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Metabolite profiles and antibacterial and antioxidant activities of leaf extracts of five *Lonicera* species: a comparative study

Yu-Han Feng[1†], Guo-Dong Zhang[1†], Ping-Chuan Zhu[1†], Wen-Hu Zhu[1], You-Zhi Li[1] and Xian-Wei Fan[1*]

Abstract

Background *Lonicera japonica* Flos is widely used as a medicinal plant in China owing to its various pharmacological activities. However, little is known about the metabolic profile and antibacterial properties of the leaves of *Lonicera* species. The present study aimed to determine and compare the metabolite profiles and antibacterial and antioxidant activities of leaf extracts of five *Lonicera* species.

Results 598 metabolites were identified based metabolomics using UHPLC-Q Exactive Orbitrap MS/MS in the five *Lonicera* species leaves. Among them, flavonoids and phenols compounds accounted for 13%. In *Lonicera dasystyla*, 110 differential metabolites were found compared to those in the other *Lonicera* species, of which flavonoids and phenols accounted for 20% and 10%, respectively. Compared to other *Lonicera* species leaves, the contents of total phenol, total flavonoid, antioxidant activities and anti-bacterial capacities were considerably higher in *Lonicera hypoglauca* and lower in *L. dasystyla*. The contents of total phenol and total flavonoid in *L. hypoglauca* were 95.86% and 201.64% higher than those in *L. dasystyla*. The RPA value in *L. hypoglauca* was 93.27% higher than the value of *L. dasystyla*, respectively. The content of 4, 5-dicaffeoylquninic acid in *L. hypoglauca* was 4.814 mg g^{-1}, which was 302.85% higher than that of *L. dasystyla*. Besides, Chlorogenic acid was most high in leaves of *L. japonica* and reached 15 mg g^{-1}, which was 209.34% higher than that of *L. dasystyla*. Moreover, correlation analysis showed most flavonoids, phenols, and coumarins were positively correlated with antioxidant activities and antibacterial capacities.

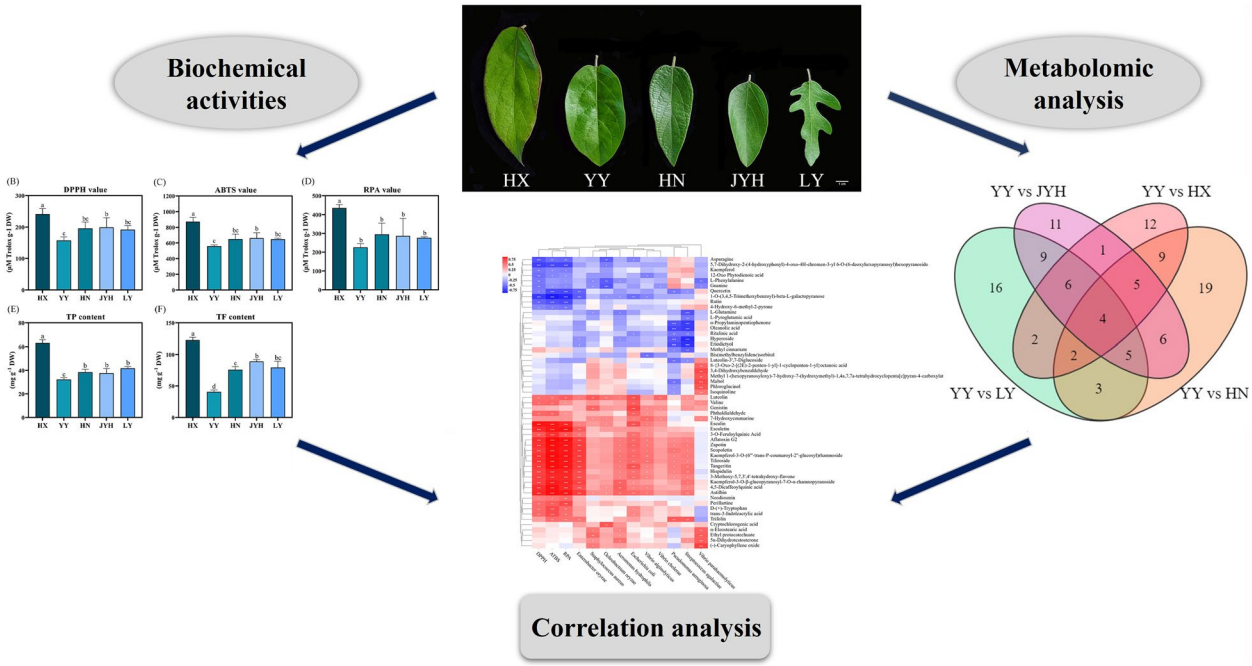
Conclusions This study identified the difference of metabolites in leaves of five *Lonicera* species, antioxidant abilities and inhibition effects of leaf extracts on pathogens, which provides a potential information for further application of *Lonicera* leaves.

Keywords Antibacterial activity, Antioxidant capacity, Comparative metabolomics, Flavonoids, *Lonicera* species, UHPLC-Q Exactive

†Yu-Han Feng, Guo-Dong Zhang and Ping-Chuan Zhu have contributed equally to this work.

*Correspondence:
Xian-Wei Fan
fanxw@gxu.edu.cn
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Lonicera japonica Thunb., a species of the Caprifoliaceae family, known as "Jin Yin Hua (JYH)" in China, is recognized as edible and medicinal food [1]. The flower, buds and caulis of JYH is determined as only plant sources widely used for in Chinese Pharmacopoeia [2]. Modern pharmacological studies have shown that JYH has broad pharmacological properties, including antioxidant, antiviral, antiseptic, and anti-inflammatory effects [3–5]. More than 300 medicine compounds have been reported in the buds and flowers of JYH and investigated their biosynthesis and pharmacological activities [6].

Hydroxycinnamic acid, flavonoids, and iridoids have been also found in leaves and stems of JYH, and the contents of these active compounds significantly vary in species [7–9]. Metabolomics revealed the energy stored in starch and sucrose metabolism contributed to accumulate the higher levels of flavonoids in fresh bud of JYH while low level of phenolic acid compounds [10]. The quality of *L. japonica* Flos was superior to *Lonicerae* Flos based on grey relational analysis of multiple bioactive constituents, and flower buds were better than flowers [11]. Moreover, the abundant chlorogenic acids (CGAs) are important medicine ingredients in JYH and play an essential role in pharmacological activities, such as anti-inflammation and anti-bacteria [12]. The flower and buds of some other *Lonicera* species, such as *Lonicera hypoglauca* Miq. (HX), *Lonicera confusa* DC. (HN), *Lonicera dasystyla* Rehd. (YY) and *L. dasystyla* via. (LY), are serve as the substituents of JYH due to the similarity of their morphological properties and the high price of JYH [13]. Chlorogenic acid and luteoloside are two major active compounds and used for the quality evaluation marker of JYH in Chinese Pharmacopoeia [2]. The leaves of JYH and other *Lonicera* species are also valuable for the potential applications in livestock and poultry breeding industry as feed additive [14]. However, most studies have focused on identifying the medicine constituents of flower/buds in JYH and other *Lonicera* species [15, 16], little is known about the compounds and biological activities of leaf extracts in *Lonicera* species. Mass spectrometry combined with high performance liquid chromatography (HPLC–MS) is increasingly used for qualitative and quantitative analysis of complex matrices, and has been widely used to identify natural compounds due to its high sensitivity and high resolution [17]. In this study, ultra-high performance liquid chromatography quadrupole exactive Orbitrap MS/MS (UHPLC-Q Exactive Orbitrap MS/MS)-based metabolomics was used to screen and identify leaf metabolites of five *Lonicera* species. At the same time, combined with biochemical experiments, the contents of phenolic

acid and flavonoid, antioxidant activity and antibacterial ability of different *Lonicera* species leaf extracts were systematically studied. Moreover, the correlation between biochemical index and metabolite accumulation was analyzed. The results will deepen our understanding of the chemical constituents of *Lonicera* plants in Guangxi, and provide reference for the development of medicinal and health care resources of *Lonicera* leaves.

Materials and methods

Materials and reagents

The leaves of five *Lonicera* species were collected from the Guangxi Medical Botanical Garden in Nanning, Guangxi Zhuang Autonomous Region, PR China (N22° 51′ 32.07″, E108° 23′ 0.32″) in April 2022. Each sample was randomly collected from the leaves of each *Lonicera* species. Five species of *Lonicera* were determined; these were *L. dasystyla* Rehd. (YY), *L. confusa* (Sweet) DC. (HN), *L. hypoglauca* Miq. (HX), *L. japonica* Thunb. (JYH), and *L. dasystyla* via. (LY) (Additional file 1: Table S1).

Ethanol, methanol, acetonitrile (ACN), and formic acid were of HPLC grade with purity ≥ 99.9% (Thermo Fisher Scientific, Waltham, MA, USA). CGA, 4,5-dicaffeoylquinic acid, neochlorogenic acid, caffeic acid, scopoletin, syringic acid, resveratrol, hispidulin, luteolin, neodiosmin, hyperoside quercetin, gallic acid, and rutin standard samples were of HPLC grade with purity ≥ 98% (Shanghai yuan-ye Bio-Technology Co., Shanghai, China). All other solvents were of analytical grade with purity ≥ 99% (Guangdong Guanghua Sci-Tech Co., Guangdong, China).

Preparation of metabolite extraction samples and standard solutions

The leaves of *Lonicera* were dried overnight in an oven at 50 °C and ground into a powder. After screening through a 60-mesh sieve, 200 mg sample was accurately weighed. 4 mL of 75% ethanol was added to the sample, and the mixture was extracted by ultrasonication at 40 °C for 30 min. Then, the mixture was centrifuged at 10,000 rpm for 10 min at room temperature, and the supernatant was collected in a new test tube. The sample was extracted three times with the same volume of 75% ethanol and the supernatant was collected. The collected supernatant was concentrated to dryness with an evaporation concentrator (RV10 BASIC; IKA-Werke, Staufen, Germany), re-dissolved in 2 mL methanol, ultrasonicated for 10 min, and centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was filtered through an organic filter (0.22 μm) and stored at − 20 °C as a sample extract. After dilution, the sample solution was used for subsequent biochemical analysis and metabolomic analysis.

CGA, 4,5-dicaffeoylquinic acid, neochlorogenic acid, caffeic acid, scopoletin, syringic acid, resveratrol, hispidulin, luteolin, neodiosmin, quercetin, and hyperoside were accurately weighed and, respectively, dissolved in methanol in a volumetric bottle. The stock solution of each compound was prepared at 1.0 mg mL^{-1} and then diluted to the appropriate concentration.

Determination of TP and TF contents

The content of total phenol in leaf extracts of *Lonicera* species was determined according to the Folin–Ciocalteu method [18] with some modifications. Briefly, 0.2 mL of sample solution, 0.2 mL of folinphenol and 0.6 mL of 7.5% sodium carbonate solution were added to 2 mL centrifuge tube, replenished with distilled water, and incubated in the dark for 30 min. The absorbance of the solution was then measured at 765 nm. The standard curve was constructed by gallic acid with different concentration gradients. The equation for the standard curve was $y = 0.0053x + 0.079$ ($R^2 = 0.9995$). The TP content was calculated using a standard curve. The results were showed in mg per gram of dried weight (mg g^{-1} DW).

The reference $NaNO_2$–Al $(NO_3)_3$ [19] was used to determine TF content. Briefly, 0.4 mL sample solution, 0.7 mL methanol and 0.05 mL of 5% $NaNO_2$ solution were added to a test tube and mixed evenly for 6 min. Then, 0.05 mL of 10% Al $(NO_3)_3$ solution was added and incubated for 6 min, followed by 0.8 mL of a 4% NaOH solution and incubated for 15 min. The absorbance of the solution was then measured at 510 nm. The standard curve was constructed by rutin standard with different concentration gradients. The standard curve equation was $y = 0.0007x + 0.0593$ ($R^2 = 0.9993$). The TF content was calculated according to a standard curve. The results were showed in mg g^{-1} DW.

Determination of antioxidant activity

Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) activity

The DPPH radical scavenging assay was performed as described previously [20], with some modifications. 0.2 mmol L^{-1} DPPH solution was prepared in anhydrous ethanol. Then, 0.25 mL sample solution and 0.75 mL DPPH solution were mixed evenly in a centrifuge tube, followed by a 30-min incubation at 25 °C and a 5-min centrifugation at 8000 r min^{-1}. The absorbance of the solution was measured at 517 nm:

$$\text{DPPH scavenging rate}(\%) = \left[1 - \left(\frac{A1 - A2}{A0}\right)\right] 100\%.$$

where *A*0 is the 0.25 mL absolute ethanol + 0.75 mL DPPH absorbance value. *A*1 is the 0.25 mL sample + 0.75 mL DPPH absorbance value. *A*2 is the 0.25 mL sample + 0.75 mL absolute ethanol absorbance value. Trolox was used as a quantitative criterion, and the results were expressed as μM Trolox g^{-1} DW.

Determination of azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) activity

The ABTS scavenging assay was performed using a previously described method [21] with minor modifications. 5 mL ABTS (7.4 mmol L^{-1}) and 5 mL $K_2S_2O_8$ (2.6 mmol L^{-1}) were mixed and incubated in the dark for 16 h at room temperature. The ABTS mixture was diluted 40–45 times with a phosphoric acid buffer solution to obtain a final ABTS solution with the optical density (OD) value of 0.7 ± 0.02 at 734 nm. Finally, 0.2 mL sample solution and 0.8 mL of the ABTS solution were added to the centrifuge tube, mixed evenly, and incubated in the dark for 6 min. The absorbance of the solution was determined at 734 nm.

$$\text{ABTS scavenging rate}(\%) = \left[1 - \left(\frac{A1 - A2}{A0}\right)\right]100\%.$$

*A*0 is the 0.2 mL distilled water + 0.8 mL ABTS solution absorbance value. *A*1 is the 0.2 mL sample solution + 0.8 mL ABTS solution absorbance value. *A*2 is the 0.2 mL sample solution + 0.8 mL methanol absorbance value. Trolox was used as a quantitative criterion, and the results were expressed as μM Trolox g^{-1} DW.

Reducing power assay (RPA)

The RPA of *Lonicera* samples was conducted using a modified method [22]. Briefly, 0.25 mL of sample solution, 0.25 mL of phosphate buffer (0.2 mol L^{-1}, pH = 6.6) and 0.25 mL of 1% potassium ferricyanide solution ($K_3Fe(CN)_6$) were added to a 2 mL centrifuge tube. The mixture was thoroughly mixed and placed in a water bath at 50 °C for 20 min, followed by rapid cooling in an ice water bath, and 0.25 mL of 10% trichloroacetic acid was immediately added. After centrifugation at 4000 r min^{-1} for 10 min, the supernatant was collected, and then equal volumes of supernatant (0.25 mL), distilled water (0.25 mL), and 0.1% $FeCl_3$ solution (0.05 mL) were added to a new centrifuge tube. After mixing, the absorbance was determined at 700 nm. Trolox was used as a quantitative criterion, and the results were expressed as μM Trolox g^{-1} DW.

Determination of antibacterial activity

Antibacterial activity was determined using the inhibition zone method [23] with minor modifications. The test pathogenic strains were *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter oryzae*, *Ochrobactrum oryzae*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, *Vibrio cholerae*, *Vibrio Parahaemolyticus*, *Aeromonas hydrophila*, and *Streptococcus agalactiae*. The pathogens are collected in the laboratory. The *Lonicera* sample extract was diluted 10 times and then inoculated in LB medium at 37 °C while shaking at 200 r min^{-1} for 12 h. The bacterial suspension was diluted with sterile water to approximately 0.6 (OD_{600}) and then diluted 20 times. The test strain suspension (100 μL) was added to a Petri dish containing LB solid medium and spread evenly. A 6-mm filter paper was placed on a solid petri dish, and 10 μL of the diluted sample solution was dropped onto the middle of the filter paper. After incubation at 37 ℃ for 24 h, the diameter of the inhibition zone was measured.

200 mg L^{-1} CGA solution was used as the positive control instead of sample solution, and methanol solution was used as the negative control. The diameter of the antibacterial zone is positively correlated with the antibacterial effect [24].

Metabolomic profiling of *Lonicera* metabolite extracts

Metabolite profiling was performed using the ultra-high-performance liquid chromatography–quadrupole exactive Orbitrap tandem mass spectrometer (UHPLC-Q Exactive Orbitrap MS/MS) (Thermo Fisher Scientific), equipped with a heated electrospray ionization (HESI) source, in the positive and negative ionization modes. All samples were separated using ACQUITY UPLC BEH C18 (50 mm × 2.1 mm, 1.7 μm column, Waters, Co., USA) with mobile phase A (water and 0.1% formic acid) and mobile phase B (ACN). The linear gradient elution conditions were as follows: 0–2 min, 5% B; 2–13 min, 5–100% B; 13–16 min, 100–100% B; and 16.1–18 min, 5% B. The injection volume was 2 μL, and the flow rate was 0.3 mL min^{-1}.

The column chamber and sample tray were maintained at 30 and 10 °C, respectively. The optimal mass spectrometry (MS) parameters were as follows: spray voltage, 3 kV; capillary temperature, 320 °C. Nitrogen was used as the sheath and auxiliary gas at flow rates of 30 and 10 psi, respectively. The auxiliary gas was then heated to 350 °C. The scanning mode was Full MS and Full MS/DD–MS^2, the mass range was 70–1000 m/z, and the resolutions of the primary and secondary scans were 70,000 and 17,500, respectively.

Raw data were analyzed using the software compound discover 3.2 (Thermo Fisher Scientific). Metabolite data

were logarithmically transformed for statistical analysis to improve normality and normalize the data.

Quantification of 12 important components for metabolite extraction

The curves for the 12 reference standards were linear between the highest and lowest concentrations. The 12 reference standards for metabolite extraction were quantified using UHPLC-Q Exactive (Thermo Fisher Scientific), due to its strong qualitative and quantitative abilities [25]. Chromatographic and mass spectrometric conditions were set as described above in "Metabolomic profiling of *Lonicera* metabolite extracts" (Materials and methods).

Statistical analysis

The differential metabolites of the leaf extracts of *Lonicera* were analyzed using SIMCA (V14.1.0, Sartorius Stedim Data Analytics AB, Umea, Sweden) and annotated using the PubChem Substance and Compound databases (https://pubchem.ncbi.nlm.nih.gov/). HCA was conducted online (http://www.ehbio.com/Image

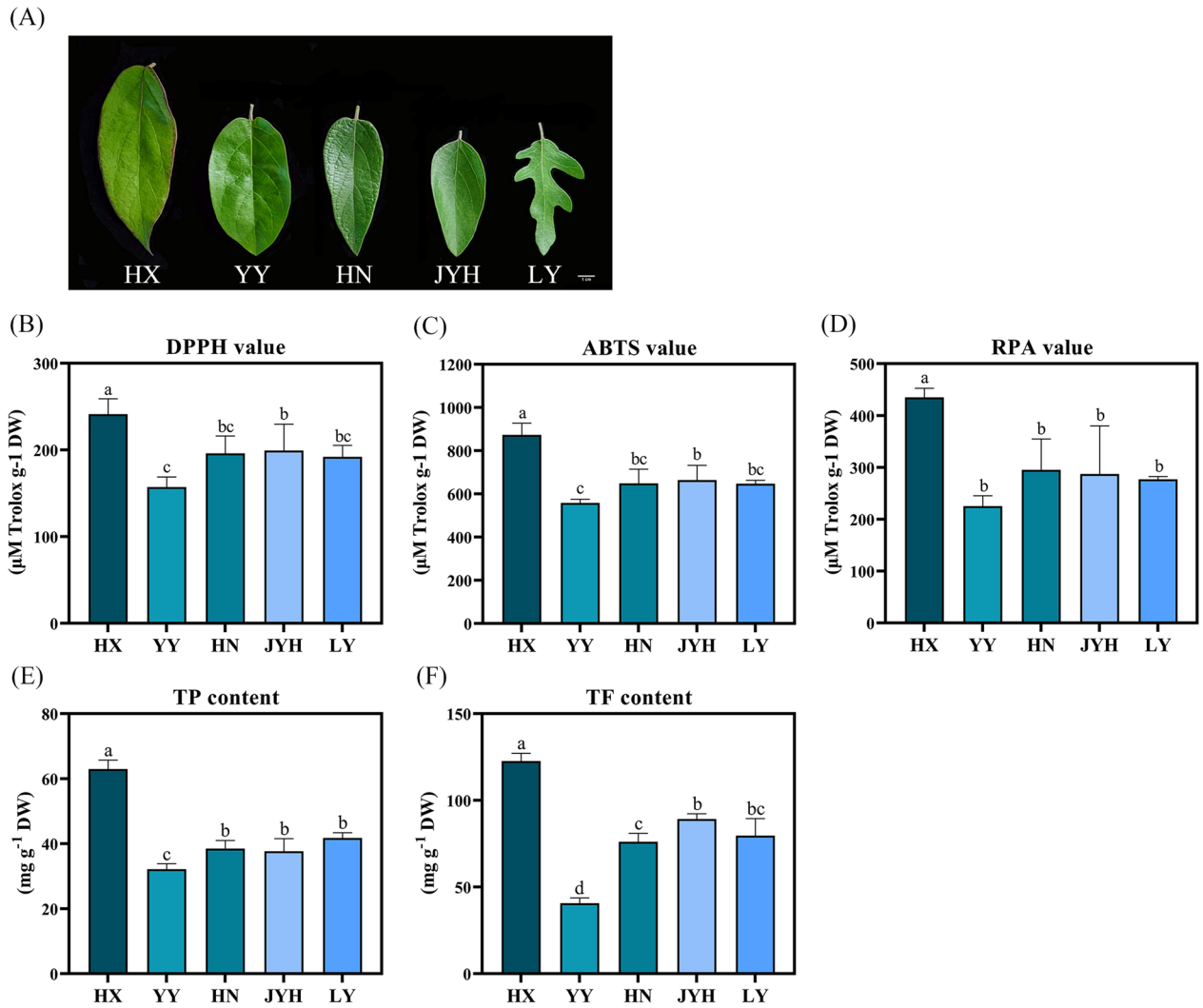


Fig. 1 Leaf morphology and effect of *Lonicera* species leaves extraction on antioxidant activity and biological activities. **A** Leaf morphology of five *Lonicera* species; scale = 1 cm. **B** Difference of antioxidant value of DPPH in five *Lonicera* species leaves. **C** Antioxidant value of ABTS. **D** Antioxidant value of RPA. **E**, **F** Total phenol (TP) and total flavonoid (TF) contents in *Lonicera* species leaves. The results were showed by means ± SD. For multiple comparisons, One-way ANOVA-test was used followed by post hoc test and Waller–Duncan method. Different lowercase letters indicate significant differences at $P < 0.05$. DPPH, 1, 1-Diphenyl-2-picrylhydrazyl; ABTS, azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); RPA, reducing power assay; YY, *Lonicera dasystyla* Rehd.; HN, *Lonicera confusa* (Sweet) DC.; HX, *Lonicera hypoglauca* Miq.; JYH, *Lonicera japonica* Thunb.; LY, *Lonicera dasystyla* via

GP/). PCA, OPLS-DA, Venn diagrams, and KEGG enrichment bubble plot were constructed online (https://cloud.metware.cn/#/tools/tool-list). Pearson correlation coefficient was calculated by SPSS 26.0 and plotted online (https://www.omicshare.com/tools/). Other data were plotted by GraphPad Prism V6.01 (GraphPad Software, La Jolla, CA, USA). ANOVA-test was used followed by post hoc test and Waller–Duncan method.

Results

Biochemical index analysis

Morphology, antioxidant activity, and TP and TF contents of the five Lonicera species

Significant differences were observed in leaf morphology and appearance among the five *Lonicera* species (Fig. 1A; Additional file 1: Table S1). The leaf extract of HX exhibited a significantly ($P<0.05$) strong DPPH-scavenging ability, while that of YY showed a weak DPPH-scavenging ability (Fig. 1B). The DPPH value in HX was 241.338 μM Trolox g^{-1} DW and was 21.13%, 23.11%, 25.67%, and 53.58% higher than the values in JYH, HN, LY, and YY, respectively (Fig. 1B). The ABTS value of the leaf extract in HX reached 873.093 μM Trolox g^{-1} DW, which was higher than that in JYH, HN, LY, and YY by 31.43%, 34.59%, 34.86%, and 56.49%, respectively (Fig. 1C). The RPA value of the leaf extract in HX was 435.046 μM Trolox g^{-1} DW and was 47.43%, 51.55%, 57.14%, and 93.27% higher than that in HN, JYH, LY, and YY, respectively (Fig. 1D). Overall, these results indicate that the antioxidant ability of HX leaf extract was significantly ($P<0.05$) higher than that of the extracts of other four species.

We also analyzed the TP content in leaf extracts of the five *Lonicera* species. The highest TP content (62.963 mg g^{-1} DW) was observed in the leaf extract of HX, which was higher than that in the leaf extracts of LY, HN, JYH, and YY by 50.74%, 63.54%, 67.09%, and 95.86%, respectively (Fig. 1E). The TF content of the leaf extract of HX was 122.632 mg g^{-1} DW, which was higher by 37.43%, 54.02%, 61.19%, and 201.64% than that in JYH, LY, HN, and YY, respectively (Fig. 1F). These results showed that the TP and TF contents of HX leaf extract were significantly ($P<0.05$) higher than those of the other four *Lonicera* extracts.

Differences in the antibacterial activity of leaf extracts

Leaf extracts of *Lonicera* showed strong antibacterial activities against both gram-negative and gram-positive bacteria, which were higher than those of CGA (Table 1). The diameter of inhibition zone of HX leaf extract against *A. hydrophila* was 13.09 mm, and that of CGA was 10.75 mm. The inhibitory rate of HX extract against *A. hydrophila* was 21.7% higher than that of CGA. While the inhibition zone diameter of HX extract on *V. cholerae* was 12.01 mm, and the inhibitory rate was 16.9% higher than that of CGA (Table 1). The antibacterial activity of HX leaf extract on ten pathogen species was stronger than that of the other four *Lonicera*; the leaf extracts of the other four species exhibited relatively similar inhibitory effects (Table 1).

The growth of two gram-positive bacteria, *Streptococcus aureus* and *S. agalactiae*, was inhibited by all five leaf extracts of *Lonicera* and CGA. The strongest inhibition was observed at 13.39 mm with HX leaf extract, which was 17.2% higher than that of CGA (Table 1). In addition,

Table 1 Different *Lonicera* species extract for antibacterial activity

Microbial species	Diameter ofpathogen inhibition zone /mm						
	HX	YY	HN	JYH	LY	CGA	Methanol
Escherichia coli	11.71 ± 0.41^{a}	10.76 ± 0.21^{b}	10.74 ± 0.23^{b}	11.56 ± 0.33^{a}	11.12 ± 0.15^{ab}	9.89 ± 0.44^{c}	9.13 ± 0.16^{d}
Enterobacter oryzae	11.8 ± 0.31^{a}	10.94 ± 0.07^{b}	10.89 ± 0.44^{b}	11.06 ± 0.1^{ab}	11.27 ± 0.53^{ab}	10.52 ± 0.43^{b}	8.56 ± 0.18^{c}
Ochrobactrum oryzae	10.47 ± 0.07^{a}	10.1 ± 0.18^{bc}	10.27 ± 0.03^{abc}	10.38 ± 0.25^{ab}	10.39 ± 0.34^{ab}	9.89 ± 0.03^{c}	9.04 ± 0.05^{d}
Vibrio alginolyticus	12.99 ± 0.36^{a}	12.02 ± 0.69^{ab}	12.12 ± 0.04^{ab}	12.75 ± 0.51^{a}	12.44 ± 0.5^{ab}	11.67 ± 0.42^{b}	10.5 ± 0.13^{c}
Vibrio cholerae	12.01 ± 0.62^{a}	11.09 ± 0.87^{ab}	11.31 ± 0.19^{ab}	11.92 ± 0.57^{a}	11.6 ± 0.4^{ab}	10.27 ± 0.85^{bc}	9.04 ± 0.38^{c}
Vibrio Parahaemolyticus	12.2 ± 0.25^{ab}	11.58 ± 0.75^{b}	12.24 ± 0.23^{ab}	12.34 ± 0.34^{ab}	12.9 ± 0.44^{a}	11.75 ± 0.33^{b}	9.55 ± 0.08^{c}
Aeromonas hydrophila	13.09 ± 0.04^{a}	11.85 ± 0.23^{ab}	11.87 ± 0.47^{ab}	12.21 ± 0.12^{ab}	12.9 ± 0.27^{a}	10.75 ± 0.96^{b}	8.99 ± 0.42^{c}
Pseudomonas aeruginosa	10.85 ± 0.34^{a}	10.77 ± 0.28^{a}	10.18 ± 0.29^{bc}	10.49 ± 0.13^{ab}	10.45 ± 0.26^{ab}	9.68 ± 0.1^{c}	8.83 ± 0.06^{d}
Streptococcus agalactiae (G+)	13.39 ± 0.49^{a}	13.01 ± 0.13^{ab}	12.08 ± 0.63^{bc}	12.68 ± 0.17^{ab}	13.13 ± 0.06^{ab}	11.42 ± 0.92^{b}	10.5 ± 0.49^{c}
Staphylococcus aureus (G+)	11.86 ± 0.23^{a}	10.54 ± 0.5^{bc}	10.92 ± 0.69^{abc}	11.61 ± 0.25^{ab}	11.63 ± 0.74^{ab}	10.46 ± 0.24^{c}	9.17 ± 0.12^{d}

Different superscripts represent significant differences ($P<0.05$)

Lonicera extracts were diluted ten times. Antibacterial activity was expressed as the mean diameter of the inhibition zone ± SD, in mm ($n=5$), including the diameter of the filter paper (6 mm). CGA concentration was 200 mg/L

YY, *Lonicera dasystyla* Rehd.; HN, *Lonicera confusa* (Sweet) DC.; HX, *Lonicera hypoglauca* Miq.; JYH, *Lonicera japonica* Thunb.; LY, *Lonicera dasystyla* via

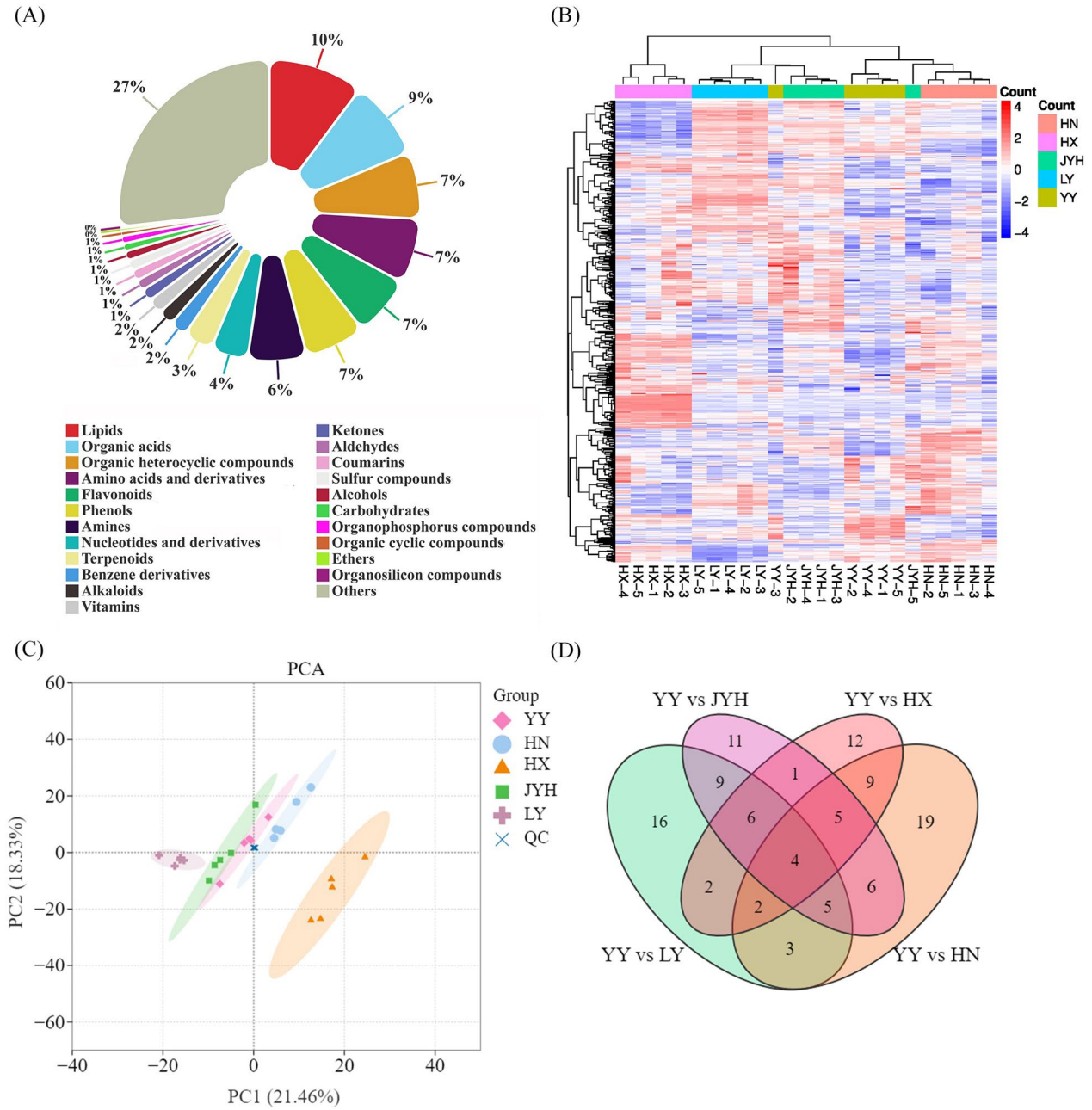


Fig. 2 Metabolites analysis of five *Lonicera* species. **A** Pie chart of the total metabolites distribution. **B** Cluster heatmap of metabolites in five *Lonicera* species. Each column represents a sample, each row represents a metabolite, and the colors of the heatmap indicate the relative content of each metabolite, from low (blue) to high (red). **C** Principal component analysis. QC is quality control. **D** Venn diagram showing the number of DMs in YY vs. HN, YY vs. HX, YY vs. JYH, and YY vs. LY

leaf extract from YY plants exhibited the strongest antibacterial activity against *P. aeruginosa*, with an inhibition zone of 10.77 mm in diameter (Table 1). JYH extracts showed the strongest antibacterial activity against *E. coli*, *V. alginolyticus*, and *V. cholerae*, with inhibition diameters of 11.56, 12.75, and 11.92 mm, respectively. The extracts of LY plants showed the best inhibition profile against *V. Parahaemolyticus* and *A. hydrophila.*

Metabolome of *Lonicera* leaf extracts

Using the UHPLC-Q Exactive Orbitrap MS/MS-based metabolomics, 598 metabolites were identified in the leaf extracts of five *Lonicera* plants; these included 61 lipids,

51 organic acids, 43 organic heterocyclic compounds, 42 amino acids and derivatives, 39 phenols, 39 flavonoids, 38 amines, 24 nucleotides and its derivatives, 19 terpenoids, 11 benzene derivatives, 10 alkaloids, 10 vitamins, 8 ketones, 7 aldehydes, 7 sulfur compounds, 7 coumarins, 6 alcohols, 5 carbohydrates, 5 organophosphorus compounds, 3 organic cyclic compounds, 2 ethers, 1 organosilicon compound, and 160 other metabolites (Fig. 2A; Additional file 1: Table S2).

Among the 598 identified metabolites, lipids and organic acids accounted for high proportions of approximately 10.2% and 8.5% of the total identified compounds, respectively (Fig. 2A). Flavonoids and phenols were beneficial secondary metabolites in plants and accounted for 6.5% of the total detected metabolites, respectively (Fig. 2A). Based on the peak area of the leaf extracts, the relative contents of flavonoids and phenols were also higher than those of other compounds among the five *Lonicera* species (Additional file 1: Table S2). The peak areas of 4,5-dicaffeoylquinic acid and CGA in the leaf extracts of the five *Lonicera species* ranged from 1.47×10^{10} to 2.49×10^{10} and from 1.81×10^{10} to 3.48×10^{10}, respectively, and those of neochlorogenic acid (5-caffeoylquinic acid) and cryptochlorogenic acid (4-caffeoylquinic acid) ranged from 1.89×10^{9} to 1.39×10^{10} and from 3.99×10^{9} to 6.35×10^{9} (Additional file 1: Table S2), respectively. Cynaroside, a flavonoid glycoside compound, had a peak area of 2.31×10^{9}–4.65×10^{9} among the five *Lonicera* extracts (Additional file 1: Table S2). In addition, the peak area of 7-hydroxycoumarine exceeded 10^{10} and that of the organic acids triethyl phosphate and 4-dodecylbenzenesulfonic acid reached 10^{9} (Additional file 1: Table S2).

The biological repetitions of the five *Lonicera species* were clustered together based on the PCA analysis, and significant differences were observed among the five groups. All samples were grouped into distinct clusters, indicating distinct metabolites characteristics of *Lonicera species* (Fig. 2C). PC1 and PC2 explained 21.46% and 18.33% of the total variance in the samples, respectively (Fig. 2C). For JYH, YY, and HN, a similar tendency, without overlap, was observed in the PCA plot (Fig. 2C); however, significant differences were noted in the OPLS-DA score plot (Additional file 1: Figure S1A). The differences were analyzed to evaluate the metabolite contents of YY and HN based on the OPLS-DA model (R^2 $X=0.609$, R^2 $Y=0.999$, $Q^2=0.97$) (Additional file 1: Figures S1B, S2A), and a similar comparison was conducted between YY and HX (R^2 $X=0.668$, R^2 $Y=0.995$, $Q^2=0.964$) (Additional file 1: Figures S1C, S2B), YY and JYH (R^2 $X=0.656$, R^2 $Y=0.997$, $Q^2=0.954$) (Additional file 1: Figures S1D, S2C), and YY and LY, R^2 $X=0.627$, R^2 $Y=0.998$, $Q^2=0.956$) (Additional file 1: Figures S1E, S2D). The $Q2$ value for all comparisons was more than 0.9 in the group pairs, indicating that these models are stable (Additional file 1: Figure S2). The OPLS-DA score was well-separated in pairs among the five species, suggesting significant differences in the metabolites of the five species (Additional file 1: Figure S1). Together, the classification and preliminary metabolomic analysis of metabolites showed marked differences among five *Lonicera* species.

Screening, functional annotation, and enrichment analysis of differential metabolites among the five species

Based on a fold change > 2 or < 0.5 and variable importance for the projection (VIP) > 1, 53 differential metabolites (DMs) were identified between YY and HN (27 upregulated and 26 downregulated) (Additional file 1: Table S4; Figure S3A), 41 DMs between YY and HX (30 upregulated and 11 downregulated) (Additional file 1: Table S5; Figure S3B), and 47 DMs between YY and JYH (34 upregulated and 13 downregulated) (Additional file 1: Table S6; Figure S3C), and 47 DMs between YY and LY (25 upregulated and 22 downregulated) (Additional file 1: Table S7; Figure S3D). There were 110 DMs in YY compared to those in other *Lonicera species*, which can be classified into 15 categories (Additional file 1: Table S3). The DMs in the four comparison groups (YY and HN/HX/JYH/LY) were classified into 14, 8, 12, and 12 categories, respectively (Additional file 1: Tables S4–S7). More than half of these DMs were secondary metabolites, of which flavonoids, lipids, phenols, organic acids, amino acids and derivatives, and terpenoids accounted for 20%, 10%, 10%, 9.1%, 9.1%, and 4.5%, respectively. The upregulated DMs in HN compared to those in YY plants included seven flavonoids, three organic acids, two terpenoids, and two coumarins and flavanone eriodictyol (upregulated by 50.9-fold) (Additional file 1: Table S4). The DMs upregulated in YY compared to those in HN included seven flavonoids, six lipids, and four amino acids and derivatives. In the HX plants, twelve flavonoids, four coumarins, four phenolic acids, and three amino acids and derivatives were upregulated compared to those in YY plants; in addition, flavonol kaempferol-3-*O*-(6‴-trans-*P*-coumaroyl-2″-glucosyl) rhamnoside and tangeritin were also upregulated by 2695- and 865-fold, respectively (Additional file 1: Table S5). In YY leaf extract, four flavonoids and four organic acids were upregulated compared to those in HX leaf extract (Additional file 1: Table S5). In JYH leaf extract, four terpenoids, four phenols, four lipids, and three flavonoids were upregulated and xanthene rhodamine 6G was upregulated by 538.8-fold (Additional file 1: Table S6). Compared to those in YY leaf extract, whereas four amino acids and derivatives and five flavonoids (Additional file 1: Table S6) were upregulated in YY leaf extract

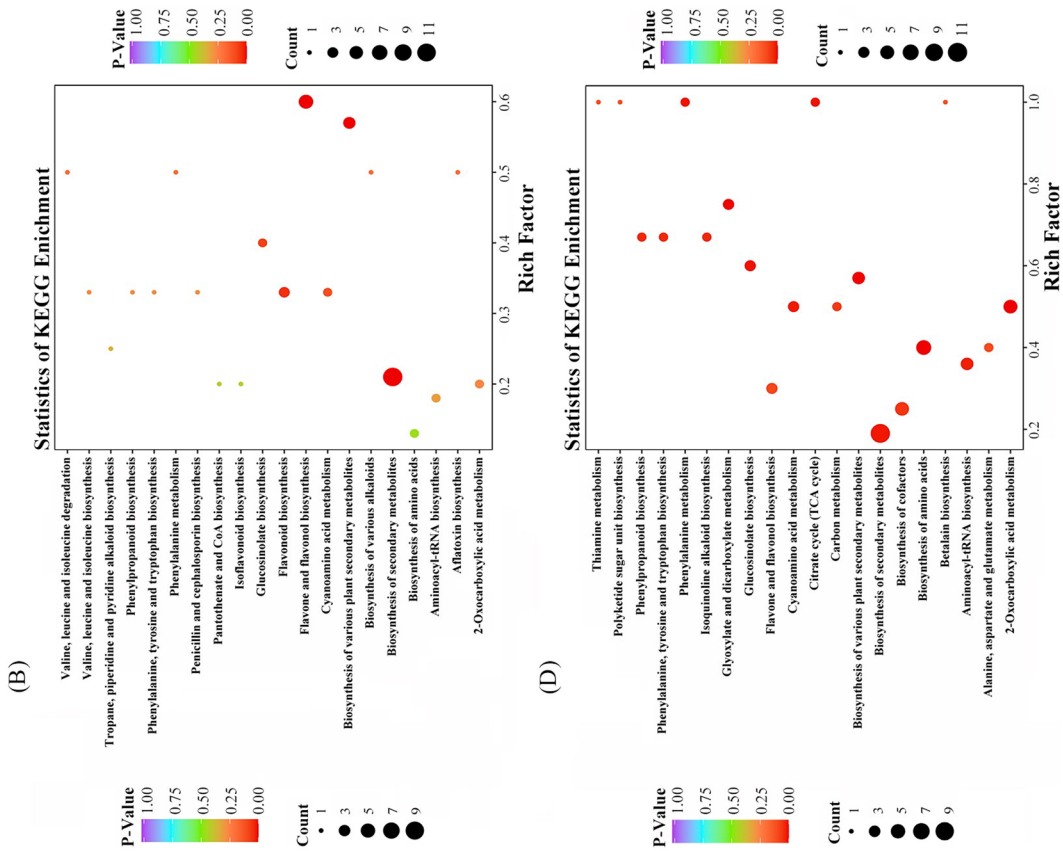


Fig. 3 KEGG annotations and enrichment of differential metabolites in five *Lonicera* species. **A** YY vs. HN. **B** YY vs. HX. **C** YY vs. JYH. **D** YY vs. LY

compared to those in JYH leaf extract. Moreover, four phenols, three lipids, two terpenoids, and flavonoids were upregulated, and sesquiterpene (−)-caryophyllene oxide was upregulated by 24.3-fold in LY leaf extract compared to those in YY leaf extract (Additional file 1: Table S7), whereas seven amino acids and derivatives, five flavonoids, three organic acids, and three phenols where upregulated in YY leaf extracts compared to those in LY leaf extract (Additional file 1: Table S7). At the intersection of each control group in the Venn diagram, only four metabolites were common to each control group (YY and HN/HX/JYH/LY) (Fig. 2D). Therefore, the DMs among YY, HN, HX, JYH, and LY were considerably different.

KEGG annotation and enrichment analyses were conducted for differentially expressed metabolites in each comparison group. These metabolites were involved in 32 metabolic pathways in YY vs. HN (Additional file 1: Table S8), 24 pathways in YY vs. HX (Additional file 1: Table S9), 36 pathways in YY vs. JYH (Additional file 1: Table S10), and 40 pathways in YY vs. LY (Additional file 1: Table S11). The primary pathways were represented using bubble charts (Fig. 3). Metabolic pathways and biosynthesis of secondary metabolites were significantly enriched in each comparison group (YY and HN/HX/JYH/LY) ($P<0.05$). Flavone and flavanol biosynthesis were significantly enriched in YY and HN, YY and HX ($P<0.05$), whereas flavonoid biosynthesis was significantly enriched in YY and HX ($P<0.05$) (Fig. 3A, B). In the enrichment analysis comparing YY and JYH or YY and LY, the biosynthesis of amino acids and 2-oxocarboxylic acid metabolism was significantly enriched ($P<0.05$) (Fig. 3C, D). Besides, cyanoamino acid metabolism was significantly enriched between YY and JYH ($P<0.05$) (Fig. 3C). The biosynthesis of cofactors was significantly enriched between YY and LY ($P<0.05$) (Fig. 3D). The flavonoid contents in HN and HX was significantly higher than that in YY plants, and the content of organic acids and amino acids was related to the DMs between YY and JYH and between YY and LY ($P<0.05$) (Fig. 3).

Correlation analysis

We performed a correlation analysis between the classified DMs with the antioxidant and antibacterial activities of the extracts against ten pathogenic strains. The antioxidant capacity (DPPH, ABTS, and RPA) was significantly positively correlated with 5 flavonoids [zapotin, tangeritin, tiliroside, kaempferol-3-*O*-(6‴-trans-*P*-coumaroyl-2″-glucosyl) rhamnoside, astilbin], 3 coumarins (aflatoxin G2, esculin, and esculetin), 2 phenolic acids (scopoletin, 4,5-dicaffeoylquinic acid), ($R>0.7$, $P<0.001$) (Fig. 4; Additional file 1: Table S12). Kaempferol-3-*O*-β-glucopyranosyl-7-*O*-α-rhamnopyranoside, 3-methoxy-5,7,3′,4′-tetrahydroxy-flavone and hispidulin were significantly positively correlated with ABTS and RPA ($R>0.7$, $P<0.001$) (Fig. 4; Additional file 1: Table S12). Perillartine ($R=0.667$, $P<0.001$) and 3-*o*-feruloylquinic acid ($R=0.611$, $P<0.01$) were significantly positively correlated with RPA. Hispidulin ($R=0.689$, $P<0.001$), 3-Methoxy-5,7,3′,4′-tetrahydroxy-flavone ($R=0.697$, $P<0.001$), Kaempferol-3-*O*-β-glucopyranosyl-7-*O*-α-rhamnopyranoside ($R=0.698$, $P<0.001$) and 3-*o*-feruloylquinic acid ($R=0.602$, $P<0.01$) were significantly positively correlated with DPPH. D-(+)-tryptophan ($R=0.654$, $P<0.001$), 3-o-feruloylquinic acid ($R=0.744$, $P<0.001$) and trans-3-indoleacrylic acid ($R=0.648$, $P<0.001$) were significantly and positively correlated with ABTS. However, a significant negative correlation was observed between DPPH/ABTS/RPA and the content of a tannin [1-*O*-(3,4,5-Trimethoxybenzol)-beta-L-galactopyranose] ($R<-0.75$, $P<0.001$) (Fig. 4; Additional file 1: Table S12). Asparagine ($R=-0.648$, $P<0.001$) was also significantly negatively correlated with DPPH levels. Quercetin and rutin were also significantly negatively correlated with ABTS and RPA levels ($R<-0.6$, $P<0.001$) (Fig. 4; Additional file 1: Table S12).

Correlation analysis of DMs with the antibacterial activity revealed that six flavonoids [zapotin, kaempferol-3-*O*-(6‴-trans-*P*-coumaroyl-2′-glucosyl) rhamnoside, tiliroside, 3-methoxy-5,7,3′,4′-tetrahydroxy-flavone, tangeritin, astilbin, and hispidulin], 2 phenolic acid (scopoletin, 4,5-Dicaffeoylquinic acid), and coumarin (aflatoxin G2) were significantly positively correlated with antibacterial activities against *E. oryzae* ($R>0.6$, $P<0.01$) (Fig. 4; Additional file 1: Table S12). In addition, the enhancement of luteolin content was significantly positively correlated with antibacterial activities against *E. coli* ($R=0.653$, $P<0.001$) and *S. aureus* ($R=0.602$, $P<0.01$). Three flavonoids [genistin ($R=0.679$, $P<0.001$), tangeritin ($R=0.625$, $P<0.01$), and luteolin ($R=0.653$, $P<0.001$)], and esculin ($R=0.638$, $P<0.01$) showed a significant positive correlation with the antibacterial activities against *E. coli*. In addition, two phenols [phloroglucinol ($R=0.623$, $P<0.01$), 3,4-dihydroxybenzaldehyde ($R=0.666$, $P<0.001$)], two terpenoids {(−)-caryophyllene oxide ($R=0.691$, $P<0.001$), methyl 1-(hexopyranosyloxy)-7-hydroxy-7-(hydroxymethyl)-1,4a,7,7a-tetrahydrocyclopenta[c]pyran-4-carboxylate ($R=0.605$, $P<0.01$)} were significantly positively correlated with the inhibition of *V. parahaemolyticus*. However, two flavonoids (hyperoside, eriodictyol), a terpenoid (oleanolic acid), and α-propylaminopentiophenone were significantly negatively correlated with the inhibition of *P. aeruginosa* and *S. agalactiae* ($R<-0.6$, $P<0.01$). Besides, l-glutamine ($R=-0.654$, $P<0.001$) and methyl cinnamate ($R=-0.6$, $P<0.01$) levels were significantly negatively

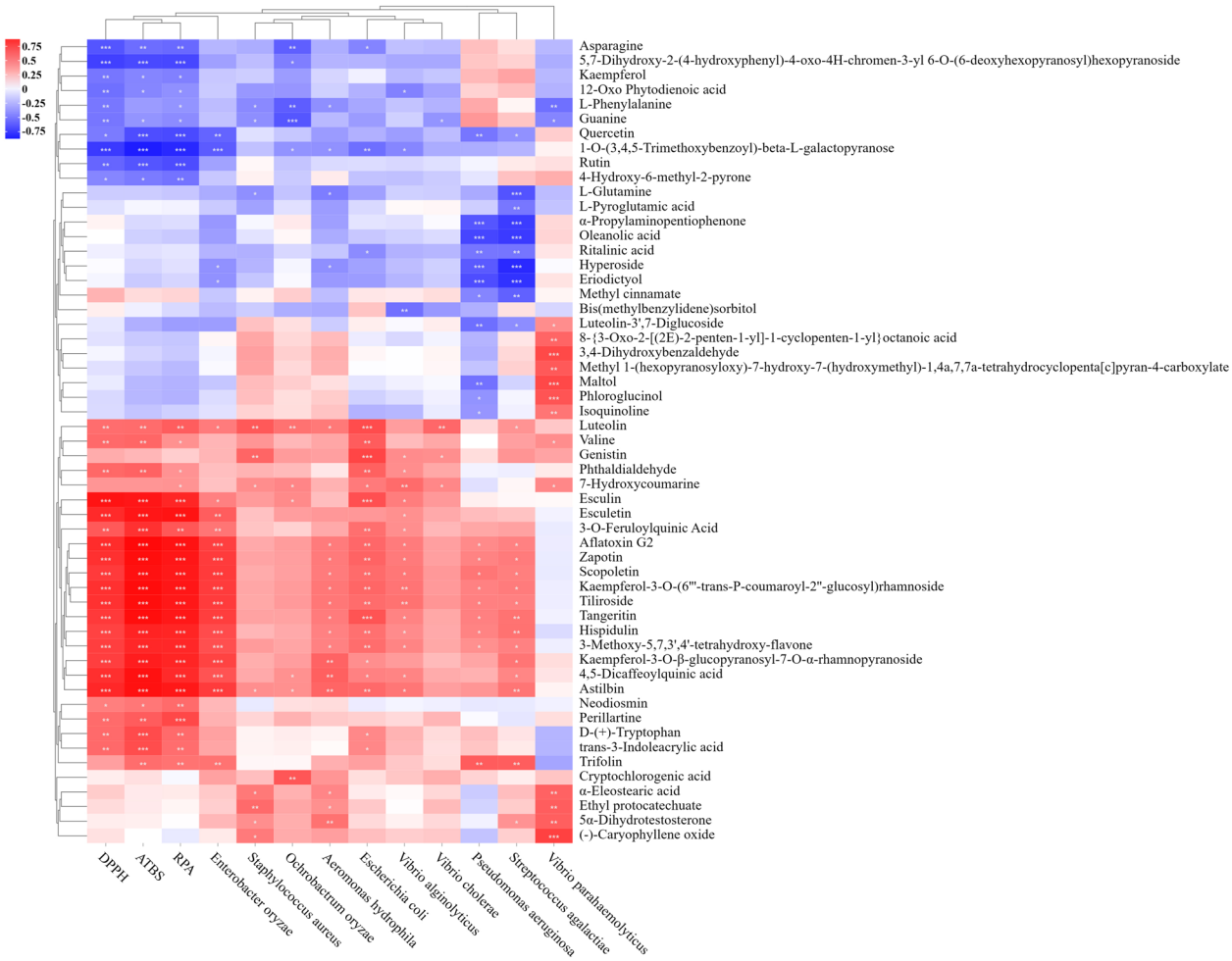


Fig. 4 Correlation analysis between the DMs and the DPPH, ABTS, and RPA values, and the antibacterial activity against ten pathogenic bacteria. The right panel shows the names of the classified DMs, and the bottom indicates DPPH, ABTS, RPA values and the antibacterial activity against ten pathogenic bacteria. Each grid represents the correlation between the two attributes, and the different colors represent the sizes of correlation coefficients between the attributes. The correlation coefficient was calculated by Pearson correlation (*t* test), with * indicating $P < 0.05$, ** indicating $P < 0.01$, and *** indicating $P < 0.001$

correlated with the antibacterial activity against *S. agalactiae*. Asparagine ($R = -0.607$, $P < 0.01$), l-phenylalanine ($R = -0.617$, $P < 0.01$), and guanine ($R = -0.643$, $P < 0.01$) were significantly negatively associated with the antibacterial activity against *Ochrobactrum oryzae* (Fig. 4; Additional file 1: Table S12).

Analysis of metabolic profiles and key metabolites contents

Twelve important phenolic acid and flavonoid metabolites among the five species were quantified using UHPLC-Q Exactive. The peak areas of seven DMs (4, 5-dicaffeoylquinic acid, luteolin, hyperoside, quercetin, hispidulin, neodiosmin, and scopoletin) and the other five metabolites were similar to those determined using UHPLC-Q Exactive (Fig. 5). The content of CGA ranged from 7.09 mg g^{-1} to 15 mg g^{-1} among the five species, whereas that of 4, 5-dicaffeoylquinic acid was higher than 1 mg g^{-1}, especially in HX species (4.81 mg g^{-1}) (Fig. 5). The total content of the three CGAs (chlorogenic acid; neochlorogenic acid; 4, 5-dicaffeoylquinic acid) was higher than 21.12 mg g^{-1} in HX compared to that in the other four species. Scopoletin content was highest (36.47 μg g^{-1}) in HX and lowest (0.09 μg g^{-1}) in LY. The highest quercetin and hyperoside contents were 61.71 μg g^{-1} in JYH and 16.93 μg g^{-1} in HN, respectively (Fig. 5).

Biosynthetic pathways of key metabolites

According to the heatmap of metabolic pathways, 10 metabolites were detected in the synthetic pathway of

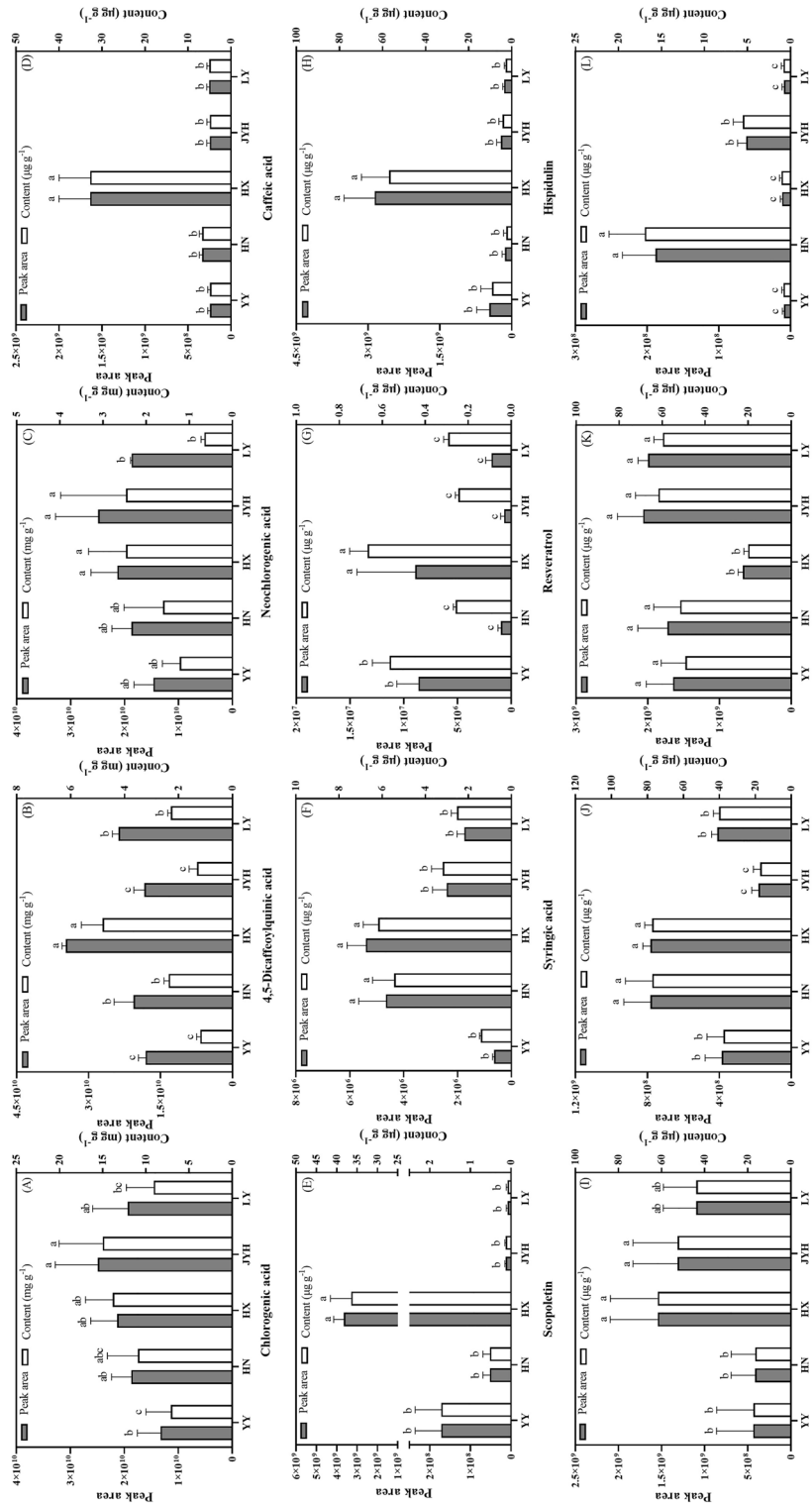


Fig. 5 Relationship between the peak area of some metabolites and their contents in five *Lonicera* species. **A**–**G** Chlorogenic acids and important phenolic acids. **H**–**L** Important flavonoids differential metabolites. The results were showed by means ± SD. For multiple comparisons, One-way ANOVA-test was used followed by post hoc test and Waller–Duncan method. Different lowercase letters indicate significant differences at $P < 0.05$

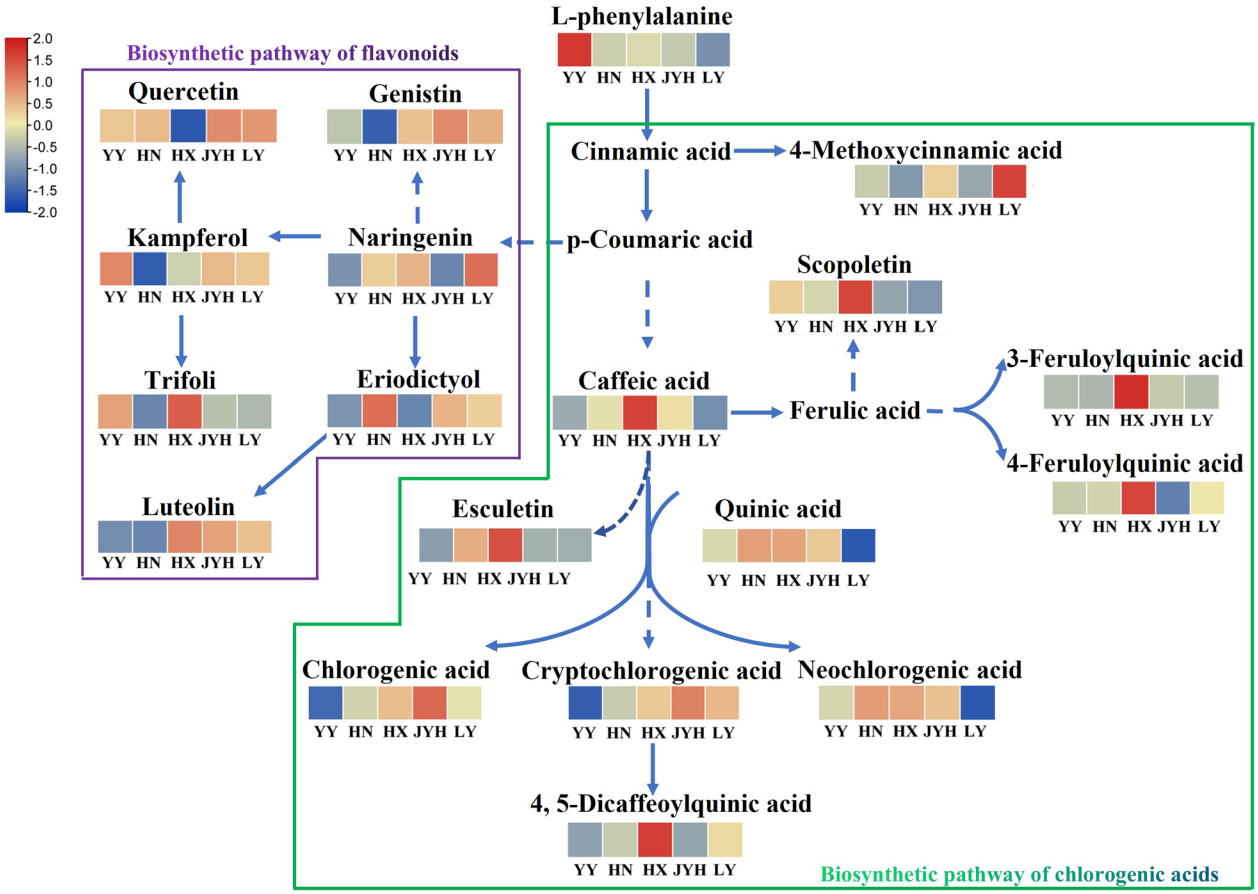


Fig. 6 Biosynthetic pathway of chlorogenic acids and flavonoids (in purple line frame), and heatmap of their relative abundance in five *Lonicera* species leaves

CGAs, of which six were significantly ($P<0.05$) upregulated in HX compared to those in the other four *Lonicera species*, including 4,5-dicaffeoylquinic acid, caffeic acid, 3-feruloylquinic acid, 4-feruloylquinic acid, and 2 phenolic acids, scopoletin and esculetin (Fig. 6). Neochlorogenic acid and quinic acid contents were higher in both HX and HN than in the other species. The highest contents of CGA and cryptochlorogenic acid were observed in JYH. Seven metabolites were detected in the flavonoid metabolic pathway, among which the contents of trifolin and luteolin in HX were higher than those in the other four species. Eriodictyol was the most abundant compound in HN. In addition, the naringenin and quercetin contents in LY were higher than those in the other *Lonicera species* (Fig. 6).

Discussion

Metabolic profiling-based methods are commonly used to identify metabolites during the development of *Lonicera* flowers, petal color, and buds [26, 27]. In the present study, we identified a series of valuable secondary metabolites, such as flavonoids, phenols, terpenoids, and alkaloids, in *Lonicera* leaves. CGA is the most abundant phenolic acid in *Lonicera* species and has been used as an indicator of the chemical quality of JYH [15, 28, 29]. Recently, 27 CGAs, including chlorogenic acid, neochlorogenic acid, isochlorogenic acid A, isochlorogenic acid B, and isochlorogenic acid C, have been isolated and identified from *Lonicera* species [15, 30–32]. In the present study, chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, and 4,5-dicaffeoylquinic acid were identified in *Lonicera* leaves. The peak area of CGAs was highest in HX (6.30×10^{10}), followed by JYH (5.06×10^{10}), LY (4.94×10^{10}), HN (4.43×10^{10}), and YY (3.62×10^{10}) (Additional file 1: Table S2). High CGA and luteoloside contents are present in HX buds, with a chemical fingerprint similar to that of JYH [16]. Therefore, HX flower buds are a potential alternative to JYH buds in traditional medicine. However, the leaves contain higher levels of hydroxycinnamic acids than the flower buds and stems [33], warranting further investigation of the medicinal value of *Lonicera* leaves.

Previous studies have shown that the differences in the chemical fingerprint of *Lonicera* are mainly due to distinctions in the contents of organic acids and flavonoids [16]. In the present study, a paired analysis of DMs revealed that the most upregulated DMs in HX compared to those in YY were flavonoids. Moreover, there were differences in the flavonoid content between HN and YY plants. Analysis of the KEGG metabolic pathway revealed that flavone and flavanol biosynthesis pathways were significantly ($P < 0.05$) enriched in YY vs. HX and YY vs. HN. Our results suggest that the high antioxidant and antibacterial abilities of HX are related to its high flavonoid content. HN shows a stronger antibacterial and antioxidant capacity than YY owing to the enriched flavonoid metabolism pathway. For YY vs. JYH and YY vs. LY, phenols and amino acids accounted for most DMs. Compared with YY, phenols were up-regulated in JYH and LY, while amino acids were down-regulated. Biosynthesis of amino acid and the 2-oxocarboxylic acid pathways were significantly ($P < 0.05$) enriched between YY and JYH and between YY and LY. Furthermore, cyanoamino acid metabolism was significantly ($P < 0.05$) enriched between YY and LY. These results indicate that the content of phenolic compounds may contribute to the higher biochemical indexes of JYH and LY than those of YY and that amino acids make little contribution to the antioxidant and antibacterial abilities of *Lonicera*.

In a previous study, antioxidant assays demonstrated that all five *Lonicera* flowers exhibit a strong antioxidant capacity in the following order: *Lonicera macranthoides* > *Lonicera fulvotomentosa* > *L. japonica* Thunb. > *L. hypoglauca* Miq. > *Lonicera confuse* [34]. The antioxidant properties of *Lonicera japonica* are closely related to polyphenols, including phenolic acids and flavonoids [35, 36]. In addition, 5-*O*-caffeoylquinic acid, 4-*O*-caffeoylshikimic acid, and methyl-5-*O*-caffeoylquinate also contribute to the antioxidant activity [34]. Moreover, JYH can extensively inhibit gram-negative and gram-positive bacteria, including *Bacteroides fragilis*, *Bacteroides ovatus*, *Propionibacterium acnes*, *S. aureus*, *Shigella*, *Salmonella*, *and E. coli* [9, 37–39]. Consistent with these results, in our study, the extracts of the leaf of five species of *Lonicera* exhibited strong antibacterial properties against *E. coli*, *S. aureus*, *P. aeruginosa*, two rice pathogenic bacteria (*E. oryzae* and *Ochrobactrum oryzae*), and five zoonotic pathogenic bacteria (*V. alginolyticus*, *V. cholerae*, *A. hydrophila*, and *S. agalactiae*). In addition, similar DMs among the species were significantly ($P < 0.05$) correlated with both antioxidant capacity and antibacterial activities against *E. coli* and *E. oryzae*. Among these DMs, most flavonoids, phenols, and coumarins were positively correlated with antioxidant and antibacterial activities, whereas some amino acids, flavonoids, lipids, and organic acids showed negative correlations. These results reveal that in the leaves of *Lonicera*, plant secondary metabolites, especially flavonoids and polyphenols, contribute to antioxidant and antibacterial activities.

CGAs flavonoids is a series of phenylpropanoids produced by shikimic acid pathway during aerobic respiration [40]. The present study indicated that in the leaves, the dominant components of flavonoids and CGAs highly varied among the five *Lonicera* species. The contents of CGA, neochlorogenic acid, and 4, 5-dicaffeoylquinic acid ranged between 7.09–15 mg g^{-1}, 0.65–2.47 mg g^{-1}, and 1.19–4.81 mg g^{-1} in the extracts of leaf, respectively. These variations are likely related to gene regulation involved in the metabolic pathway of CGAs, such as *C3'H*, *HCT*, and *H3K9/H3K4* [31, 41]. This observation is also supported by the higher contents of CGA and neochlorogenic acid in *L. japonicae* Flos than in *L japonicae* caulis [42]. Similarly, the contents of flavonoids vary with the different genera in the Caprifoliaceae family. In our study, the content of luteolin was 61.82 μg g^{-1} in HX and that of quercetin was 61.71 μg g^{-1} in LY. The antioxidant and antibacterial activities also showed significant differences among the *Lonicera* species, with *L. hypoglauca* Miq. exhibiting the most prominent antibacterial capacity against pathogens, which was consistent with high contents of neochlorogenic acid, 4,5-dicaffeoylquinic acid and scopoletin in its leaves. Therefore, it is necessary to combine more transcriptional analyses to examine the variation in polyphenol compounds in different *Lonicera* species.

Conclusion

This study combined metabolomic and biochemical analyses to investigate the antioxidant and antibacterial abilities of five different *Lonicera* species in vitro. This study is the first report on the metabolomics of leaves of different *Lonicera* species. We revealed that the TP and TF contents and antioxidant and antibacterial abilities of HX plants were significantly higher than those of the other four *Lonicera* species. We identified a total of 598 metabolites in whole leaves and revealed that the metabolites considerably varied among the five species; the most significant difference in flavonoids was found between HX and YY. Besides, these DMs affected the antioxidant and antibacterial activities of the five *Lonicera* species, with flavonoid and phenolic compounds being the main contributors to the differential biochemical activities of these plants. In summary, we confirmed that flavonoids and phenolic compounds make a major contribution to the antioxidant and antibacterial

properties of *Lonicera* leaf extracts. Moreover, we showed that *L. hypoglauca* Miq. (HX), a unique species in Guangxi, has great potential value owing to its high 4, 5-dicaffeoylquinic acid/scopoletin content and outstanding biochemical activity. Multiomics analysis of metabolic pathway of *L. hypoglauca* Miq. should be considered in the future.

Abbreviations

CGAs	Chlorogenic acids
TP	Total phenol
TF	Total flavonoid
YY	*Lonicera dasystyla* Rehd.
HN	*Lonicera confusa* (Sweet) DC.
HX	*Lonicera hypoglauca* Miq.
JYH	*Lonicera japonica* Thunb.
LY	*Lonicera dasystyla* Via.
ACN	Acetonitrile
DW	Dried weight
RPA	Reducing power assay
DMs	Differential metabolites
DPPH	1, 1-Diphenyl-2-picrylhydrazyl
ABTS	Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)
OD	Optical density
KEGG	Kyoto Encyclopedia of Genes and Genomes
OPLS-DA	Orthogonal partial least squares-discriminant analysis
HCA	Hierarchical cluster analysis
PCA	Principal component analysis
VIP	Variable importance for the projection

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00460-1.

Additional file 1. Table S1. Difference of leaf morphology and appearance among five *Lonicera* species. **Table S2.** Compounds of five *Lonicera* species by LC-MS/MS. **Table S3.** Differential metabolites between YY and other four *Lonicera* species (HN/HX/JYH/LY). **Table S4.** Differential metabolites between YY and. **Table S5.** Differential metabolites between YY and HX. **Table S6.** Differential metabolites between YY and JYH. **Table S7.** Differential metabolites between YY and LY. **Table S8.** Metabolic pathways in the comparison groups (YY vs HN). **Table S9.** Metabolic pathways in the comparison groups (YY vs HX). **Table S10.** Metabolic pathways in the comparison groups (YY vs JYH). **Table S11.** Metabolic pathways in the comparison groups (YY vs LY). **Table S12.** Correlation analysis between the DMs and the DPPH, ABTS, and RPA values, and the antibacterial activity against ten pathogenic bacteria. **Table S13.** Analysis of variance of identified differential metabolites. **Figure S1.** OPLS-DA analysis among five *Lonicera* species. **Figure S2.** The OPLS-DA analysis permutation among five *Lonicera* species. **Figure S3.** The volcano plots of five *Lonicera* species.

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Author contributions
YHF: Methodology, investigation, writing—original draft. GDZ: antioxidant and antibacterial investigation. PCZ: methodology, comparative metabolites. WHZ: partial investigation. YZL: methodology. Writing—review and editing. XWF: conceptualization, methodology, writing—review and editing, supervision, project administration.

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Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The research can be published with the consent of all authors.

Competing interests
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Author details
[1]State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Life Science and Technology, Guangxi University, 100 Daxue Road, Nanning 530004, Guangxi, China.

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