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Research Article

Metabolomics Reveals Distinct Metabolites between *Lonicera japonica* and *Lonicera macranthoides* Based on GC-MS

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Lonicera japonica Thunb. (LJ) and Lonicera macranthoides Hand. -Mazz. (LM) have been widely used in Chinese medicine for thousands of years. Although the morphological characteristics of LJ and LM are quite similar, there are significant distinctions of medicinal ingredients (mainly the secondary metabolites) and clinical indications between them. However, the in-depth differences of primary metabolites have not thoroughly been studied yet. Therefore, gas chromatography-mass spectrometry- (GC-MS-) based metabolomics method combined with chemometric methods were performed to analyze the distinction in this study. The results showed that LJ and LM were obviously classified into two groups. 10 metabolites were obtained as biomarkers on account of their p values, $|p(\mathrm{corr})|$ values, and differing variable importance in projection (VIP) values. Metabolic pathway analysis showed that the galactose metabolism and starch and sucrose metabolism gathered as potential pathways caused these extraordinary differences of primary metabolites between LJ and LM. Further, we found that the differences of main medicinal ingredients between LJ and LM could be interpreted from these metabolites according to the analysis of mainly related pathways. The metabolites involved in the starch and sucrose metabolism presented upregulated in LJ, while almost all metabolites in the galactose metabolism, the TCA cycle, and the phenolic acid part of phenylpropanoid metabolism were downregulated in LJ. Therefore, the energy stored in the starch and sucrose metabolism may be saved to produce flavonoid, which could be the reason that the level of flavonoid of phenylpropanoid metabolism is higher in LJ compared to LM. Consequently, this study presented an effective tool for quality evaluation of LJ and LM and laid a foundation for further studies of the metabolic mechanisms and high-quality manufacturing of them.

1. Introduction

Lonicera japonica Flos, the flower bud of *Lonicera japonica* Thunb. (LJ), has been widely used in Chinese medicine and food for thousands of years [1]. It is mainly cultivated in Shangdong and Henan provinces of China, and it is widely applied for the prevention and treatment of H1N1 influenza, severe acute respiratory syndromes, exopathogenic wind-heat, and hand-foot-mouth diseases [2, 3]. Therefore, LJ is a Chinese medicine with great demand. Moreover, it is frequently used as important raw material to produce Chinese patent drugs and functional food additives, such as *Yinqiao Jiedu/Shuanghuanglian* tablets, *Tanreqing/Reduning* injection, and *Heqizheng/Qingchunbao* herb teas [4, 5]. However, some flower buds of relevant species are confused or treated as LJ sometimes due to their similar appearance [6]. *Lonicera macranthoides* Hand. -Mazz. (LM), mostly cultivated in Hunan and Guangxi provinces of China, is one of them. Though the morphological characteristics of LJ and LM are pretty similar, there are significant differences of

medicinal ingredients (mainly the secondary metabolites) and clinical indications between them [7]. The main chemical constituents of LJ and LM include organic acids, flavonoids, triterpenoidal saponins, iridoids, volatile oils, and trace elements. However, the chemical composition and content are different between them. Chlorogenic acid is the main active organic acid shared by LJ and LM, but LM has a high level of chlorogenic acid, which is almost two times higher than that in LJ [7]. The flavonoids, including rutin, luteolin, and luteolin-7-O-glucoside, are almost undetectable in LM, but are abundant in LJ [4]. Triterpenoidal saponins are also the components contributed to the distinction between LJ and LM. LM contains more kinds of triterpenoidal saponins which proved to have strong hemolytic activities compared to LJ [8, 9]. Besides, both LJ and LM contain Fe, Mn, Zn, Cu, Ni, and other trace elements. LJ contains a wealth of Fe and Ni compared to LM, while LM has a higher content of Mn [7]. Therefore, there are significant differences of major effective compounds between LJ and LM. In accordance with the principle of "gradual classification of multisource varieties of Chinese medicinal materials with significant component differences," LM used to list in *Lonicerae Flos* was separated from *Lonicerae japonicae Flos* after the 2005 edition of the Chinese pharmacopoeia [10]. Hence, it is necessary to study the in-depth differences between them and the reason behind these remarkable distinctions, which will be helpful for discrimination, quality evaluation, and high-quality manufacturing.

Numerous experiments correlated with the distinctions of LJ and LM were established during the past two decades, which were mainly focused on the secondary metabolites [11–13]. However, metabolite profiles can be classified into two categories: primary and secondary metabolites [14]. Primary metabolites were directly involved in the growth, development, and reproduction of a plant, and it also played an important role in spanning the interface between primary and secondary metabolism [15, 16]. The differences in primary metabolites between LJ and LM may be the basis that caused the dissimilitude of medicine quality. Therefore, it is indispensable to analyze the distinction of primary metabolites, as well as the contact between primary and secondary metabolite profiles.

Metabolomics, an emerging tool used to analyze metabolite networks and regulatory mechanisms [17], has been proved to be an effective and meaningful method in medical analysis, quality evaluation of the medicine, and other analytical fields [18]. The main analytical platforms for plant metabolomics research are nuclear magnetic resonance (NMR) and methods based on mass spectrometry (MS), including GC-MS (gas chromatography-mass spectrometry), LC-MS (liquid chromatography-mass spectrometry), and CE-MS (capillary electrophoresis-mass spectrometry) [19–21]. GC-MS is one of the most suitable and versatile methods in metabolomics, and it is widely applied on account of its repeatability, wide measurement range, and direct peak identification [22].

Therefore, the GC-MS method combined with chemometric methods was performed to analyze metabolite profiles of LJ and LM in this study. Moreover, the relationship and mechanism between primary and secondary metabolic pathways were also explored according to the metabolic net analysis.

2. Materials and Methods

2.1. Plant Materials and Reagents. Fresh bud samples of LJ and LM were collected from the main habitats of the original plants in China (Shandong and Hunan Province, respectively). Two voucher specimens (X20180421 for LJ and X20180516 for LM) were deposited in the Key Laboratory for Quality Evaluation of Bulk Herbs of Hunan Province, China. After samples were identified by Prof. Li-min Gong at the Hunan University of Chinese Medicine, Hunan, China, they were immediately frozen in liquid nitrogen and stored at −80°C to inhibit the decline of enzyme activity before sample preparation. In brief, bud samples were divided into two groups (S_{LJ} and S_{LM}), each group consisted of thirteen biological replicates. The standard substances (ribitol), pyridine, methoxyamine hydrochloride, and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) were provided by Sigma-Aldrich Trading Co., Ltd. (St. Louis, Missouri, USA). Methanol and all the other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Sample Preparation. The freeze-dried samples were ground into uniform powder and stored at −80°C until the metabolic analysis [23]. A quality control (QC) sample was acquired by a representative average of samples pooled from different samples. The extraction procedure was in line with the previous research with a little modification [23]. 100 mg samples were transferred to a 10 mL centrifuge tube, and 1400 μL chilled methanol (precooled at −20°C) was added, reacting at 70°C for 30 min. After sufficient cooling, the mixture was centrifuged for 5 min (13000 rpm/min). 400 μL supernatant and 50 μL ribitol (1 mg/mL) as an interior label was placed into a 1.5 mL centrifuge tube and shaken for approximately 1 min with a vortex mixer (Wiggen Hauser, Malaysia). After adequate evaporation under N_2, the dry extracts were derivatized by 50 μL of methoxyamine pyridine solution (20 mg/mL) for 1 h at 30°C. Then, 100 μL BSTFA was added and the following reaction was carried out at a temperature of 45°C for 2 h. Finally, the solution was transferred into a vial to be analyzed. In addition, the QC samples were tested at the beginning, end, and randomly throughout the analytical run.

2.3. GC-MS Method. The extract (1 μL) was injected into GCMS-QP2010 (Shimadzu, Japan) equipped with HP-5 MS capillary column (5% phenyl methyl silox: 30 m × 250 μm, 0.25 μm; Agilent J&W Scientific) for primary metabolite profiling. Injection temperature was set at 280°C and the split ratio was 25 : 1. Helium was the carrier gas delivered at a constant flow rate of 1 mL/min. The temperature of the ion source and transmission line was 230°C and 280°C, respectively. The initial GC temperature was set at 70°C for 2 minutes, raised to 185°C at a rate of 10°C/min and maintained for 3 min. Then, the temperature was increased to

220°C by 3°C/min and held constant for 3 min. Finally, the temperature was elevated to 280°C with a ratio of 10°C/min and lasted for 10 min. The mass spectrometer was programmed under electron impact (EI) in a total ion chromatography mode (m/z 35–550) and electron ionization was applied at 70 eV.

2.4. Data Preprocessing. Peaks were picked up with the signal to noise ratio (S/N) >6. Raw data of GC-MS were transformed into Net CDF format by the Shimadzu postrun workstation and then processed by XCMS software to detect metabolic features and align all metabolite peaks. Metabolites with a similarity of more than 85% were identified and screened by the Automated Mass Spectral Deconvolution and Identification Software (AMDIS) combined with the National Institute of Standards and Technology (NIST) 14 database. Finally, all peak areas of identified metabolites were normalized to the internal standard.

2.5. Statistical Analysis. Heat-map analysis and clustering analysis were also performed to visualize the overall difference of metabolite profiles in LJ and LM. Then, multivariate statistical analysis including PCA and OPLS-DA were applied to detect the distinction of metabolite profiles between LJ and LM using SIMCA-P 15.0 (Umetrics AB, Umea, Sweden) and the MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/). Significant metabolites as biomarkers were evaluated based on their p value, $|p(\text{corr})|$ value, and differing variable importance in projection (VIP) values calculated with OPLS-DA. The related pathways of these biomarkers were interpreted and visualized according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/), a free and web-based tool. Consequently, a metabolite net was constructed to visualize the connections between the various pathways.

3. Results

3.1. Metabolite Profiles of LJ and LM Samples. Raw data with retention time, mass-to-charge ratio (m/z), and peak intensity were acquired by GC-MS. According to the NIST database, 63 metabolites were identified and matched with a similarity of more than 85% listed in Table 1. These metabolites could be classified into several categories, including sugar, polyol, fatty acid, organic acid, and amino acid, and others accounted for 26%, 19%, 5%, 25%, 11%, and 14% of all identified metabolites, respectively (supplementary materials Figure S1). The main components of the metabolites were sugar (26%), organic acid (25%), and polyol (19%). Sugar as a basic compound played a key role in plant growth, adaptation to the environment, and adjustment to nutrient availability [24]. Organic acid with various and powerful pharmacological activities was the major active component in LJ and LM [2]. Therefore, the high percentage of these two kinds of metabolites was understandable. The characteristic GC-MS total ion current chromatograms showed visually differences between LJ and LM encouraging further explorations (Figure 1(a)). In order to make the overall discrepancies in the metabolite profiles of LJ and LM more external, heat map analysis was performed. As shown in Figure 1(b), a clear segregation, once again observed through heat map analysis and cluster analysis, further illustrated the prominent distinctions between LJ and LM.

3.2. PCA Analysis and QC Evaluation. PCA is an unsupervised method compressing multidimensional data into several major components and displaying the internal structure of datasets in an unprejudiced manner [25]. PCA with unit variance (UV) scaling was performed to evaluate QC samples and generate an overview of the metabolic profiles. As shown in Figure 2(a), there were no outlier samples and the QC samples were clustered together near the center of the score plot, which illustrated the stability of the analytical apparatus and the reproducibility of pretreatment. The score plot showed a promising separation, suggesting the primary metabolites in LJ were different from that in LM.

3.3. OPLS-DA Analysis and Identification of Biomarkers. OPLS-DA is a supervised technique built from both predictive and orthogonal components to assess the maximum class separations [26]. In order to highlight the discrepancies between LJ and LM, OPLS-DA was consequently utilized to locate the radically different metabolites. As indicated in Figure 2(b), the metabolites of LJ and LM were distributed on two sides obviously. The variation in X (R^2Xcum)c was 0.706 and the variation in Y (R^2Ycum) was 0.943, predicting 90.4% of the variation in response to Y (Q^2cum = 0.904). The value of these parameters illustrated that the model had high explanatory and predictive abilities. The permutation test with a permutation number of 200 times was also performed to validate the model (Figure 2(c)). The result indicated that the intercepts of R^2 and Q^2 were 0.377 and −0.765, respectively. Furthermore, all R^2 and Q^2 values were lower than the original ones, suggesting a high degree of reliability.

Moreover, the S-plot was applied to identify the chief metabolites that contributed highly to the distinctions among samples. As detailed in Figure 2(d), the dots at the edges of the plots translated a bigger difference than those shown near the center. The VIP value ≥1.0, $|p(\text{corr})| > 0.3$, and $p < 0.05$ were regarded as the screening criteria. As a result, 10 spots were filtered as potential biomarkers listed in Table 2, including the p value, the VIP value, and the most related metabolic pathway of these biomarkers. Starch and sucrose metabolism, galactose metabolism, and fructose and mannose metabolism were the major pathways involved in these biomarkers. In view of the complexity and association of these metabolisms, the analysis still needs to go further.

3.4. Correlation Network Analysis of Potential Biomarkers and Biosynthesis of Main Active Components in LM and LJ. The 10 compounds screened by the previous OPLS-DA analysis were further confirmed and analyzed through the KEGG metabolic database and MetaboAnalyst. As shown in Figure 3, the y-axis represented the p value, and the x-axis

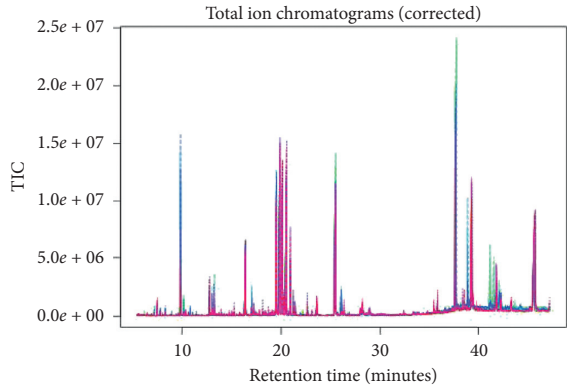
Table 1: Metabolites data based on gas chromatography-mass spectrometry (GC-MS).

No.	RT	HMDB	KEGG	Name	Type	Similarity (%)
1	15.30	HMDB0000283	C00121	D-(-)-Ribose	Sugar	92
2	17.04	N/A	C06474	3,6-Anhydro-D-galactose	Sugar	86
3	18.94	HMDB0003418	C00795	D-(-)-Tagatose	Sugar	90
4	19.89	HMDB0000660	C00095	D-Fructose	Sugar	95
5	20.15	N/A	C06468	Psicose	Sugar	85
6	20.36	HMDB0000122	C00031	D-(+)-Glucose	Sugar	86
7	20.54	HMDB0000143	C00124	D-(+)-Galactose	Sugar	89
8	22.72	N/A	N/A	1,2,3,4,6-O-Pentatrimethylsilylglucopyranose	Sugar	89
9	26.12	HMDB0000975	C01083	D-(+)-Trehalose	Sugar	91
10	26.91	HMDB0001151	C01487	D-Allose	Sugar	90
11	33.36	HMDB0033750	N/A	D-Glycero-D-gulo-heptose	Sugar	85
12	35.96	HMDB0000163	C00208	Maltose	Sugar	86
13	36.45	HMDB0000055	C00185	D-(+)-Cellobiose	Sugar	86
14	37.33	HMDB0029933	C01728	2α-Mannobiose	Sugar	85
15	37.64	HMDB0000258	C00089	Sucrose	Sugar	96
16	39.22	HMDB0000169	C00159	Mannose	Sugar	88
17	5.69	HMDB0031645	N/A	Acetamide	Organic acid	95
18	6.56	HMDB0000237	C00163	Propanoic acid	Organic acid	93
19	6.81	HMDB0000115	C00160	Glycolic acid	Organic acid	89
20	10.40	HMDB0000254	C00042	Succinic acid	Organic acid	93
21	10.70	HMDB0000139	C00258	Glyceric acid	Organic acid	93
22	12.81	HMDB0000156	C00149	L-(-)-Malic acid	Organic acid	97
23	13.76	HMDB0000943	C01620	Threonic acid	Organic acid	93
24	17.31	HMDB0000094	C00158	Citric acid	Organic acid	85
25	17.49	HMDB0003290	C00800	Gulonic acid	Organic acid	91
26	18.18	HMDB0003070	C00493	(-)-Shikimic acid	Organic acid	87
27	18.51	N/A	C00311	DL-Isocitric acid	Organic acid	85
28	19.57	HMDB0003072	C00296	Quinic acid	Organic acid	89
29	23.17	HMDB0000625	C00257	Gluconic acid	Organic acid	90
30	26.41	HMDB0001964	C01197	Caffeic acid	Organic acid	97
31	37.05	HMDB0000944	C08281	Behenic acid	Organic acid	85
32	45.69	HMDB0003164	C00852	Chlorogenic acid	Organic acid	88
33	6.26	HMDB0003156	C20657	2,3-Butanediol	Polyol	96
34	9.75	HMDB0000149	C00189	2-Aminoethanol	Polyol	88
35	9.88	HMDB0000131	C00116	Glycerol	Polyol	98
36	13.08	HMDB0004136	C16884	Threitol	Polyol	95
37	15.88	N/A	N/A	pentitol	Polyol	85
38	16.10	HMDB0002917	C00379	Xylitol	Polyol	87
39	16.34	HMDB0001851	C00532	Arabitol	Polyol	95
40	17.72	N/A	C01507	L-Iditol	Polyol	90
41	21.24	HMDB0000765	C00392	Mannitol	Polyol	93
42	21.46	HMDB0000247	C00794	D-Glucitol	Polyol	96
43	21.60	HMDB0000107	C01697	Galactitol	Polyol	87
44	25.52	HMDB0000211	C00137	Myo-inositol	Polyol	93
45	7.24	HMDB0000161	C00041	L-Alanine	Amino acid	98
46	9.01	HMDB0000883	C00183	L-Valine	Amino acid	96
47	10.16	HMDB0000172	C16434	Isoleucine	Amino acid	87
48	10.21	HMDB0000162	C00148	L-(-)-Proline	Amino acid	95
49	13.22	HMDB0000191	C00049	Aspartic acid	Amino acid	89
50	13.28	HMDB0000267	C01879	L-5-Oxoproline	Amino acid	95
51	13.32	HMDB0000112	C00334	4-Aminobutyric acid	Amino acid	95
52	23.66	HMDB0000220	C00249	Palmitic acid	Fatty acid	88

Table 1: Continued.

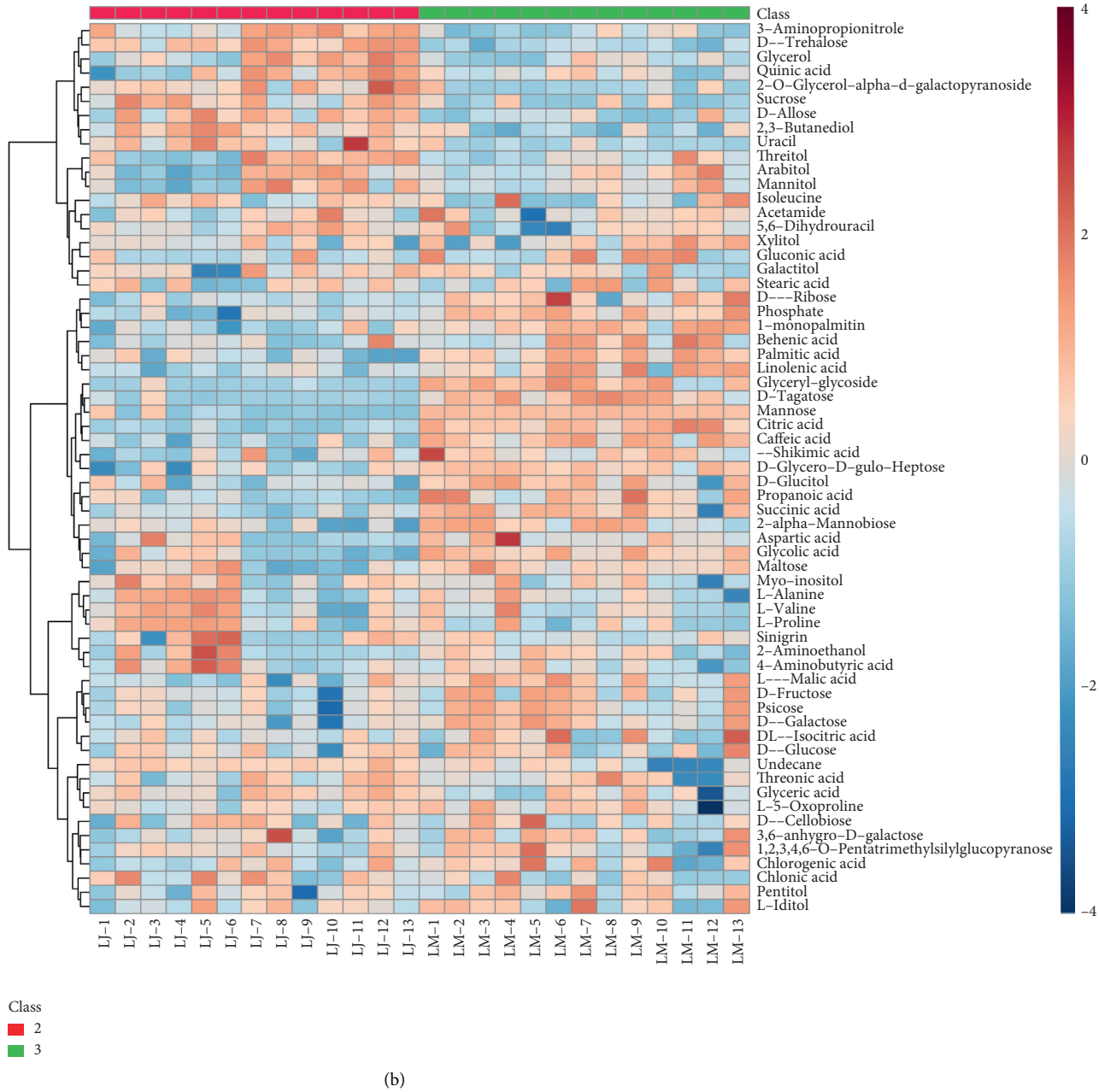
No.	RT	HMDB	KEGG	Name	Type	Similarity (%)
53	28.28	HMDB0001388	C06426	Linolenic acid	Fatty acid	85
54	28.98	HMDB0000827	C01530	Stearic acid	Fatty acid	91
55	6.47	HMDB0031445	N/A	Undecane	Others	92
56	7.86	HMDB0000076	C00429	5,6-Dihydrouracil	Others	86
57	9.92	HMDB0001429	N/A	Phosphate	Others	93
58	10.81	HMDB0000300	C00106	Uracil	Others	91
59	10.87	HMDB0004101	C05670	3-Aminopropionitrile	Others	95
60	32.43	N/A	N/A	Glyceryl-glycoside	Others	88
61	33.51	N/A	N/A	2-O-Glycerol-α-d-galactopyranoside	Others	89
62	36.58	N/A	N/A	1-Monopalmitin	Others	94
63	41.87	HMDB0034070	C08427	Sinigrin	Others	88

N/A: not available.



(a)

Figure 1: Continued.



(b)

Figure 1: (a) GC-MS TIC chromatograms. (b) Heatmap obtained after hierarchical cluster analysis of the metabolite profiles of LJ and LM. Columns and rows represent different samples and individual metabolites, respectively.

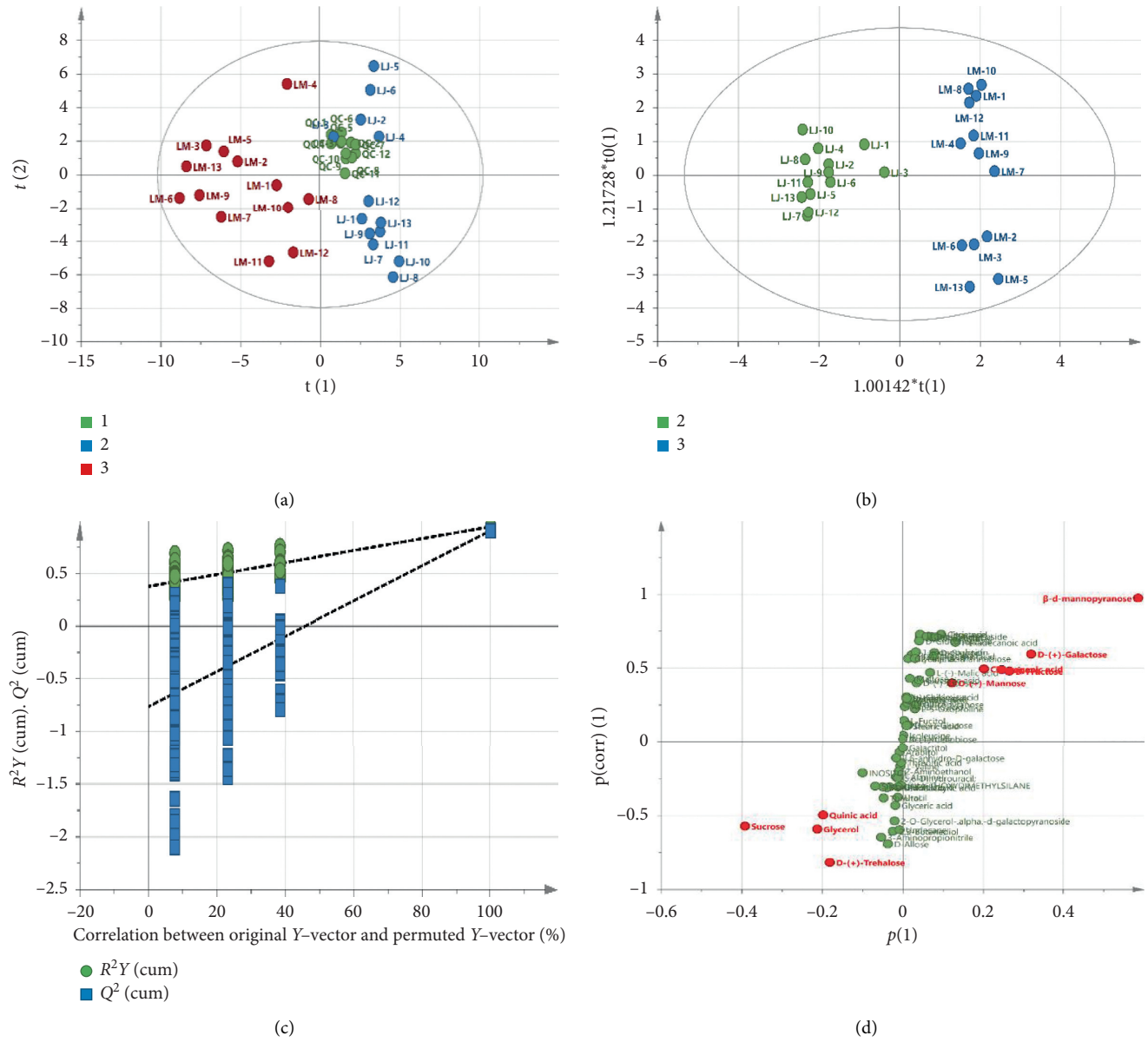


Figure 2: Multivariate statistical analysis of LJ and LM including PCA and OPLS-DA. (a) PCA score plot. (b) OPLS-DA score plot of LJ and LM. (c) The corresponding validation plots based on 200 times permutation tests of the OPLS-DA model. (d) S-plot screening of biomarkers.

Table 2: Potential biomarkers of LJ in comparison to LM.

No	VIP	p value	Name	Metabolic pathway
1	4.08	$1.01E-13$	Mannose	Amino sugar and nucleotide sugar metabolism
2	3.47	0.0019	Sucrose	Starch and sucrose metabolism
3	2.77	0.0028	D-(+)-Galactose	Galactose metabolism
4	2.57	0.0190	D-Fructose	Fructose and mannose metabolism
5	2.39	0.0130	Psicose	Unclear
6	1.49	0.0074	Glycerol	Glycerolipid metabolism
7	1.45	0.0128	Chlorogenic acid	Phenylpropanoid biosynthesis
8	1.41	0.0289	Quinic acid	Phenylpropanoid biosynthesis
9	1.34	0.0421	D-(+)-Glucose	Glycolysis
10	1.29	$1.21E-05$	D-(+)-Trehalose	Starch and sucrose metabolism

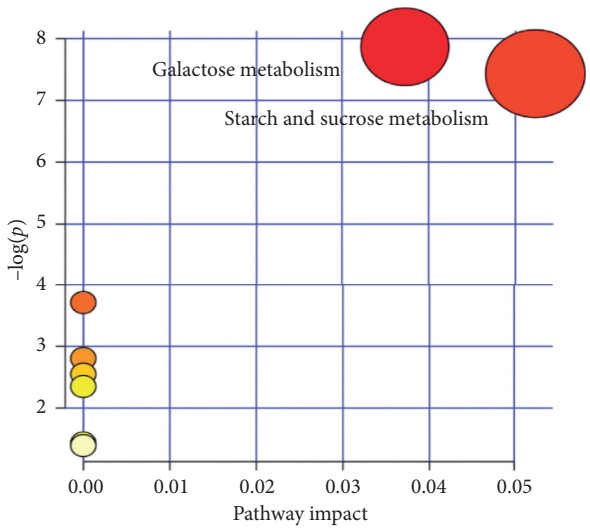


Figure 3: Metabolic pathway analysis of potential biomarkers by MetaboAnalyst 4.0.

represented the pathway impact values. The color and size of each circle graph had a positive relationship with their p value and pathway impact value, respectively. In consequence, the galactose metabolism and the starch and sucrose metabolism gathered as potential pathways caused the remarkable distinctions between LJ and LM. However, the mechanism of how these pathways contributed to the distinction between LJ and LM was still unclear. Therefore, a network of metabolic pathways was constructed to show the changes in the key metabolic pathway based on these information and related literature [27–29].

As displayed in Figure 4, sucrose and trehalose, involved in the starch and sucrose metabolism, were at a higher level in LJ than that of LM. While galactose, mannose, maltose, and glucitol, derived from the hydrolysis of sucrose and involved in the galactose metabolism, were more abundant in LM than those in LJ. The two metabolisms presented absolutely different activities. Citric acid, malic acid, and succinic acid in the TCA cycle showed a distinct metabolism pattern. All of them showed obvious upaccumulation in LM compared with LJ. Caffeic acid and chlorogenic acid in the phenylpropanoid pathway were obviously upregulated in LM as well. However, the quinic acid, which is also involved in the phenylpropanoid pathway, presented downregulation in LM. All in all, metabolites in the starch and sucrose metabolism, galactose metabolism, TCA cycle, and the phenylpropanoid pathway showed significant distinctions and different metabolism patterns between LJ and LM.

4. Discussion

According to OPLS-DA analysis and MetaboAnalyst, the starch and sucrose metabolism and the galactose metabolism were obtained as potential pathways contributed to the remarkable distinctions of primary metabolites between LJ and LM.

In the starch and sucrose metabolism, sucrose and trehalose showed higher content in LJ compared to LM (Figure 4). The metabolites involved in the starch and sucrose metabolism showed upregulation in LJ. Starch and sucrose are the primary products of photosynthesis of most plants [30]. Starch represents the major plant storage carbohydrate providing energy during the times of heterotrophic growth [31]. Sucrose is a nonreducing sugar playing an eminent role in sugar translocation in the phloem, sugar storage, the syntheses of cell wall polysaccharides [32], and the tolerance to various stresses [33, 34]. Trehalose, synthesized via trehalose 6-phosphate, is essential for plant metabolism, growth, and stress responses. It is implicated in responses to cold, salinity, and in regulation of stomatal conductance and water use efficiency [35]. The higher content of sucrose and trehalose in LJ compared to LM revealed that LJ may perform better in stress responses, development, growth, and other aspects. In the galactose metabolism, most metabolites (galactose, mannose, glucitol, and ribose) were downregulated in LJ. Galactose can be obtained from the raffinose pathway and the breakdown of glucose (Figure 4). Some studies presented that galactose could inhibit cell wall biosynthesis in plants [36] and decrease the level of sucrose [37]. Mannose can affect gas exchange and decrease the photosynthetic capacity in plants [38]. Hence, the lower level of galactose and mannose in LJ compared to LM is understandable.

Metabolites in the phenylpropanoid pathway play a crucial role in the pharmacological effect of LJ and LM [9, 39–41]. These metabolites could be divided into two categories, phenolic acids and flavonoids.

Caffeic acid, quinic acid, and chlorogenic acid, as secondary metabolites, are the main functional phenolic acids in LJ and LM. Caffeic acid and chlorogenic acid presented lower levels in LJ compared with LM (Figure 4). Chlorogenic acid has been regarded as one of the most significant constitutes of LJ and LM for its multiple pharmacological activities, such as antibacterial, antioxidant, and antidiabetic [2, 42, 43]. Moreover, chlorogenic acid is viewed as a significant factor to evaluate the quality of LM and LJ [1]. Caffeic acid synthesized by p-coumaric acid has strong antioxidative and anti-inflammatory activities [44]. Quinic acid is a powerful antioxidant and has been reported to have antiviral and antihuman immunodeficiency virus activities [45, 46]. In this study, an interesting phenomenon was found. Caffeic acid and chlorogenic acid presented lower levels in LJ compared with LM, while quinic acid showed an opposite trend (Figure 4). The level of these components in LJ and LM was similar to previous studies [12, 47]. Such a case also occurred to different subtypes of white tea [48], which is interesting and thought-provoking. In fact, the level of phenolic acids can be influenced by many factors. Previous research showed that gene expressions [49, 50], different breeding processes, producing process, and manufacture process all had significant impacts to the level of chlorogenic acid and other phenolic acids [51, 52]. Therefore, the reason for such case requires more specific studies.

Flavonoid is another category of secondary metabolites in the phenylpropanoid pathway. These compounds are demonstrated to exhibit significant pharmacological activities and understood to be influential targets for metabolic engineering [53–57]. Naringenin generated from the

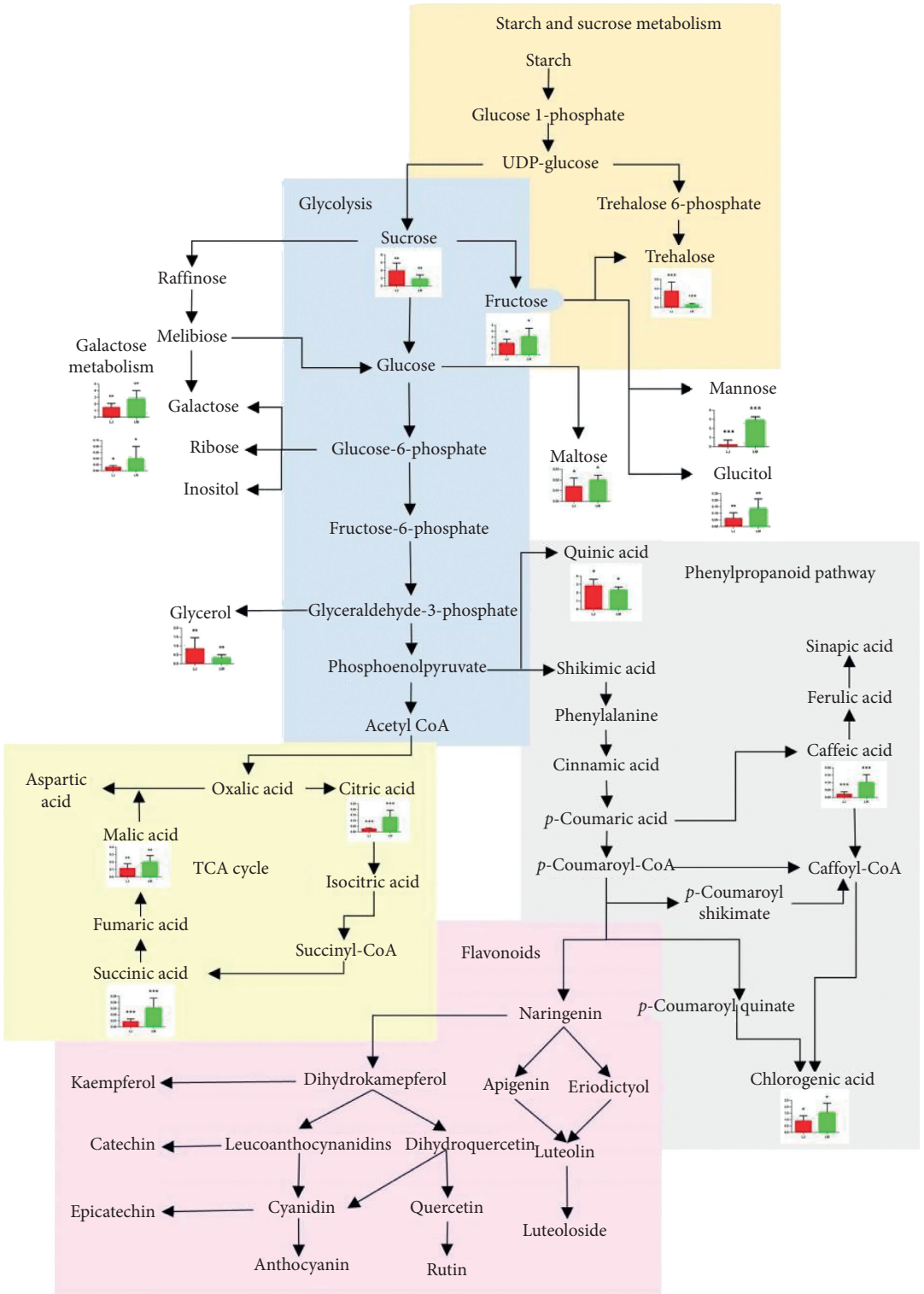


Figure 4: The metabolic pathway map, including the galactose metabolism, the starch and sucrose metabolism, the glycolysis, the phenylpropanoid pathway, the flavonoids metabolism, and the TCA cycle. In the relative peak area figure, red represents LJ and green represents LM.

conversion of *p*-coumaroyl-CoA is the precursor of most flavonoids [58]. It can be catalyzed to apigenin and eriodictyol through flavones synthase or flavonoid 3-hydroxylase [29]. Then, luteolin can be synthesized from these two flavones. Luteolin, a flavonoid mainly found in fruits and vegetables, is regarded as a representative metabolite in LJ. It has antioxidant [59], anti-inflammatory [60], antitumor [61], and anti-5-lipoxygenase [62] activities. Moreover, luteoloside can be further synthesized from luteolin via UDP-glucose flavone 7-O-β-glucosyltransferase [28]. Luteolin-7-O-glucoside was viewed as the standard compound to evaluate the quality of LJ [1]. Previous studies showed that flavonoids had a higher level in LJ compared with LM [7, 63, 64], but a few studies about the inner causes of it were established. Liu et al. revealed that miRNAs play a key role in the flavonoid biosynthesis of LJ [53]. Wu et al. found that the weak catalytic activity and low expression of LmFNSII-1.1 in flowers might be responsible for the low levels of flavone accumulation in LM [4]. In this study, we found that the high level of flavonoids in LJ can be interpreted from the primary metabolite aspect. The metabolites involved in the starch and sucrose metabolism exhibited upregulation in LJ (Figure 4). These substances were then broken down and altered through various enzymes and different metabolic pathways. However, almost all metabolites in the galactose metabolism, the TCA cycle, and the phenolic acid part of phenylpropanoid metabolism were downregulated in LJ. Therefore, the high level of flavonoid in LJ could be explained by the factor that the energy stored in the starch and sucrose metabolism may be saved to produce flavonoid.

5. Conclusions

In summary, the combination of metabolomics based on GC-MS with chemometric analysis could be a useful tool for discrimination, quality evaluation, and exploring metabolic regulation mechanisms of LJ and LM. The result suggested great variations in the metabolome between LJ and LM, and 10 metabolites (mannose, sucrose, D-(+)-galactose, D-fructose, psicose, glycerol, chlorogenic acid, quinic acid, D-(+)-glucose, and D-(+)-trehalose) were obtained as characteristic ingredients contributed highly to the difference. The starch and sucrose metabolism and the galactose metabolism were obtained as potential pathways contributed to the remarkable distinctions of primary metabolites between LJ and LM. Moreover, the metabolites involved in the starch and sucrose metabolism presented upregulation in LJ. While almost all metabolites in the galactose metabolism, the TCA cycle, and the phenolic acid part of phenylpropanoid metabolism were downregulated in LJ, the high level of flavonoid in LJ could be explained that the energy stored in the starch and sucrose metabolism may be saved to produce flavonoid.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors. The authors confirm compliance with ethical standards.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Rui-ying Liu and Jing Deng contributed equally to this work.

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Supplementary Materials

Figure S1: 6 different chemical classes of the 63 metabolites. (*Supplementary Materials*)

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