Different varieties of Congou black tea secondary metabolites antidisease activity

Cancer, diabetes, cardiovascular disease, hypertension, thrombosis, atherosclerosis, and Parkinson's disease are major diseases that threaten human health. Of the 657 metabolites identified by searching the TCMSP database, 183 metabolites had active ingredients in TCM, including 56 phenolic acids, eight alkaloids, 89 flavonoids, 16 lignans and coumarins, five tannins, and eight terpenoids. A total of 132, 46, 101, 108, 41, 28, and 22 metabolites corresponded to anticancer/antitumor, antidiabetic, anti-cardiovascular, antihypertensive, antithrombotic, anti-atherosclerotic, and anti-Parkinson's diseases, respectively (Fig. 6), among which 73.22% had multiple antidisease activities at the same time.

Based on the parameters OB $\geq$ 0.5% and DL $\geq$ 0.14 as the screening criteria (Medeiros & Simoneit, 2007; Zheng, Zhang, Quan, Zheng, & Xi, 2016), the key active TCM substances were further identified. A total of 113 were screened as key active TCM substances (Fig. 6).

3.5. The correlation between specific differential metabolites and their antidisease activity

On the basis of the TCMSP database, the specific differential metabolites of each group were mainly flavonoids and phenolic acids. These metabolites have been found to possess anti-cancer, anti-cardiovascular, and multiple anti-disease activities. Particularly. It is worth noting that most of the specific differential metabolites of flavonoids are considered the key active TCM substances (Table 1).

The correlation network diagram (Fig. 7) clearly shows that the flavonoids and phenolic acids of the differential metabolites correlated. These metabolites have been found to possess well anticancer, anti-cardiovascular, and other antidisease activities and were the key active TCM substances. Lignans and coumarins, alkaloids, and tannins also correlated with anticancer, antihypertensive, and anti-cardiovascular disease activities.

4. Discussion

Black tea is a class of fully fermented teas in which the fresh leaves undergo enzymatic changes to form a series of secondary metabolites, of which flavonoids are considered to be the most predominant feature, with a variety of biological activities (Li et al., 2022). Luo and Jiang (2021) discovered that the main anticancer molecular targets of flavonoids are cell-cycle regulatory proteins, apoptosis-related proteins, cell migration-related proteins, and growth transcription factors. The flavonoids quercetin-3-O-rutinoside, kaempferol 3-O-rutinoside, kaempferol 3-O-glucoside, and patulin O-glucoside of flavonoids are considered active ingredients in black tea that have anticancer and antihypertension properties (Chen, Sun, et al., 2022; Xue et al., 2018). And then the flavonoid kaempferol-3-O-rutinoside was the unique differential metabolite in this study. It was upregulated in the FD vs Q and FD vs Q601 groups, and it is a key active herbal ingredient. Quercetin is a prominent flavonol in tea (Shabana Jaysree & Rajendran, 2020), and

quercetin inhibits RANKL-mediated osteoclastogenesis, osteoblast apoptosis, oxidative stress, and inflammatory response (Shamsudin, Ahmed, & Mahmood, 2022). The shared differential metabolites of quercetin (a key active herbal ingredient) in the four groups in this study, which were all upregulated and annotated by multiple metabolic pathways as key differential metabolites, were present at relatively high levels in JGY.

Phenolic acid is also an important characteristic metabolite of black tea. Rena, Moemi, Amane, Yuri, and Hitoshi (2021) showned that phenolic acid can effectively cross the blood-brain barrier. In this study, the phenolic acid, Caffeic acid, 5-O-p-coumaroylquinic acid, sinapinaldehyde, and chlorogenic acid were also screened as specific differential metabolites and key metabolites with some antidisease activity. Caffeic acid and chlorogenic acid were screened as key active ingredients in TCM. In this paper, the correlation between the differences in secondary metabolites in black tea and the disease resistance activity was investigated by metabolome analysis technology. In the future, the accumulation mechanism of flavonoids and phenolic acids in black tea and the identification of key functional funds can be further explored by transcriptomics.

5. Conclusion

The types of JGY and Q601 Congou black tea of the relative contents show large differences in secondary metabolites. Flavonoid and phenolic acid secondary metabolites were identified as the primary factors contributing to the variation in secondary metabolites among different varieties of Congou black tea. These compounds also exhibited a stronger correlation with disease resistance activity.

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CRediT authorship contribution statement

Zhongying Liu: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Qiansong Ran:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Jinlong Luo:** Methodology, Investigation. **Qiang Shen:** Formal analysis. **Tuo Zhang:** Resources, Project administration. **Shimao Fang:** Software, Resources. **Ke Pan:** Funding acquisition. **Lin Long:** Funding acquisition.

Declaration of competing interest

There are no conflicts to declare.

Data availability

Data will be made available on request.

References

Banerjee, A., Kanwar, M., & Maiti, S. (2021). Theaflavin-3'-O-gallate a black-tea constituent blocked SARS CoV-2 RNA dependant RNA polymerase active-site with better docking results than Remdesivir. *Drug Research., 71*(08), 462–472. https://doi.org/10.1055/A-1467-5828

Bhagwat, S., Beecher, G. R., Haytowitz, D. B., Holden, J. M., & Balentine, D. A. (2015). Flavonoid composition of tea: Comparison of black and green teas. *Johanna T Dwyer., 4*(15), 1–4. DOI: https://doi.org/.

Bond, J. T., & Derbyshire, J. E. (2020). Black tea flavonoids: A focus on thearubigins and their potential roles in diet & health. *Nutr Food Technol Open Access., 6*(2), 1–8.

Chen, D., Sun, Z., Gao, J. J., Peng, J. K., Wang, Z., Zhao, Y. N., Lin, Z., & Dai, W. D. (2022). Etabolomics combined with proteomics provides a novel interpretation of the compound differences among Chinese tea cultivars (Camellia sinensis var. sinensis) with different manufacturing suitabilities. *Food Chemistry, 09*(12). https://doi.org/10.1016/j.foodchem.2021.131976, 131976–131976.

Chowdhury, P. (2020). Tea bioactive modulate innate immunity: In Perception to COVID-19 pandemic. *Frontiers in Immunology, 28*(11), 590716.

Dogan, K., Pelvan, E., Aksu, S., & Akalin, K. (2019). In vivo antidiabetic activities of green and black tea polysaccharides using streptozotocin- induced diabetic mice fed with a high-fat diet. *Journal of Food Bioactives, 8*, 74–83. https://doi.org/10.31665/JFB.2019.820

El-Mekkawy, H., Al-Kahtani, M., Shati, A., et al.Alshehri, M. A., Al-Doaiss, A. A., Elmansi, A. E., & Ahmed, A. E. (2020). Black tea and curcumin synergistically mitigate the hepatotoxicity and nephropathic changes induced by chronic exposure to aflatoxin-B1 in Sprague–Dawley rats. *Journal of Food Biochemistry, 44*(9). https://doi.org/10.1111/jfbc.13346

Fang, S., Huang, W., Yang, T., Pu, L., Ma, Y., Zhu, X., Pan, K., & Fang, W. (2024). Ancient tea plants black tea taste determinants and their changes over manufacturing processes. *LWT- Food Science and Technology, 193*, Article 115750. https://doi.org/10.1016/J.LWT.2024.115750

Ge, G., Yang, S., Hou, Z. Y., Gan, M. F., & Geng, D. C. (2021). Theaflavin-3,3′-Digallate promotes the formation of osteoblasts under inflammatory environment and increases the bone mass of Ovariectomized mice. *Frontiers in Pharmacology, 23*(3), 12–15. https://doi.org/10.3389/fphar.2021.648969

Itoh, N., Toda, H., & Wakita, T. (2021). Anticancer effect of Epitheaflagallin 3- O -Gallate, a very minor constituent of black tea extract. *Foods, 10*(23), 231–235.

Li, B., Mao, Q., Xiong, R., Zhou, D., Huang, S., Saimaiti, A., Shang, A., Luo, M., Li, H., Li, H., & Li, S. (2022). Preventive effects of different black and dark teas on obesity and non-alcoholic fatty liver disease and modulate gut microbiota in high-fat diet fed mice. *Foods, 11*(21), 3457.

Li, H. Y., Lv, Q. Y., Liu, A., Wang, J. R., Sun, X. Q., Deng, J., Chen, Q. F., & Wu, Q. (2022). Comparative metabolomics study of Tartary (Fagopyrum tataricum (L.) Gaertn) and common (Fagopyrum esculentum Moench) buckwheat seeds. *Food Chemistry, 371* (Mar.1). https://doi.org/10.1016/j.foodchem.2021.131125, 131125.1–131125.11.

Lim, H. Y., Kim, E., & Sang, H. P. (2021). Antimelanogenesis effects of theasinensin A. *International Journal of Molecular Sciences, 22*(14), 7453. https://doi.org/10.3390/IJMS22147453

Liu, Y. R., Tang, Z. S., & Wang, M. (2020). Virtual screening of novel coronavirus 3CL hydrolase inhibitors based on TCMSP database of traditional Chinese medicine components. *Chinese Herbal Medicines, 51*(07), 1694–1703. https://doi.org/10.7501/j.issn.0253-2670.2020.07.003

Liu, Z., Chen, Q., Zhang, C., & Ni, L. (2022). Comparative study of the anti-obesity and gut microbiota modulation effects of green tea phenolics and their oxidation products in high-fat-induced obese mice. *Food Chemistry, 367*(Jan.15). https://doi.org/10.1016/j.foodchem. 2021.130735, 130735.1-130735.10.

Liu, Z., Ran, Q., Yuan, C., Fang, S., Pan, K., et al. (2023). Differences in taste characteristics and antioxidant properties of four substitute teas based on a targeted metabolomics approach. *Beverage Plant Research., 3*, 33. https://doi.org/10.48130/BPR-2023-0033

Luo, T., & Jiang, J. (2021). Anticancer effects and molecular target of theaflavins from black tea fermentationin vitroandin vivo. *Journal of Agricultural and Food Chemistry, 69*(50), 15052–15065. https://doi.org/10.1021/acs.jafc.1c05313

Medeiros, P. M., & Simoneit, B. R. (2007). Analysis of sugars in environmental samples by gas chromatography–mass spectrometry. *Journal of Chromatography. A, 1141*(2), 271–278. https://doi.org/10.1016/j.chroma. 2006.12.017

Ohgitani, E., Shin-Ya, M., & Ichitani, M. (2020). Rapid inactivation in vitro of SARS-CoV-2 in saliva by black tea and green tea. *Cold Spring Harbor Laboratory., 6*, 102–105. https://doi.org/10.1101/2020. 12.28.424533

Pan, K., Li, Q., Fang, S. M., Dai, Y. Q., Ran, Q. S., Liu, Z. Y., & Yang, T. (2021). Analysis and evaluation of free amino acid dynamics during withering of different varieties of tea green in Guizhou. *Food and Fermentation Industry., 47*(08), 82–89. https://doi.org/10.13995/j.cnki.11-1802/ts.026073

Qiao, D. H., Guo, Y., Yang, C., & Chen, Z. W. (2019). Construction of fingerprint maps and genetic structure analysis of major cultivated tea tree varieties in Guizhou Province. *Journal of Plant Genetic Resources., 20*(02), 412–425. https://doi.org/10.13430/j.cnki.jpgr.20180801002

Rena, S., Moemi, A., Amane, M., Yuri, N., & Hitoshi, M. (2021). Evaluation of blood-brain barrier permeability of polyphenols, anthocyanins, and their metabolites. *Journal of Agricultural and Food Chemistry, 69*(39), 11676–11686. https://doi.org/10.1021/acs.jafc.1c02898

Ru, J., Li, P., Wang, J., Zhou, W., Li, B., & Chao, H. (2014). TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. *Journal of Cheminformatics, 6*(1), 13–18. https://doi.org/10.1186/1758-2946-6-13

Shabana Jaysree, R. C., & Rajendran, N. (2020). Review on the bioactivities of quercetin. *International Journal of ChemTech Research, 13*(3), 181–194. https://doi.org/10.20902/IJCTR.2019.130315

Shamsudin, N. F., Ahmed, Q. U., & Mahmood, S. (2022). Flavonoids as antidiabetic and anti-inflammatory agents: A review on structural activity relationship-based studies and Meta-analysis. *International Journal of Molecular Sciences, 23*(20). https://doi.org/10.3390/IJMS232012605, 12605–126.

Shi, H. L., Huang, Y., & Cheng, Y. (2022). Virtual screening of SARS-CoV-2 main protease inhibitors from the TCMSP database of herbal chemical components. *New Chinese Medicines and Clinical Pharmacology., 33*(02), 219–227.

Su, S., Long, P., Zhang, Q., Wen, M., Han, Z., Zhou, F., Ke, J., Ho, X., & Zhang, L. (2024). Chemical, sensory and biological variations of black tea under different drying

temperatures. *Food Chemistry, 446*, Article 138827. https://doi.org/10.1016/J.FOODCHEM.2024.138827

Sun, J., Cai, W., Feng, T., Chen, D., Lu, J., et al. (2024). Revealing the flavor profile of citrus Pu-erh tea through GC-MS-O and untargeted metabolomics. *Beverage Plant Research., 4*, Article e009. https://doi.org/10.48130/bpr-0024-0001

Sun, L., Zhang, Y., Zhang, W., Lai, X., & Sun, S. (2020). Green tea and black tea inhibit proliferation and migration of HepG2 cells via the PI3K/Akt and MMPs signalling pathway. *Biomedicine & Pharmacotherapy, 125*, Article 109893. https://doi.org/10.1016/j.biopha.2020.109893

Sun, S. H., Wang, H., Xie, J. P., & Yue, S. (2016). Simultaneous determination of rhamnose, xylitol, arabitol, fructose, glucose, inositol, sucrose, maltose in jujube (Zizyphus jujube Mill.) extract: Comparison of HPLC-ELSD, LC-ESI-MS/MS and GC-MS. *Chemistry Central Journal, 10*. https://doi.org/10.1186/s13065-016-0171-2, 25,1021–1026.

Truong, V. L., & Jeong, W. S. (2022). Antioxidant and anti-inflammatory roles of tea polyphenols in inflammatory bowel diseases. *Food Science and Human Health., 11*(3), 502–511. https://doi.org/10.1016/J.FSHW.2021.12.008

Tsiani, E. (2021). Anti-cancer properties of theaflavins. *Molecules, 26*(4), 987. https://doi.org/10.3390/molecules26040987

Wan, X. C., & Xia, T. (2015). *Secondary metabolism of tea plants* (pp. 1–3). Beijing, China: Science Publishers.

Wang, J. (2021). Study on the biological activity of Yunnan black tea extract in regulating lipid metabolism. *Hunan Agricultural University, Changsha China.*. https://doi.org/10.27136/d.cnki. ghunu. 2021.000073

Wang, X. H., Cai, M. B., & You, Q. H. (2022). Virtual screening of JAK3-specific inhibitors based on the TCMSP database for molecular docking. *Natural Product Research and Development, 34*(03), 484–490. https://doi.org/10.16333/j.1001-6880.2022.3.016

Wu, L. Y., Wang, Y. H., Liu, S. H., Sun, Y., Li, C. X., Lin, J. K., & Wei, S. (2022). The stress-induced metabolites changes in the flavor formation of oolong tea during enzymatic-catalyzed process: A case study of Zhangping Shuixian tea. *Food Chemistry, 391*, Article 133192. https://doi.org/10.1016/j.foodchem.2022.133192

Xu, L. L., Chen, Y., Chen, Z. Q., Xudong Gao, X. D., Wang, C. L., Panichayupakaranant, P., & Chen, H. X. (2020). Ultrafiltration isolation, physicochemical characterization, and antidiabetic activities analysis of polysaccharides from green tea, oolong tea, and black tea. *International Journal of Molecular Sciences, 85*(10/12), 4025–4032. https://doi.org/10.1111/1750-3841.15485

Xue, Z., Wang, J., Chen, Z., Ma, Q., Guo, Q., Gao, X., & Chen, H. (2018). Antioxidant, antihypertensive, and anticancer activities of the flavonoid fractions from green, oolong, and black tea infusion waste. *Journal of Food Biochemistry, 42*(6), 1153–1161. https://doi.org/10.1111/jfbc.12690

Yan, Z. M., Zhong, Y. Z., Duan, Y. H., Chen, Q. H., Li, F. N., Yan, Z. M., Zhong, Y. Z., & Duan, Y. H. (2020). Antioxidant mechanism of tea polyphenols and its impact on health benefits. *Animal Nutrition, 2*(6), 115–123. https://doi.org/10.1016/j.aninu.2020.01.001

Yang, C., Chen, J., Guo, Y., Qiao, D. H., & Chen, Z. W. (2019). New high-yielding and high-quality tea tree variety 'Qiancha No.1'. *Journal of Horticulture., 46*(S2), 2930–2931 (DOI: CNKI:SUN: YYXB.0.2019-S2-102).

Yue, C., Wang, Z., Peng, H., Li, W., & Yang, P. (2023). UPLC–QTOF/MS-based non-targeted metabolomics coupled with the quality component, QDA, to reveal the taste and metabolite characteristics of six types of congou black tea. *LWT- Food Science and Technology, 185*, Article 115197. https://doi.org/10.1016/J.LWT.2023.115197

Zeng, L., Jin, S., Fu, Y., Chen, L., Yin, J., & Xu, Y. (2022). A targeted and untargeted metabolomics analysis of 'Oriental Beauty' oolong tea during processing. *Beverage Plant Research., 2*, 20. https://doi.org/10.48130/BPR-2022-0020

Zhan, J., Cao, H., Hu, T., Shen, J., Wang, W., Wu, P., Yang, G., Ho, C., & Li, S. (2021). Efficient preparation of black tea extract (BTE) with the high content of theaflavin mono- and digallates and the protective effects of BTE on CCl4-induced rat liver and renal injury. *Journal of Agricultural and Food Chemistry, 69*(21), 5938–5947. https://doi.org/10.1021/acs.jafc.1c01851

Zhang, K., Ren, T., Liao, J., Wang, S., Zou, Z., et al. (2021). Targeted metabolomics reveals dynamic changes during the manufacturing process of Yuhua tea, a stir-fried green tea. *Beverage Plant Research., 1*, 6. https://doi.org/10.48130/BPR-2021-0006

Zheng, H., Zhang, Q., Quan, J., Zheng, Q., & Xi, W. (2016). Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps. *Food Chemistry, 205*, 112–121. https://doi.org/10.1016/j.foodchem.2016.03.007

Chen, C., Zhu, D., Zhang, S., & Zhang, W. (2022). Identification of circRNA/miRNA/mRNA regulatory net-work involving(+)-catechin ameliorates diabetic nephropathy mice. *Food Science and Human Health., 11*(3), 660–668. https://doi.org/10.1016/j.fshw.2021.12.023

Huajie, W., Wen, O., & Yaya, Y. (2022). Analysis of non-volatile and volatile metabolites reveals the influence of second-drying heat transfer methods on green tea quality. *Food Chemistry: X, 14*, 100354–100355. https://doi.org/10.1016/J.FOCHX.2022.100354

Van Dorsten, F. A., Daykin, C. A., & Mulder, T. (2006). Metabonomics approach to determine metabolic differences between green tea and black tea consumption. *Journal of Agricultural and Food Chemistry, 54*(18), 6929–6938. https://doi.org/10.1021/jf061016x