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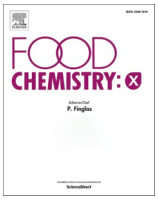

Chemical profile and quantitative comparison of constituents in different medicinal parts of *Lactuca indica* during varied harvest periods using UPLC-MS/MS method

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ABSTRACT

Lactuca indica L. cv. Mengzao (LIM), acknowledged as a pivotal "One Root of Medicine and Food", boasts dual applications in both culinary and medicinal domains. This research delves into the influence of various harvest periods (vegetative, budding, blossom, and fruiting) on distinct medicinal parts (roots, stems, leaves, flowers, and seeds) of LIM, employing plant metabolomics to assess its chemical constituents. A total of 66 chemical constituents were identified in LIM, with 11 chemical components emerging as potential markers for distinguish medicinal parts. Notably, nutritional organs exhibited elevated levels of cichoric acid, rutin and chlorogenic acid. Specifically, leaves during the budding stage displayed the highest chicoric acid content at 11.70 mg·g^{-1}. Conversely, reproductive organs showed heightened concentrations of cichoric acid, rutin and chlorogenic acid, with seeds exhibiting the peak cichoric acid content at 4.53 mg g^{-1}. This study enriches our understanding of LIM by offering novel insights into quality assessment and the comprehensive utilization of its diverse parts.

1. Introduction

In recent years, the burgeoning interest in plant secondary metabolites has been fueled by their recognized health functions and valuable nutritional benefits (Ceh et al., 2020; Deseo et al., 2020; Hao et al., 2021; Lee et al., 2023; Sahu, 2021; Muhammad et al.,2018). These secondary metabolites, encompassing phenols, terpenes, anthocyanins and alkaloid, are prevalent in a wide array of crops (Ha et al., 2021; Muhammad et al., 2022; Subhani et al., 2020), fruits (Celli et al., 2011), vegetables (Del Baño et al., 2003), and food items (Dai et al., 2015). Beyond their nutritional significance, these metabolites play a pivotal role in safeguarding plants against insects, microorganisms, and herbivores, concurrently serving as crucial sources of nutrients and medicines for humans (Thakur et al., 2019; Muhammad et al., 2018). Notably, the composition of metabolites varies substantially across different growth periods and medicinal parts of the same plant. Therefore, a comprehensive analysis and identification of diverse metabolites in distinct growth periods or medicinal parts not only enhances our understanding of metabolite production of metabolites in these parts but also unveils specific metabolites with health-promoting functions unique to particular medicinal parts (Li et al., 2022).

Lactuca indica L. holds a dual distinction as both a significant food source and a medicinal plant, contributing positively to human health. Renowned for its antioxidant properties, it is known to potentially reduce serum total cholesterol levels, while exhibiting hepatoprotective and anti-mutagenic properties (Kim et al., 2012; Lüthje et al., 2011).

Abbreviations: UPLC-QE-MS/MS, ultra-high performance liquid chromatography coupled with quadrupole tandem Q-Exactive mass spectrometry; UPLC-QqQ-MS, ultra-high performance liquid chromatography coupled with a triple quadrupole mass spectrometer; LOQ, Limit of quantification; LOD, Limit of detection; HCA, hierarchical cluster analysis; OPLS-DA, orthogonal partial least-squares discriminat analysis; VIP, variable importance in projection; EIC, extraction ion chromatography; EI, electron impact; TIC, total ion chromatograms; MRM, multiple reaction monitoring.

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Furthermore, its delicate leaves and rich nutritional profile have made it a popular kitchen herb, frequently incorporated into salads, sushi, and soups (Oliya et al., 2018; Richard et al., 1991). The pharmacological of *L. indica* are intricately linked to its bioactive compounds, such as flavonoids, triterpenes, and quinic acid derivatives) (Hao et al., 2023; Kim et al., 2007; Li et al., 2020). Notably, flavonoids play a pivotal role in preventing chronic human diseases, owing to their anticancer, antioxidant, antibacterial, and enzyme inhibition properties (Hao et al., 2023; Gawlik-Dziki et al., 2016; Goesaert et al., 2005). Despite the plethora of pharmacological effects associated with these bioactive compounds, their varied mechanisms of action may be attributed to the intricate diversity of active ingredients found in *L. indica*.

The procurement of medicinal plant materials, including those *L. indica*, primarily relies on wild harvesting, leading to a concerning depletion and fragmentation of wild populations due to escalating demand and land conversion. The escalating pressure on wild plant populations necessitates a shift toward domestication of medicinal plants as a sustainable solution to mitigate overexploitation. Presently, the varieties of *L. indica* cultivated in China are Mengzao, Longmu, Gongnong, Chuanxuan 1., SA96, and Chuanxu 1., distributed across regions such as Heilongjiang, Sichuan, and Inner Mongolia, with an annual production exceeding 5000 kg/667 m^2(Ban et al., 2016). In a perior study, we introduced on a cultivated variant of *Lactuca indica* L. cv. Mengzao (LIM) (Zhang et al., 1986) and explored its optimal extraction processes and antioxidant activity of its active components (Rong, 2020). However, there remains a dearth of research on the chemical components of different parts of LIM. Given the abundant active ingredients in LIM beneficial to human health, a more comprehensive investigation is warranted to unlock its clinical applications and potential benefits.

Although some studies have highlighted the excellent functional properties of flavonoids in LIM (Hao et al., 2021; Rong, 2020), a comprehensive and detailed analysis of the active component profiles, particularly in different medicinal parts, is lacking. Therefore, a large-scale endeavor to identify, quantify, and comparatively analyze the chemical components of LIM holds the potential not only to discover new components with health-promoting functions, but also to facilitate the development of nove functional products. Moreover, the timing for harvest traditional Chinese medicines is an critical factor influencing plant quality, as different harvest periods may significantly impact compounds contents in plants (Fan et al., 2022; Huang et al., 2014; Luo et al., 2017). To the best of our knowledge, the variation in the quality of LIM based on the harvest period remains to be completely elucidated.

In this study, a comprehensive qualitative and quantitative analysis of five distinct medicinal parts of LIM was conducted using ultra-high performance liquid chromatography coupled with quadrupole tandem Q Excative mass spectrometry (UPLC-QE-MS/MS) and ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry (UPLC-QqQ-MS/MS). The global characterization of flavonoids, organic acids, amino acids, fatty acids and other components was initially carried out using UPLC-QE-MS/MS. Subsequently, multivariate statistical methods were employed to analyze the chemical distinctions among samples from different parts of LIM. Furthermover, a total of 11 effective ingredients served as evaluation indicators to assess the effects of differences in medicinal parts and harvest periods on the quality of LIM. The findings of this study offer a theoretical foundation and valuable reference for the research and development of new functional foods, the formulation of quality standards for LIM, and the optimization of its harvest period.

2. Material and methods

2.1. Plant materials and chemicals

The LIM materials were sourced from the medicinal plant's planting base of Inner Mongolia Agricultural University. Initial observations of the growth period of LIM populations in the early stages indicated an approximate duration of 10 days from the vegetative period to the fruiting stage. Consequently, the sampling interval time for each growth period was set at 10 days. A total of 18 batches of roots, stems, leaves, flowers, seeds, and whole plants of LIM were systematically collected at distinct harvest periods. Detailed information on each sample is listed in Table S1, and visual representations of roots, stems, leaves, flowers, seeds, and whole plants of LIM are presented in Fig. 1.

Methanol, acetonitrile, and formic acid (HPLC grade) were all procured from Thermo Fisher Scientific (San Jose, CA, USA). Chemical standards, comprising 4,5-dicaffeoylquinic acid (lot B21541, purity > 98 %), β-sitosterol (lot B21972, purity > 98 %), quercetin (lot B20527, purity > 98 %), chlorogenic acid (lot B20782, purity > 98 %), apigenin (lot B20981, purity > 98 %), oleanolic acid (lot B72739, purity > 98 %), kaempferol (lot B20888, purity > 98 %), cichoric acid (lot B20647, purity > 98 %), rutin (lot B20771, purity > 98 %), cynaroside (lot B20887, purity > 98 %), luteolin (lot B20888, purity > 98 %), and 2-chloro-L-phenylalanine (lot B25643, purity > 98 %), were all obtained from Yuanye Biotechnology Co. Ltd. (Shanghai, China).

2.2. Sample preparation

A mixed standard stock solution comprising reference compounds 1–11 was meticulously prepared in 90 % methanol, with individual analyte concentrations set as follows: 95.1, 162.3, 104.4, 98.7, 98.5, 108.8, 98.2, 114.4, 116.1, 101.4, and 95.9 $\mu g \cdot g^{-1}$. Calibration curves were established by crafting working standard solutions, achieved through dilution of the mixed standard stock solution with 80 % methanol to various concentrations.

The samples, each weighing 1.0 g, underwent ultrasonically extracted (400 W) for 30 min with 25 mL of 60 % ethanol. To the whole plant sample, 2-Chloro-L-phenylalanine of 1 μg/mL was added. Subsequently, the mixture was centrifuged at 3000 rpm for 10 min and filtered through a 0.22 μm membrane filter.

2.3. Instrumentation and conditions

2.3.1. UPLC-Q-Exactive-MS/MS analysis

The UPLC-QE-MS/MS system was composed of a Thermo Vanquish UHPLC system coupled with a Q-Exactive HF mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Analysis was performed using a Zorbax C18 column (100 × 2.1 mm, 1.8 μm, Agilent, Palo Alto, CA, USA) with a 20-μL aliquot of each sample solution injected.

The mobile phase comprised 0.1 % formic acid in distilled water (A) and acetonitrile (B), employing a gradient elution program as follows: 95 % A at 0–2 min, 95–70 % A at 2–6 min, 70 % A at 6–7 min, 70–5 % A at 7–12 min, 5 % A at 12–13 min, 5–95 % A at 13–16 min, and 17 min, 95 % A. The flow rate was set at 0.3 mL/min, with sample injection volume of 5 μL. For MS analysis, electrospray ionization (ESI) and high-energy collisional dissociation (HCD) were employed in both positive and negative ion modes. The optimized conditions were as follows: heater temperature, 325 °C; sheath gas pressure, 45 Arb; auxiliary gas pressure, 15 Arb; spray voltage, 3500 V; capillary temperature, 350 °C. The mass spectrum scanning mode involved primary full scan (*m/z* 100–1500) and data-dependent secondary mass spectrometry (dd-MS2, TopN = 10).

2.3.2. UPLC-QqQ-MS analysis

The analysis utilized an UltiMate 3000 system connected to a TSQ Quantum Access Max triple-stage quadropole mass spectrometer (Thermo Fisher Scientific). Chromatographic separation was achieved using a Thermo C18 column (100 × 2.1 mm, 1.8 μm; Thermo Fisher Scientific), and the column temperature was maintained at 25 °C.

The mobile phases consisted of 0.1 % formic acid in distilled water (A) and acetonitrile (B). The gradient elution method employed was as follows: 95 % A at 0–2 min, 95–70 % A at 2–4 min, 70–60 % A at 4–7 min, 60–20 % A at 7–10 min, 20–5 % A at 10–13 min, 5 % A at 13–15

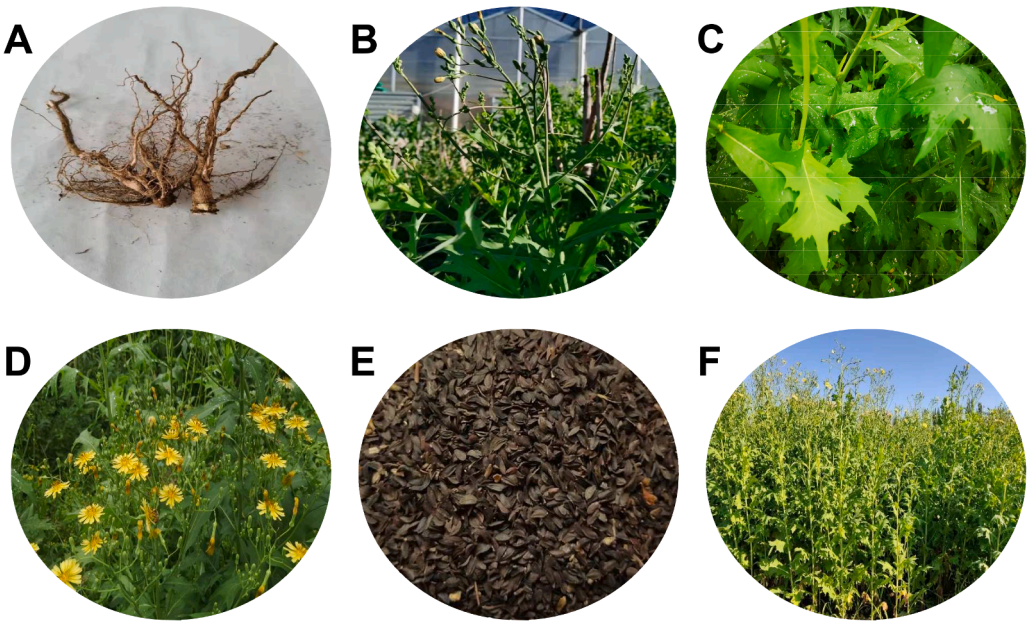

Fig. 1. Photographic representation of roots (A), stems (B), leaves (C), flowers (D), seeds (E), and whole plants (F) of selected *Lactuca indica* L. cv. Mengzao (LIM) samples collected.

min, 5–50 % A at 15–17 min, and 50–95 % A at 17–20 min. The flow rate was set at 0.3 mL/min, with a 5 μL. Mass spectrometry was conducted in both positive and negative ion modes, and the MS spectra were acquired in multiple reaction monitoring (MRM) mode. The instrumental parameters were as follows: vaporizer temperature, 350 °C; capillary temperature, 300 °C; spray voltage, 3000/2500 V; shealth gas pressure at 35 Arb; and aux gas pressure, 10 Arb. Quantitative parameters are detailed in Table S2.

2.3.3. Methods validation

The quality control (QC) sample was prepared by blending extracts from LIM samples, serving to assess the repeatability of samples subjected to the same treatment method. Throughout the instrument analysis, a standard practice involved inserting one QC sample after every 10 test and analysis samples to monitor the repeatability of the analysis process.

For each standard substance, the limit of detection (LOD) and limit of quantification (LOQ) were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. Precision was evaluated by analyzing standard solutions with six replicates, calculating the relative standard deviation (RSD) of the peak area for each standard compound. To affirm repeatability, QC samples were prepared, and the RSD of each component was calculated. To assess stability, sample solutions were injected intervals of 0, 2, 4, 8, and 12 h, and RSD values were calculated. Additionally, a spike recovery test was conducted by supplementing the corresponding standard at low (80 %), medium (100 %), and high (120) levels to the QC samples, followed by measurements in six replicates. Detailed results of the methodology are documented in Table S2.

2.4. Data analysis

The compounds found in various medicinal parts of LIM, including seeds, roots, stems, leaves, flowers, and whole plant, were s compiled based on existing literature. Molecular weights and retention times (Rt) for all identified compounds were cataloged in an Excel file and saved in. csv format. Subsequently, an in-house compound library for LIM was established and employed to efficiently locate compounds of interest within the extensive raw MS^2 data using the "find by formula" function in Compound Discover (CD) software (Thermo Fisher Scientific). Further, a thorough identification process was conducted by cross-referencing the parent ion, molecular formula, secondary mass spectrometry ion, and retention time from the extraction ion chromatography (EIC) with relevant information sourced from the literature, the mzCloud online database, and Thermo mzValut local database.

These processed data were imported into SIMCA 14.1 (Umetrics, Umea, Sweden) for comprehensive multivariate analysis, incorporating hierarchical cluster analysis (HCA), principal components analysis (PCA), and orthogonal partial least-squares discriminant analysis (OPLS-DA), These analyses were instrumental in categorizing the new matrix data, and revealing similarities, and highlighting differences among distinct samples. Employing a supervised pattern recognition method, the samples were partitioned into training and validation sets. The classification model was developed using the training set and applied to predict the validation set. In this study, the validated method was utilized to analyze 10 batches of LIM samples. The statistical properties of the models were assessed using R^2X, R^2Y, and Q^2. Variable importance in projection (VIP) analysis was employed to evaluate the significance of each component and select those with the highest discrimination potential between LIM samples (VIP > 1, $p < 0.05$).

3. Results

3.1. Untargeted metabolomics analysis of LIM in different medicinal parts by UPLC-QE-MS/MS

To investigate the chemical components of different parts of LIM, batches S9, S10, S11, S12, and S17 were specifically chosen for the chemical profiling of roots, stems, leaves, flowers, and seeds, respectively. Representative total ion chromatograms (TICs) for different medicinal parts of LIM in both positive and negative ion modes are shown in Fig. S1. Utilizing authentic compounds, available literature data, and the acquired MS data, a total of 66 chemical components were tentatively identified in roots, stems, leaves, flowers, and seeds. This including 17 shared components, with 31, 45, 53, 49, and 29 compounds in roots, stems, leaves, flowers, and seeds, respectively. The structures of these compounds were categorized into 7 groups, comprising 19 flavonoids, 13 organic acids, 10 carboxylic acids and derivatives, 5 saccharides, 5 coumarins and derivatives, 8 fatty acids, and 6 other types. Detailed information about the retention time, high accurate precursor ions, molecular formula, and characteristic fragment ions of each

compound from different parts in LIM is outlined in Table 1. Method validation results are summarized in Table S3. The data revealed showed that the RSD of all retention times (RTs) and peak area were below 15 %, indicating that the system precision, method repeatability, and sample stability met the stringent requirements of metabolomics research.

To investigate the differences in chemical components among different parts of LIM, chromatographic data from 10 batches (S1 − S3, S5 − S7, S9 − S12) obtained through UPLC-QE-MS/MS in positive and negative ion modes were subjected to Simca-P 14.1. Unsupervised pattern recognition methods, such as HCA and OPLS-DA were employed for groups of samples that have not yet been clearly classified (Cao et al., 2019). The intuitive categorization of samples based on their variable characteristics is facilitated by the dendrogram, visually illustrating the similarities and differences between the tested samples (Gao et al., 2019). In Fig. 2A, the HCA diagrams of 10 batches of LIM reveal a clear division into four categories based on different medicinal parts. Specifically, sample S12 falls into the first category (Flowers), while S11, S3, and S7 constitute the second category (Leaves). The third category (Roots) in represented by S1 and S5, and fourth category (Stems) is comprised of S10, S2 and S6.

In general, the OPLS-DA is effective in filtering random noise, enhancing model validity and analytical capabilities, and facilitating better differences among groups (Lucio-Gutiérrez et al., 2012). To identify key compounds contributing most to the differences among different medicinal parts of LIM, OPLS-DA was conducted, generating a scatter plot depicted in Fig. 2B. The results showed clearly separation among the four different medicinal parts of LIM, indicating the effectiveness of the model. Additionally, 200 iterative permutation tests demonstrated the model's robust predictability and goodness of fit (Fig. 2C). To screen the key chemical components making significant contributions to the distinctions among different medicinal parts of LIM, both HCA and OPLS-DA models were utilized. The S-plot generated from the OPLS-DA model illustrated the ion contribution to group separation, and the variable importance projection (VIP) value were analyzed for four groups of different components. Following one-way ANOVA, compounds with VIP > 1 and $p < 0.05$ were identified as potential key components contributing to the separation. Table S4 and Fig. 2D demonstrate the 11 potential key components, including cichoric acid, apigenin-7-O-glucuronide and luteolin, that played a crucial role in distinguishing different medicinal parts of LIM.

3.2. Quantitative analysis of major active ingredients in LIM

To accurately depict the dynamic changes in the main active components across different parts of LIM, 11 typical active components were selected for a targeted quantitative study. Employing the MRM method on QqQ mass spectrometer, known for its cost-effectiveness and higher selectivity (Xuan et al., 2018; Zhang et al., 2020), a new sensitive UPLC-MS/MS method was established for the simultaneous determination of the 11 compounds. The quantitative method underwent thorough validation to ensure linearity, LOQ, LOD, precision, repeatability, stability, and recovery. Table S5 illustrates the linear calibration curves of peak area (y) and concentration (x) for the 11 compounds. The precision, repeatability, and stability of the developed method were assessed across 11 compounds, yielding RSDs of less than 4.08 %, 4.55 %, and 4.56 %, respectively. The method exhibited commendable accuracy, as reflected in average spiked recoveries ranging from 93.34 % to 101.85 %. These findings unequivocally confirm the accuracy and reliability of the established method for the simultaneous quantitative determination of all 11 components.

To investigate the variations of the LIM samples at different content levels, the concentrations of the 11 active compounds in 18 batches of samples from different parts of LIM in various growth periods were determined using the established UPLC-MS method. The MRM diagram is depicted in Fig. S2. The concentration of each of the 11 compounds was obtained from the linear equation of the standard, allowing for the calculation of their contents. The contents of 11 compounds (C1: quercetin; C2: kaempferol; C3: β-sitosterol; C4: cichoric acid; C5: rutin; C6: cynaroside; C7: chlorogenic acid; C8: luteolin; C9: apigenin; C10: oleanolic acid; C11: 4,5-dicaffeoylquinic acid) in the sample are presented in Tables S6 and S7.

To visually highlight the differences among the 18 samples and 11 compounds, the contents of 11 compounds in different samples were standardized for HCA using the complete linkage algorithm based on Euclidean distance. The results are presented in a heat map and dendrogram (Fig. 3A). The color spectrum of the heat map ranges dark blue and dark red, with redder colors indicating greater difference. The 11 compounds exhibited varying trends of variation among LIM samples and were categorized into four clusters based on the dendrogram. Cluster I included C2 (β-sitosterol) and was predominantly found in samples S6, S1, S14, and S15. Cluster II encompassed most compounds, including C3 (quercetin), C9 (rutin), C7 (kaempferol), C5 (apigenin), C11 (luteolin), C4 (chlorogenic acid), and C10 (cynaroside), and was primarily gathered in sample S12. Cluster III included C6 (oleanolic acid) and mainly gathered in samples S9, S16, S12, and S18. Cluster IV included C1 (4,5-dicaffeoylquinic acid) and C8 (cichoric acid) and was mainly gathered in samples S3 and S18.

To compare the contents of the 11 compounds in 18 batches of samples, a histogram was generated (Fig. 3B). According to the results presented in Tables S5 and S6, C8 exhibited the highest content among of all samples of the 11 compounds. C9 displayed a high content in the leaves, whole plants, and flowers of LIM. Among the 18 samples, the total content of 11 compounds in S3, S7, S11, and S16 was higher than in other batches. Fig. 3C reveals that the total content of 11 compounds in the mature stage (2020.8.24) was the highest among the whole plant samples, while the total content of the 11 compounds in the bud stage (2020.7.14) was the highest in the leaf samples. Further analysis of the relative contents of the 11 compounds in different medicinal parts was conducted, focusing on the distribution of 11 compounds across various plant components. The relative content was calculated by dividing the content of a medicinal part by the sum of the contents of all five medicinal parts. The results are illustrated in Fig. 3D and Fig. S3. The findings indicate that, in comparison with other parts, the relative content of C4, C8, and C9 in leaves and whole plants was higher, suggesting that C4, C8, and C9 serve as primary indicators for distinguishing leaves and whole plants from other medicinal parts. Furthermore, the relative content of C4, C5, C7, C9, C10, and C11 in the flower of LIM was relatively high, making them key indicators for distinguishing flower from other medicinal parts.

Further cluster analysis was conducted on the 11 compounds from different medicinal parts (seeds, roots, stems and leaves). The results of HCA are presented in Fig. 4A. The analysis revealed that when the distance scale was approximately 40, the samples were divided into two groups based on the similarities and differences in the contents of 11 compounds. Specifically, the contents of leaves (S3, S7, S11, and S16) and whole plants (S4, S8, S13, and S18) were relatively similar and formed the first group, while the contents of roots (S1, S5, S9, and S14) and stems (S2, S6, S10, and S15) were relatively similar and formed the second group. Additionally, these groups were further divided based on different medicinal parts, and an OPLS-DA model was established to analyze the samples (Fig. 4B). The results in Fig. 3B show that samples within each group exhibit evident clustering by different medicinal parts, indicating that different medicinal parts of LIM have a significant impact on its intrinsic active components. Furthermore, based on VIP $>$ 1 and $p < 0.05$, key components of different medicinal parts were screened and distinguished. Preliminary results suggest that C8 (cichoric acid) and C9 (rutin) might be the reason for the difference in the content of the four medicinal parts of LIM (Fig. 4C).

Moreover, a PCA model was established to study the impact of different harvest dates (Fig. 4D). The results showed that samples harvested on different dates did not exhibit evident clustering, suggesting

Table 1
Identification of the chemical components of *Lactuca indica* L. cv. Mengzao (LIM) by ultra-high performance liquid chromatography coupled with quadrupole tandem Q-Excative mass spectrometry (UPLC-QE-MS/MS).

NO.	RT (min)	Found at *m/z*	Expected at *m/z*	Error (ppm)	Molecular formula	Ion mode	Mass (*m/z*)	MS/MS	Proposed compounds	S9	S10	S11	S12	S17
Flavonoids														
1	6.468	610.15346	610.15338	0	$C_{27}H_{30}O_{16}$	[M−H]-	609.14600	301.03488, 300.02719, 151.00256	Rutin	√	√	√	√	√
2	6.473	594.15771	594.15847	−1	$C_{27}H_{30}O_{15}$	[M + H]+	595.16504	433.10678, 287.05429	Kaempferol-3-O-rutinoside	NA	√	√	NA	NA
3	6.682	464.09430	464.09548	−3	$C_{21}H_{20}O_{12}$	[M + H]+	465.08777	304.05246, 303.04910, 257.04355, 214.91052, 145.04932	Isoquercitrin	√	√	√	√	NA
4	6.690	448.09967	448.10056	−2	$C_{21}H_{20}O_{11}$	[M + H]+	449.10696	287.05417, 210.24162, 153.01753	Cynaroside	NA	√	√	√	NA
5	6.694	448.09965	448.10056	−2	$C_{21}H_{20}O_{11}$	[M + H]+	449.10672	287.05426, 153.0157, 116.17660, 88.12576	Kaempferol-7-O-glucoside	NA	NA	√	√	NA
6	6.712	286.04765	286.04774	0	$C_{15}H_{10}O_6$	[M−H]-	285.04037	268.68231, 151.50806, 133.02843	Luteolin	NA	√	√	√	√
7	6.717	316.05818	316.05830	0	$C_{16}H_{12}O_7$	[M−H]-	315.05090	225.25003, 191.03874	3-O-Methylquercetin	NA	NA	√	NA	NA
8	6.898	448.09969	448.10056	−2	$C_{21}H_{20}O_{11}$	[M + H]+	449.10596	286.04767, 287.210.54845, 146.05214, 88.64880	Kuromanin	NA	NA	√	NA	NA
9	7.061	462.07876	462.07983	−2	$C_{21}H_{18}O_{12}$	[M−H]-	461.07242	285.04025, 217.08017, 193.48659, 113.0233, 85.02839	Scutellarin	NA	√	√	√	√
10	7.160	550.09553	550.09587	−1	$C_{24}H_{22}O_{15}$	[M−H]-	549.08826	301.03476, 213.56686, 151.00270, 104.41696	Quercetin-3-O-malonylglucoside	√	√	√	√	NA
11	7.211	448.10024	448.10056	−1	$C_{21}H_{20}O_{11}$	[M−H]-	447.09302	284.03241, 255.02945, 151.00255	Astragalin	NA	NA	√	NA	NA
12	7.380	446.08467	446.08491	−1	$C_{21}H_{18}O_{11}$	[M−H]-	447.09149	271.05927, 153.01793, 113.02331, 85.02838	Apigenin-7-O-glucuronide	NA	√	√	√	NA
13	8.861	286.04712	286.04774	−2	$C_{15}H_{10}O_6$	[M + H]+	287.05423	184.68994, 153.01776, 87.57372	Kaempferol	NA	√	√	√	NA
14	8.958	302.04184	302.04265	−3	$C_{15}H_{10}O_7$	[M−H]-	301.03506	273.04022, 178.99767, 151.00264, 121.02842, 107.01268	Quercetin	NA	√	√	√	NA
15	9.682	270.05224	270.05282	−2	$C_{15}H_{10}O_5$	[M−H]-	269.04504	151.00225, 107.01238	Apigenin	NA	√	√	√	NA
16	9.687	300.06289	300.06339	−2	$C_{16}H_{12}O_6$	[M + H]+	301.07016	286.04651, 245.09196, 205.72273,	Isokaempferide	NA	NA	√	NA	NA
17	9.834	270.05274	270.05282	0	$C_{15}H_{10}O_5$	[M−H]-	269.04547	149.02333, 117.03341, 107.43125	Genistein	NA	NA	√	√	NA
18	9.838	300.06321	300.06339	−1	$C_{16}H_{12}O_6$	[M−H]-	299.05594	284.03229, 256.03735, 215.19597, 108.85090, 72.84940	Hispidulin	NA	NA	√	NA	NA
19	9.959	446.08417	446.08491	−2	$C_{21}H_{20}O_{10}$	[M + H]+	433.11200	272.05268, 271.05936, 255.01714, 209.95494, 84.49276	Apigenin-7-O-glucoside	√	√	√	√	NA
Organic acids														

(*continued on next page*)

Table 1 (*continued*)

NO.	RT (min)	Found at *m/z*	Expected at *m/z*	Error (ppm)	Molecular formula	Ion mode	Mass (*m/z*)	MS/MS	Proposed compounds	S9	S10	S11	S12	S17
20	0.786	196.05743	196.05830	−4	$C_6H_{12}O_7$	[M−H]-	195.05014	59.01265, 75.00762, 85.02836, 99.00761, 105.01818, 129.01820, 159.02882	Gluconic acid	NA	NA	√	√	NA
21	1.122	192.02618	192.02700	−4	$C_6H_8O_7$	[M−H]-	191.01892	87.00764, 111.00763, 173.00819	Citric acid	NA	NA	√	√	NA
22	1.211	164.04715	164.04734	−1	$C_9H_8O_3$	[M + H]+	165.05446	119.04912, 147.04387	2-Hydroxycinnamic acid	NA	√	√	√	NA
23	4.416	354.09494	354.09508	0	$C_{16}H_{18}O_9$	[M−H]-	353.08755	135.04408, 179.03407, 191.05527, 216.81255	Neochlorogenic acid	NA	√	√	√	NA
24	4.466	312.04799	312.04813	0	$C_{13}H_{12}O_9$	[M−H]-	311.04056	87.00761, 135.04402, 149.00810, 179.03404	Caftaric acid	√	√	√	√	NA
25	5.236	354.09494	354.09508	0	$C_{16}H_{18}O_9$	[M−H]-	353.08762	135.04408, 179.03407, 191.05527	Chlorogenic acid	√	√	√	√	√
26	5.236	192.06251	192.06339	−5	$C_7H_{12}O_6$	[M + H]+	191.05519	85.02835	D-(-)-Quinic acid	√	√	√	√	√
27	6.662	474.07945	474.07983	−1	$C_{22}H_{18}O_{12}$	[M−H]-	473.07214	236.03198	Cichoric acid	√	√	√	√	√
28	7.113	516.12590	516.12678	−2	$C_{25}H_{24}O_{12}$	[M + H]+	517.13318	163.03865, 187.98233, 203.50183	4,5-Dicaffeoylquinic acid	√	√	√	√	√
29	9.559	328.22481	328.22497	0	$C_{18}H_{32}O_5$	[M−H]-	327.21753	129.09105, 141.09103, 171.10155, 185.11752, 197.11754, 209.11768	Fulgidic acid	√	√	√	√	√
30	12.457	210.12486	210.12559	−3	$C_{12}H_{18}O_3$	[M−H]-	209.11758	61.98721, 83.01274, 121.02842, 125.09610	Jasmonic acid	NA	NA	√	NA	NA
31	12.652	294.21949	294.21949	0	$C_{18}H_{30}O_3$	[M−H]-	293.21210	195.13829, 223.13345	9-Oxo-10(E),12(E)-octadecadienoic acid	√	√	√	√	√
32	19.066	132.04244	132.04226	1	$C_5H_8O_4$	[M−H]-	131.03520	65.01337, 89.01341	Ethylmalonic acid	NA	√	NA	NA	NA
Carboxylic acids and derivatives														
33	0.746	174.11144	174.11168	−1	$C_6H_{14}N_4O_2$	[M + H]$^+$	175.11867	60.05626, 70.06567, 116.07057, 130.09727, 158.09204	DL-Arginine	√	√	√	NA	√
34	0.771	146.06895	146.06914	−1	$C_5H_{10}N_2O_3$	[M + H]$^+$	147.07622	84.04488, 130.04980	DL-Glutamine	√	√	√	NA	NA
35	0.775	147.05296	147.05316	−1	$C_5H_9NO_4$	[M + H]$^+$	148.06018	85.04821, 102.05521, 130.04980, 131.05312	L-Glutamic acid	NA	√	√	NA	√
36	0.798	129.04243	129.04259	−1	$C_5H_7NO_3$	[M + H]$^+$	130.04980	84.04484	L-Pyroglutamic acid	NA	√	NA	√	√
37	0.809	115.06339	115.06333	1	$C_5H_9NO_2$	[M + H]$^+$	116.07068		D-(+)-Proline	√	√	√	√	√
38	0.870	228.14699	228.14739	−2	$C_{11}H_{20}N_2O_3$	[M + H]$^+$	229.15442	70.06574, 101.63992, 183.14900	Prolylleucine	√	√	√	√	√
39	1.210	181.07369	181.07389	−1	$C_9H_{11}NO_3$	[M−H]$^-$	180.06572	72.00795, 93.03344, 119.04910, 163.03900	L-Tyrosine	NA	√	√	√	√
40	1.254	293.14673	293.14745	−2	$C_{12}H_{23}NO_7$	[M + H]$^+$	294.15402	86.09685, 132.10176, 144.10165, 161.06796, 212.12772,	*N*-Fructosyl isoleucine	NA	√	√	√	NA

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Table 1 (*continued*)

NO.	RT (min)	Found at *m/z*	Expected at *m/z*	Error (ppm)	Molecular formula	Ion mode	Mass (*m/z*)	MS/MS	Proposed compounds	S9	S10	S11	S12	S17
								248.14862, 258.133						
41	1.303	131.09451	131.09463	−1	$C_6H_{13}NO_2$	[M + H]$^+$	132.10181	69.07050, 87.10032	L-Isoleucine	√	√	√	√	√
42	2.235	165.07878	165.07898	−1	$C_9H_{11}NO_2$	[M + H]$^+$	166.08603	120.08076	L-Phenylalanine	√	√	√	√	√
Saccharides														
43	0.770	260.02937	260.02972	−1	$C_6H_{13}O_9P$	[M−H]-	259.02206	79.95791, 96.96848, 241.01140	D-Glucose 6-phosphate	NA	NA	NA	√	NA
44	0.803	180.06254	180.06339	−5	$C_6H_{12}O_6$	[M−H]-	179.05521	59.01268, 85.02839, 89.02331, 94.92396, 101.02328, 113.02328	D-Galactose	√	√	NA	NA	√
45	0.823	342.11582	342.11621	−1	$C_{12}H_{22}O_{11}$	[M−H]-	341.10846	59.01267, 85.02840, 113.02328, 143.03389, 161.04462, 179.05518	α-Lactose	√	√	√	√	√
46	0.824	666.22164	666.22186	0	$C_{24}H_{42}O_{21}$	[M−H]-	665.21430	89.02327, 101.02327, 113.02325, 119.03381, 179.05513	Stachyose	√	NA	√	√	√
47	0.830	828.27440	828.27468	0	$C_{30}H_{52}O_{26}$	[M−H]-	827.26697	89.02327, 101.02328, 113.02326, 143.03386, 161.04454, 179.05515	Maltopentaose	√	NA	√	NA	NA
Coumarins and derivatives														
48	4.495	162.03143	162.03169	−2	$C_9H_6O_3$	[M + H]+	163.03870	89.03896, 107.04930, 116.95417, 139.98192, 145.02817	7-Hydroxycoumarine	NA	NA	NA	√	NA
49	4.562	206.05753	206.05791	−2	$C_{11}H_{10}O_4$	[M + H]+	207.06479	163.03865	Scoparone	NA	NA	NA	√	NA
50	4.795	340.07913	340.07943	−1	$C_{15}H_{16}O_9$	[M−H]-	339.07187	177.01840, 210.55910	Esculin	NA	√	NA	√	NA
51	6.655	162.03143	162.03169	−2	$C_9H_6O_3$	[M + H]+	163.03870	145.02818	7-Hydroxycoumarine	NA	NA	NA	√	NA
52	6.889	192.04197	192.04226	−2	$C_{10}H_8O_4$	[M + H]+	193.04929	133.02829	Scopoletin	√	√	√	NA	NA
Fatty acids														
53	7.485	188.10406	188.10486	−4	$C_9H_{16}O_4$	[M−H]-	187.09673	61.98719, 125.09606, 141.86702, 159.87762	Azelaic acid	√	√	√	√	√
54	9.559	328.22481	328.22497	0	$C_{18}H_{32}O_5$	[M−H]-	327.21756	85.02837, 97.06467, 171.10182, 211.13315, 229.14384	Fulgidic acid	√	√	√	√	√
55	12.957	292.20320	292.20384	−2	$C_{18}H_{28}O_3$	[M + H]+	293.21036	67.05486, 121.10112, 147.11653, 173.11688, 209.15302, 247.20500	9S,13R-12-Oxophytodienoic acid	√	√	√	√	√
56	13.118	278.22403	278.22458	−2	$C_{18}H_{30}O_2$	[M + H]+	279.23120	67.05484, 109.10129, 123.11676, 137.12223, 149.02309, 173.13222, 195.08012	α-Eleostearic acid	√	√	√	√	√
57	15.908	278.22396	278.22458	−2	$C_{18}H_{30}O_2$	[M + H]+	279.23126	66.41770, 93.07026, 123.11684, 135.11688,	α-Linolenic acid	√	√	√	NA	√

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Table 1 (*continued*)

NO.	RT (min)	Found at *m/z*	Expected at *m/z*	Error (ppm)	Molecular formula	Ion mode	Mass (*m/z*)	MS/MS	Proposed compounds	S9	S10	S11	S12	S17
58	15.918	278.22426	278.22458	−1	$C_{18}H_{30}O_2$	[M−H]-	277.21698	195.13768, 209.15323, 243.21021 59.01271, 130.55818, 177.97186, 217.07895	Pinolenic acid	√	√	√	NA	√
59	16.897	264.24466	264.24532	−2	$C_{18}H_{32}O$	[M + H]+	265.25198	67.05483, 109.10131, 128.92627, 135.11664, 163.14789, 214.65956	Linolenyl alcohol	NA	NA	NA	√	NA
60	18.504	282.25551	282.25588	−1	$C_{18}H_{34}O_2$	[M−H]-	281.24823	61.98717, 81.52533, 106.03990, 107.03515, 117.41459, 121.05109,	Oleic acid	NA	NA	NA	√	NA
Other types														
61	2.230	102.04703	102.04695	1	C_8H_6	[M + H]+	103.05446	53.03930, 95.04948	Phenylacetylene	NA	NA	√	√	NA
62	4.516	187.06298	187.06333	1	$C_{11}H_9NO_2$	[M + H]+	188.07028	118.06563, 146.05975	Trans-3-Indoleacrylic acid	√	√	√	√	NA
63	8.041	410.13575	410.13655	−2	$C_{23}H_{22}O_7$	[M + H]+	411.14301	109.02856, 167.08527, 195.08014, 231.10110, 260.09927	Lactucopicrin	√	NA	NA	√	√
64	12.609	314.18760	314.18819	−2	$C_{20}H_{26}O_3$	[M + H]+	315.19489	69.07052, 99.04434, 145.06462, 173.09590, 187.11136, 259.13242, 269.18	Kahweol	NA	NA	NA	NA	√
65	16.511	456.36009	456.36035	−1	$C_{30}H_{48}O_3$	[M−H]-	455.35281	167.96708, 187.76880, 209.87030, 212.39192, 359.13030	Oleanolic acid	√	√	√	√	√
66	19.139	168.04261	168.04226	2	$C_8H_8O_4$	[M−H]-	167.03525	89.01334, 99.92464, 140.02432	4-Hydroxymandelic acid	NA	√	NA	√	NA

Note: NA means that it had not been detected from the LIM.

that different harvest dates have no significant impact on the chemical content of LIM, which requires further exploration.

4. Discussion

In this study, we employed phytochemical analysis based on UPLC-QE-MS/MS and targeted metabolomics to conduct a comprehensive investigation into the chemical components of different medicinal parts of LIM. *L. indica* is widely distributed in China, and recent studies have highlighted its significant pharmacological activity (Treuren et al., 2013) Given that the quality and efficacy of traditional Chinese medicine are primarily determined by its chemical composition (Deng et al., 2016), it becomes crucial to explore and understand the chemical constituents of LIM. While previous *L. indica* has primarily focused on the whole plant, there has been limited comprehensive investigation into its other parts, including the roots, stems, leaves, flowers, and seeds (Deng et al., 2016; Hao et al., 2021; Liu et al., 2018). However, all parts of LIM, including roots, stems, leaves and, flowers, have been utilized in traditional Chinese medicine for centuries. The variation in efficacy among different medicinal parts may be attributed to differences in the content of various components (Fan et al., 2022). Therefore, conducting a thorough qualitative comparison of the chemical component among different medicinal parts of LIM becomes imperative to offer insights and serve as a reference for foundational research on the pharmacological substances present in LIM.

To the best of our knowledge, this is the first work to comprehensively investigate alterations in non-volatile components across various parts of LIM. A total of 66 non-volatile chemical components, encompassing flavonoids, organic acids, amino acids and esters, were successfully identified from the roots, stems, leaves, and flowers of LIM. Employing multivariate statistical methods, evident variations among different plant parts were observed, underscoring the significant impact that different parts can exert on the internal qualities of LIM. Furthermore, the study identified two key chemical markers that prove valuable in distinguishing between various medicinal parts of LIM.

In present study, flavonoids emerged as the predominant compounds in LIM, with a total of 19 identified flavonoid. Building upon prior studies (Hao et al., 2021; Tanaka & Ohmiya, 2008; Zhang et al., 2020), we constructed a concise metabolic pathway diagram depicting flavonoids biosynthesis in LIM, which is presented in Fig. 5. The synthesis of flavonoids in LIM initiates with the enzymatic activity of chalcone synthase, catalyzing the conversion of *p*-coumarin coenzyme A into naringin chalcone. This precursor compound then undergoes transformation into naringin through the action of chalcone isomerase. The metabolism of naringin in LIM primarily involves two pathways: conversion into dihydroquercetin and apigenin. The study revealed that

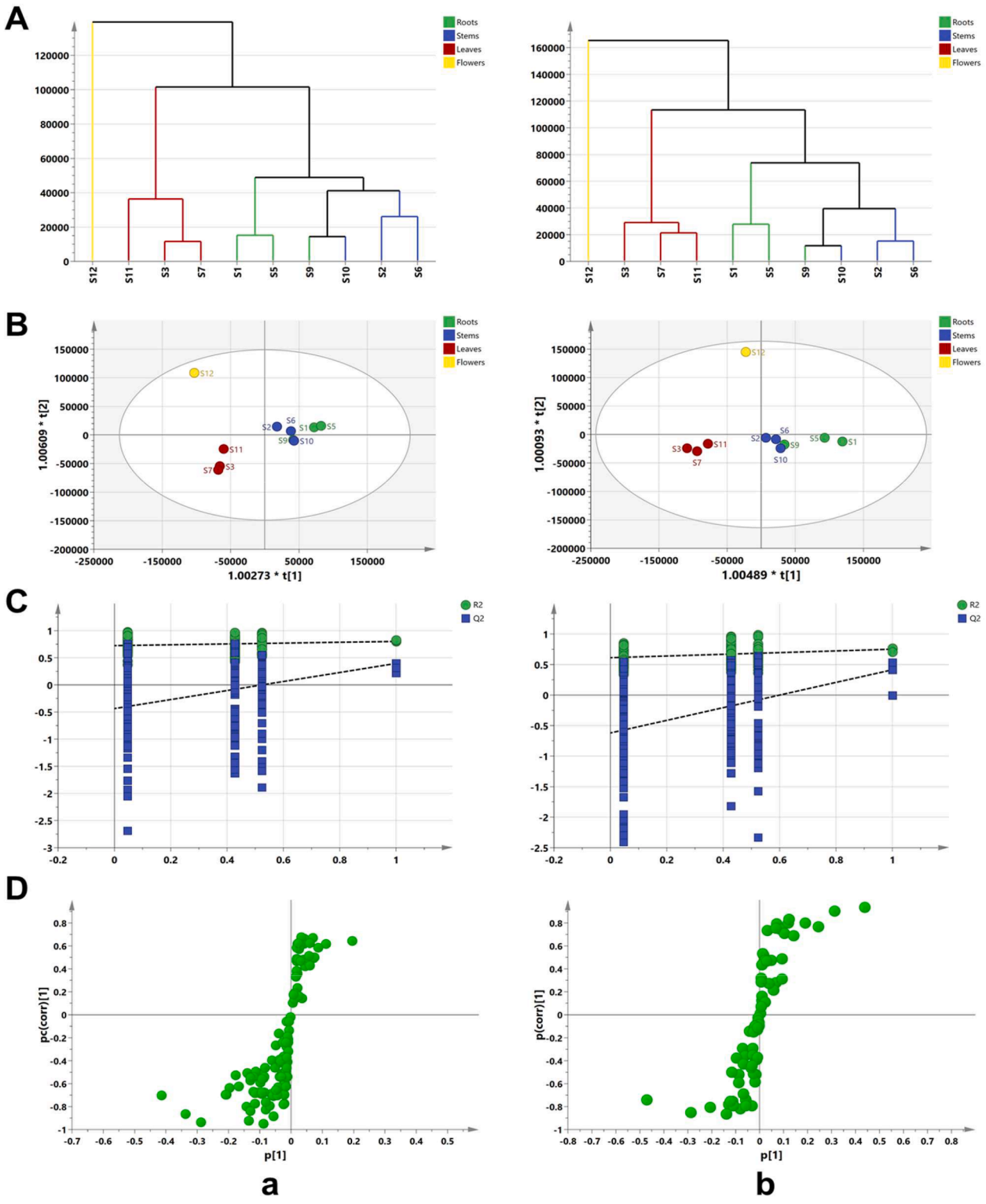

Fig. 2. Exploratory analyses of different medicinal parts of LIM (S1 − S3, S5 − S7, S9 − S12) hierarchical cluster analysis (HCA) (A), orthogonal partial least-squares discriminant analysis (OPLS-DA) (B), permutation test (PT) (C), and S-plot (D) under the mode of positive (a) and negative (b) ions. Green ions near the origin indicate little contribution to groups separation, while those furthest from the origin are crucial components. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

leaves contained the highest concentration of flavonoids among different medicinal parts, suggesting the leaves have high potential for utilization in LIM.

Previous research has utilized UPLC-MS/MS method to determine flavonoids content in various parts of LIM, including rutin, kaempferol and quercetin (Hao et al., 2021). Additionally, studies on *Ixeris chinensis* (Thunb.) Nakai have employed HPLC to determine compounds such as luteolin and cichoric acid (Zeng et al., 2021). While these studies have contributed to understanding the chemical components, the number of determination indexes for *L. indica* is limited, and the analytical methods

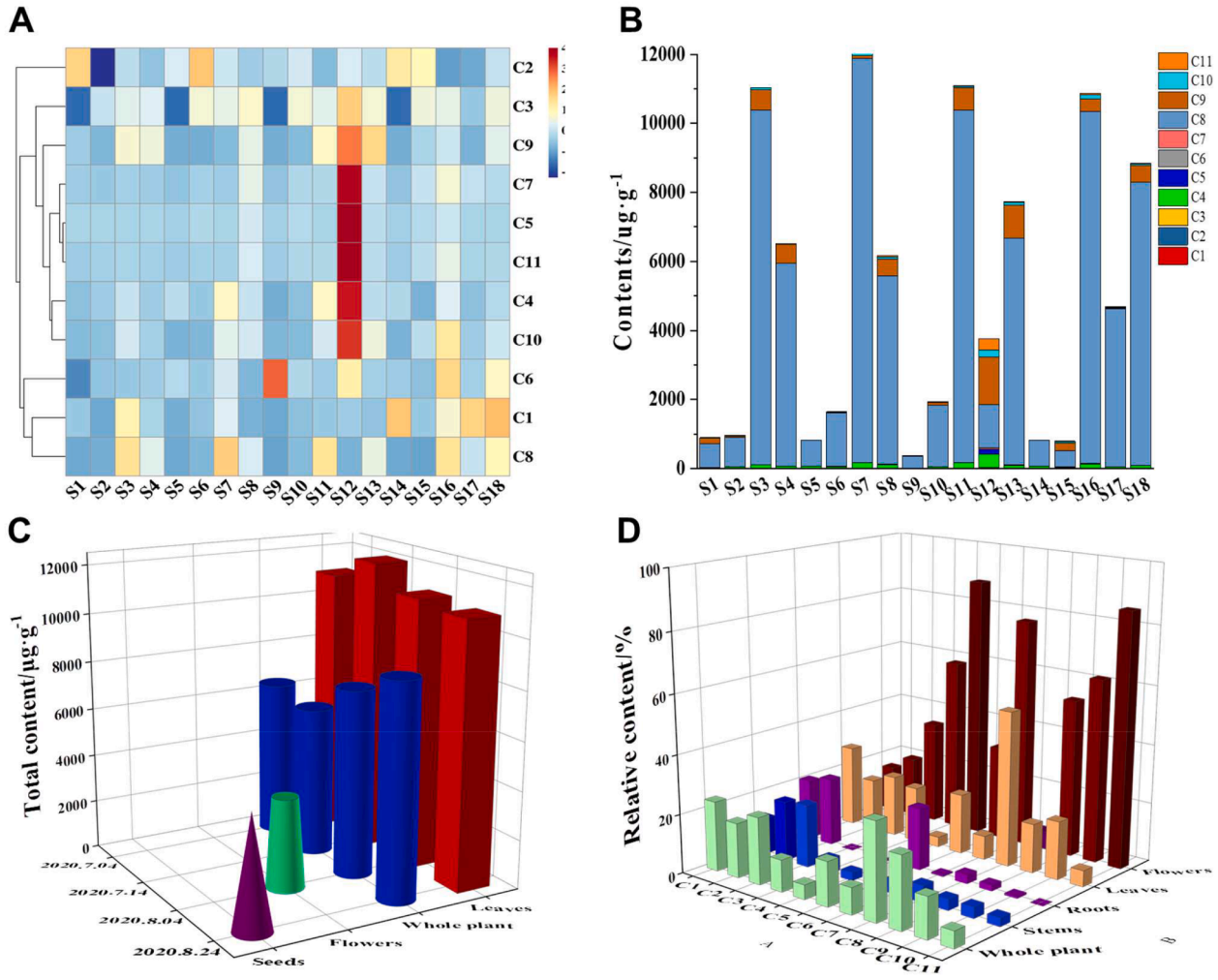

Fig. 3. Visualization of 11 components in various LIM samples: Heat map (A), stacked column chart (B), and 3D histogram of the total content of 11 components (C). Additionally, a histogram depicting the relative content of 11 components in different medicinal parts of LIM (D). C1: 4,5-dicaffeoylquinic acid; C2: β-sitosterol; C3: Quercetin; C4: Chlorogenic acid; C5: Apigenin; C6: Oleanolic acid; C7: Kaempferol; C8: Cichoric acid; C9: Rutin; C10: Cynaroside; C11: Luteolin.

vary. Previous research underscores the non-volatile components in *L. indica* mainly include flavonoids, organic acids, and phenylpropanoid compounds and act as key contributors to the medicinal value of *L. indica* (Li et al., 2020). Building on this understanding, herein, we selected 11 active ingredients—namely, 4,5-dicaffeoylquinic acid, β-sitosterol, quercetin, chlorogenic acid, apigenin, oleanolic acid, kaempferol, cichoric acid, rutin, cynaroside, and luteolin—based on the results of phytochemical analysis and the reported potential active ingredients in the literature. To facilitate a comprehensive analysis of these active ingredients, a sensitive and rapid UPLC-MS/MS method was developed and validated. This method enables the simultaneous determination of the content of 11 selected active ingredients, encompassing flavonoids, organic acids, triterpenoids, sterols, phenylpropanoids, in18 batches of LIM samples.

The holistic perspective on plant growth highlights the interconnectedness of various tissues, organs and, systems within a plant. These components operate both independently and in close relation to each other. Environmental factors, including water, temperature, and light, exert a significant impact on plant growth and the accumulation of bioactive components (Chen et al., 2012; Zeng et al., 2021). Among these factors, the harvest period stands out as a crucial determinant of the chemical content of plants. The growth period has a profound impact on the accumulation of secondary metabolites in different medicinal parts(Hao et al., 2021). Existing literature has indicated variations in the content of main active compounds in *L. indica* occurs at different harvest times, such as mid-July (flowering period) (Dong, 2008), and late July to mid-August (immature period to flowering period) (Hao et al., 2021). However, another study reported that the best harvest time was early June (flowering period) (Wang, 2011). These discrepancies in findings could be influenced by severalfactors, including environmental (such as drought), genetics differences, climatic conditions, ecotype variations, or a combination of these factors (Chen et al., 2011; Liao et al., 2010). Therefore, determining the optimal time for harvesting each medicinal part to maximize the production of active ingredients is crucial and should be based on experimental evidence rather than solely relying on published data. PCA is a commonly used method to assess the degree of sample separation (Fan et al., 2022). Therefore, it is essential to conduct a detailed analysis of the active components of samples collected during different harvest periods in a traditional way. Our study revealed that, in comparation to that in roots and stems, the content of the 11 active components in leaves, whole plants, flowers, and seeds samples was higher. Leaves and whole plants emerged as the primary harvestable parts. Subsequent investigation unveiled that the active components in leaf samples exhibited an initial increase followed by a decrease with delayed harvest time, while whole plant samples displayed a continuous increasing trend. This pattern is consistent with observations in other plants (Bai et al., 2017; Hao et al., 2021). However, these trends are influenced by various factors, including the maturity level at harvest and environmental conditions (Bai et al., 2017; Zadernowski et al., 2005).

In addition, the variability in active ingredient content can be

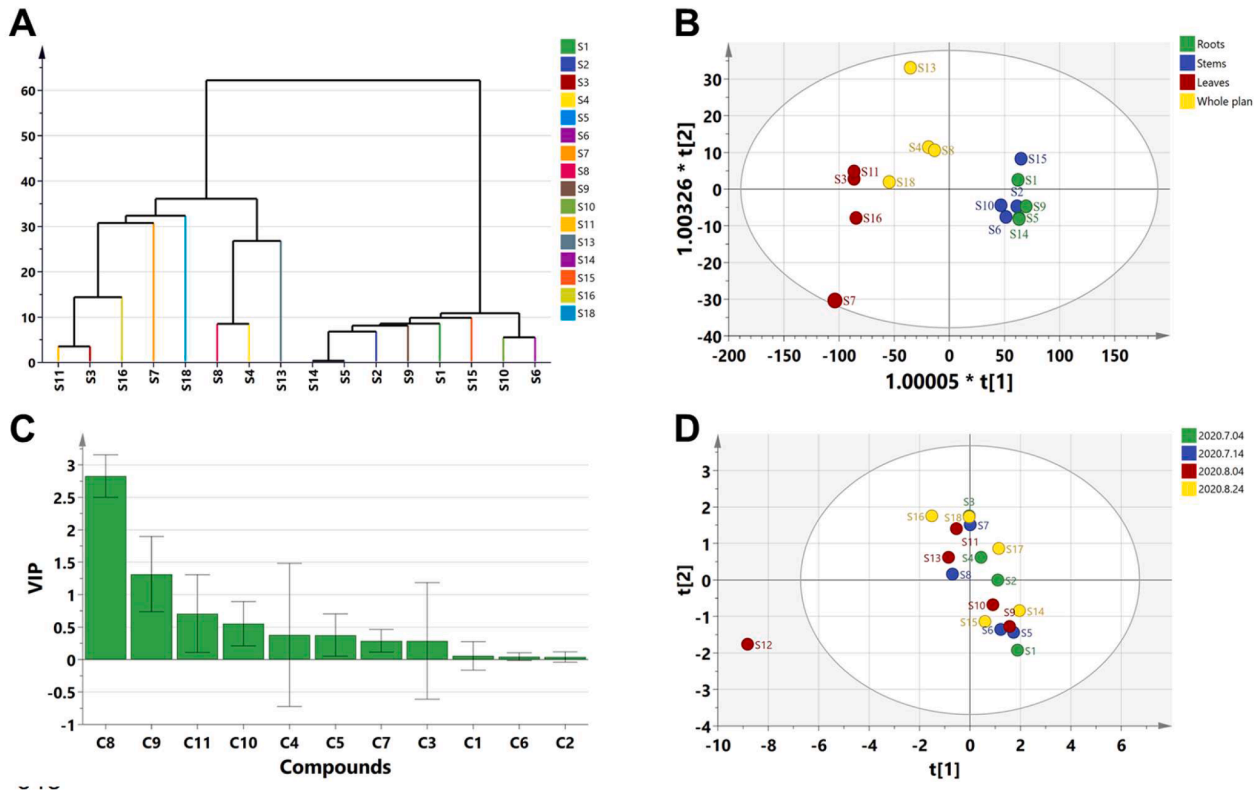

Fig. 4. Multivariate analyses of 11 components in different medicinal parts of LIM: HCA (A), OPLS-DA, and variable importance in projection (VIP) plot (B, C), and principal component analysis (PCA) in harvest poried (D). C1: 4,5-dicaffeoylquinic acid; *C2*: β-sitosterol; C3: Quercetin; C4: Chlorogenic acid; C5: Apigenin; C6: Oleanolic acid; C7: Kaempferol; C8: Cichoric acid; C9: Rutin; C10: Cynaroside; C11: Luteolin.

influenced by factors such as light exposure and the growth and development stages of LIM. Aa the plant progresses through its growth and development stages, several physiological changes occur. Notably, the leaves gradually enlarge, stomata open, carbon dioxide uptake in the intercellular space increases, and the photosynthetic rate rises. These physiological changes contribute to the enhanced synthesis of active components (Hao et al., 2021; Zobayed et al., 2005). The exposure of leaves to more light during the plant's growth and development is another contributing factor. Light serves as a crucial environmental factors influencing the formation of plant secondary metabolites, and it has a notable impact on the biosynthesis of the 11 active ingredients (Wang et al., 2022). However, the transition of LIM into the flowering stage brings about notable changes in leaf morphology. The shift is characterized by a transformation from broad-leaved to split-leaved structures, accompanied by a reduction in leaf size. As the leaves gradually yellow and age during this stage, the synthesis of active ingredients tends to decelerate. Simultaneously, the active ingredients may undergo dilution throughout leaf growth. This implies a dynamic process involving the biosynthesis, degradation, and transport of the active ingredients within different plant organs (leaves, stems, flowers, or seeds) (Bai et al., 2017; Del Baño et al., 2003; Tsao et al., 2006). In essence, the decrease in the overall amount of active ingredients in leaves might be a result of reallocating these compounds to enhance their concentration in flowers or seeds.

The intriguing observation of an increasing trend in the content of active components in whole plant samples of LIM adds complexity to the dynamics of chemical composition during the plant's growth stages. Cichoric acid, a crucial phenolic acids, reached its peak concentration during the seed-setting period (late August). This notable increase might be attributed to water deficit conditions, as studies indicate that plants respond to water deficit by accumulating terpenes and phenols (Castellarin et al., 2007), This adaptive response could potentially stimulate the elevation of cichoric acid and other substances in the whole plant of LIM. Consequently, in practice, the selection of appropriate medicinal parts and harvest times should be tailored to the specific target according to the target active ingredients, taking into account the complex interplay of environmental factors.

5. Conclusion

The comprehensive analysis of 18 LIM samples using UPLC-MS/MS method has provided valuable insights into its chemical composition and active components. The identification of 66 major chemical constituents, with a predominant presence of flavonoids, organic acid, and carboxylic acids and derivatives, contributes to a detailed understanding of the plant's phytochemical profile. Notably, 11 chemical components were identified as potential markers for distinguishing different medicinal parts of LIM. The application of a sensitive UPLC-MS/MS method to quantify 11 active components, particularly highlighting cichoric acid as the most abundant phenolic acid, especially in leaves (11695.72 μg g^{-1}), offers a quantitative perspective on the distribution of key compounds in LIM. This study's findings have implications for the quality evaluation of LIM, providing a foundation for further research and development in the field.

CRediT authorship contribution statement

Junfeng Hao: Conceptualization, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing. **Risu Na:** Conceptualization, Investigation, Methodology. **Lin Sun:** Supervision, Writing – review & editing. **Yushan Jia:** Formal analysis, Methodology, Validation. **Feng Han:** Methodology, Validation, Writing – review & editing. **Zhihui Fu:** Data curation, Methodology. **Zhijun Wang:** Supervision, Writing – original draft. **Muqier Zhao:** Formal analysis, Validation. **Cuiping Gao:** Writing – review & editing. **Gentu Ge:** Conceptualization, Funding acquisition, Methodology, Project

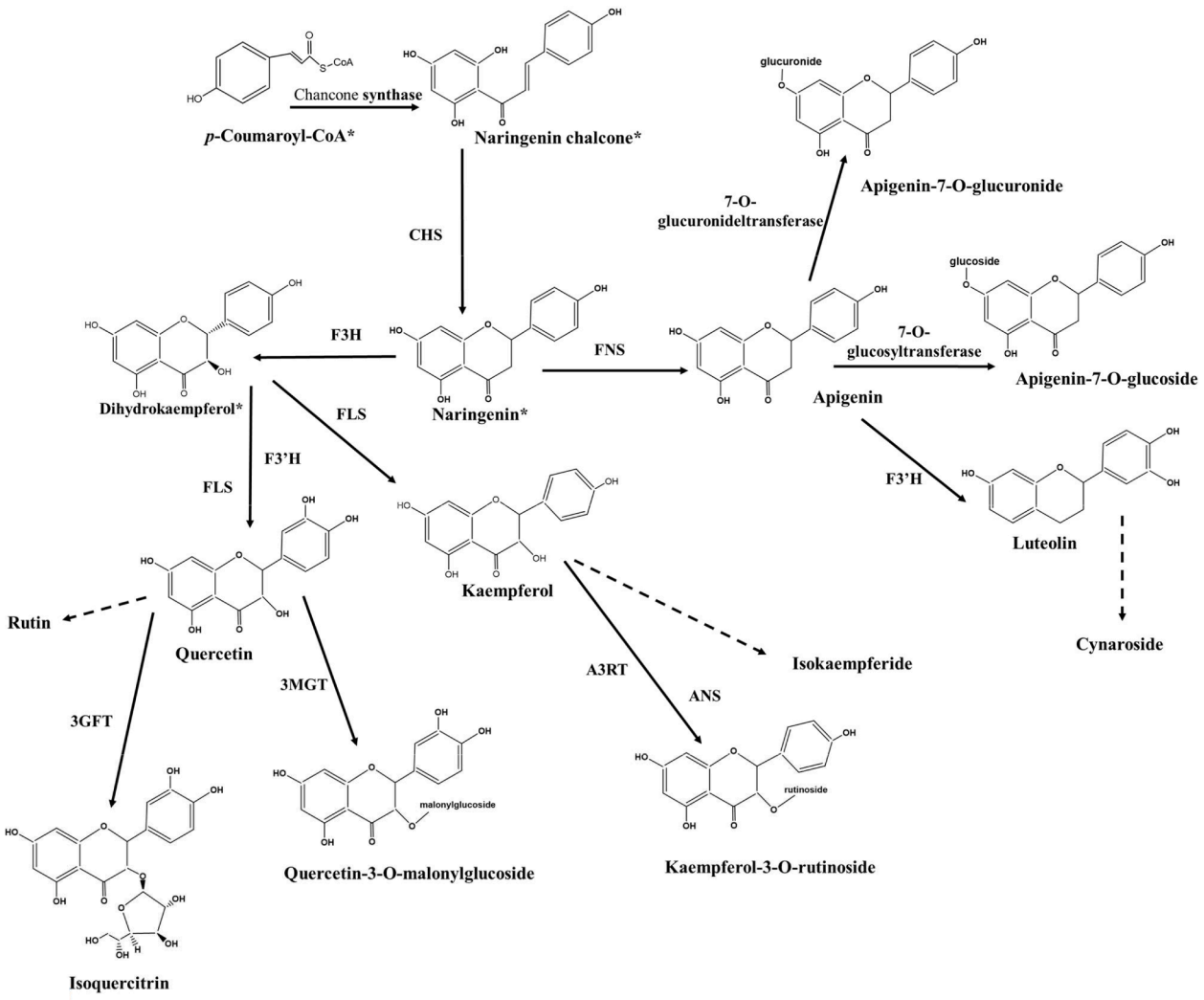

Fig. 5. Schematic representation of the metabolic pathways of flavonoids in LIM. Dotted arrows denoted a speculative reaction. * indicates components not detected in LIM. CHS: Chalcone synthase; F3H: Flavanone 3-hydroxylase; F3′H: Flavonoid 3′-hydroxylase; FNS: Flavone synthase; FLS: Flavonol synthase, 3MGT: 3-O-malonylglucoside-transferase; 3GFT: 3-O-β-D-glucofuranosidetransferase; A3RT: Anthocyanidin-3-O-glucoside-6-O-rhamnosyltransferase; ANS: Anthocyanidin synthase.

administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101031.

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