

# Supplementary Materials

## Enhanced Antioxidant Extraction from *Lonicerae Japonicae* *Flos* Based on A Novel Optimization Strategy with Tailored Deep Eutectic Solvents

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## 1. Supplementary Experimental Section

### 1.1 Determination of Content of Antioxidants

**Total Polyphenols Content** The total phenolic content (TPC) in *Lonicerae Japonicae Flos* (LJF) extract was determined by a modified Folin-Ciocalteu method [47]. In Briefly, 50  $\mu\text{L}$  Folin-Ciocalteu reagent, 840  $\mu\text{L}$  deionized water, and 10  $\mu\text{L}$  LJF sample were mixed evenly. Then, 50  $\mu\text{L}$  of 20%  $\text{Na}_2\text{CO}_3$  was added to initiate the reaction. After reaction at 25°C for 60 min, and the absorbance was measured at 760 nm by a UV spectrophotometer. Gallic acid was adopted as the reference for construction the standard curve ( $y = 0.9414x + 0.0483$ ,  $R^2 = 0.9988$ , 0.2-1.5 mM), and the results were expressed as milligram of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

**Total Flavonoids Content** The total flavonoid content (TFC) was determined by the colorimetric method based on the formation of flavonoid-aluminum compounds [47]. Specifically, 100  $\mu\text{L}$  of LJF extract was mixed with 400  $\mu\text{L}$  of deionized water and 30  $\mu\text{L}$  of  $\text{NaNO}_2$  (5% w/v) solution. After being kept at 25 °C for 5 min, 30  $\mu\text{L}$  of  $\text{AlCl}_3$  (10% w/v) solution was added and incubated for 6 min, followed by the addition of 200  $\mu\text{L}$  of  $\text{NaOH}$  (1 M) solution and 140  $\mu\text{L}$  of water. After reaction at 30 °C for 30 min, and the absorbance was measured at 510 nm. A calibration curve was constructed with rutin as the reference, the resulted calibration curve was  $y = 1.0319x - 0.0165$  ( $R^2 = 0.9988$ , 0.1-1.0 mM) and the measured results were expressed as milligram rutin equivalents (RE) per gram of sample in dried weight (mg RE/g DW).

**Total Reducing Sugar Content** The amount of the total reducing sugar (TRS) was determined by the dinitrosalicylic acid (DNS) method [52]. First, 100  $\mu\text{L}$  of LJF extract and 100  $\mu\text{L}$  of DNS (1%) were mixed thoroughly and heated in a boiling water bath for 5 min. After cooled to room temperature, 800  $\mu\text{L}$  of deionized water was added and the absorbance at 540 nm was measured. The calibration curve ( $y = 1.0855x - 0.0351$ ,  $R^2 = 0.9986$ , 0.1-1.0 mM) was constructed with glucose as the reference, and the measured results were expressed as milligram of glucose equivalents (GE) per gram of dry weight (mg GE/g DW).

### 1.2 Surface Morphology Characterization of LJF Powder

LJF powder treated with different solvents (DES, ethanol and water) was prepared. This was done as follows: 0.1 g of LJF powder was mixed with 3.5 mL water or 70% (v/v) solvents (including ethanol, ChCl-EG-1 DES). The mixture was then shaken

vigorously and heated in a water bath at 70 °C for 5 min with stirring (500 rpm). After centrifugation (10,000 rpm, 10 min), the solid residue was collected and lyophilized under vacuum (−80 °C, 24 h). Untreated LJF powder was used as a control. The obtained dry powder was immobilized on a silicon wafer and then sputtered with gold. The surface morphology was observed with a scanning electron microscope (SEM, JSM-6390LV, JEOL, Tokyo, Japan).

## 2. Supplementary Results and Discussion

### 2.1 Classification Analysis of Antioxidant Components

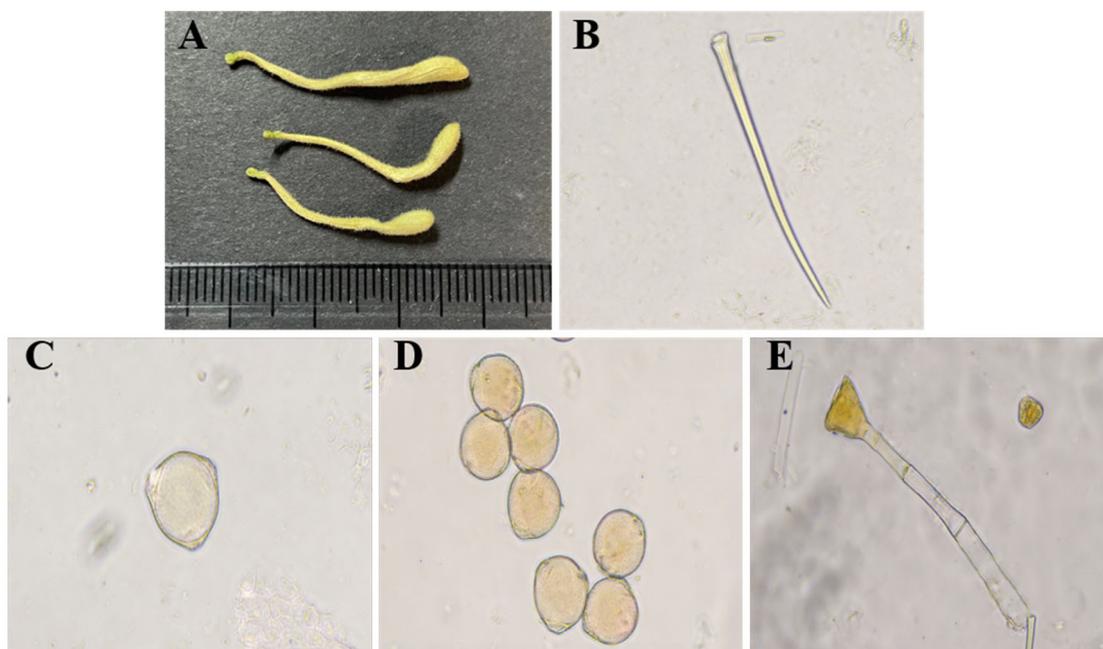
According to the predecessors' research, the antioxidant capacity of LJF was mainly derived from active components such as reducing sugars, polyphenols, flavonoids and phenolic acids [48,53]. To explore the real antioxidants of LJF, the concentration of the total reducing sugars, flavonoids and polyphenols (phenolic acids) in various batches of LJF extracts were measured, and then the gray relation analysis (GRA) was performed with antioxidant capacity of various LJF extracts as the compared sequence and antioxidants level (the total reducing sugars, flavonoids and polyphenols, Table S4) as the compared sequence. The fitting results (Table S5) showed that the degree of association ( $r$ ) of the total polyphenols (0.884) and flavonoids (0.882) were significantly greater than that of total reducing sugar (0.749), indicating the antioxidant ability of LJF extracts was more related to the contents of total flavonoids and polyphenols. The above results suggested that flavonoids and polyphenols are the main antioxidant components of LJF.

### 2.2 Construction of HPLC Fingerprints

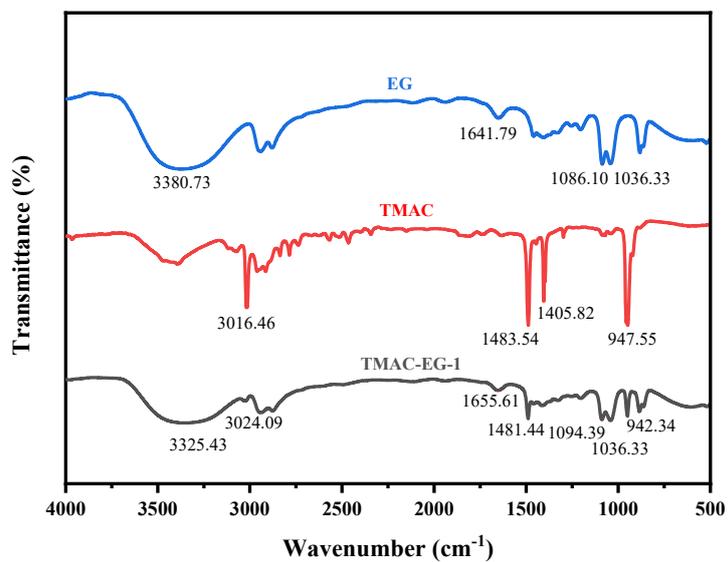
In this work, HPLC-DAD based fingerprint was adopted to explore the specific components of flavonoids and polyphenols for screening the antioxidant Q-marker. To verify the reliability of this method, the test solution (9 characteristic peaks) was adopted as the representative of LJF to investigate the precision, stability and repeatability of this method. The results (Table S6) showed that the relative standard deviation (RSD) of peak area and relative retention time of all the 9 characteristic peaks were within 4.5% and 0.8%, respectively, which demonstrated this method owned excellent precision, stability and repeatability.

Then, the fingerprints of LJF antioxidants were constructed by importing the chromatogram of 15 batches LJF samples from different origins into the Chinese Materia Medica chromatographic fingerprint similarity evaluation system (2012 version) with the time window width of 0.1 min and the median method of full spectrum peak matching. The generated chromatogram (Figure S5A and Figure 6A) showed there existed 9 common peaks on the fingerprint spectrum. Subsequently, the similarity of the samples and the corresponding reference chromatogram R were examined. As shown in Table S7, the similarity of different LJF samples were more than 0.934, suggesting that the overall quality of LJFs from different region are similar.

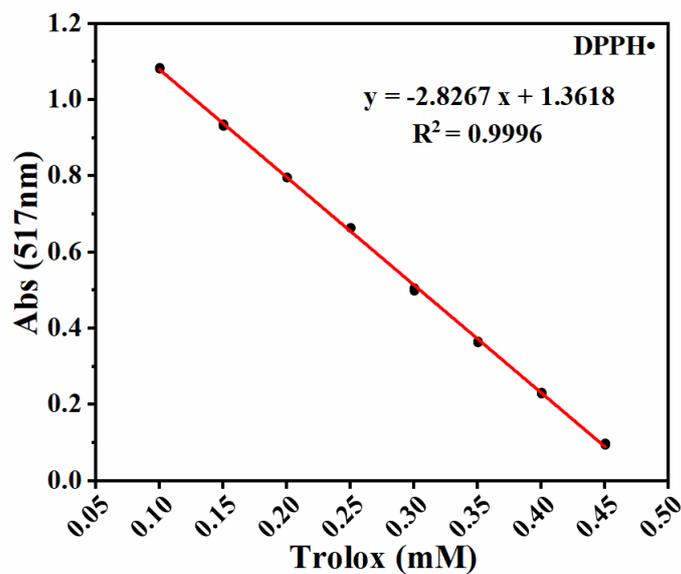
### 3. Supplementary Figures



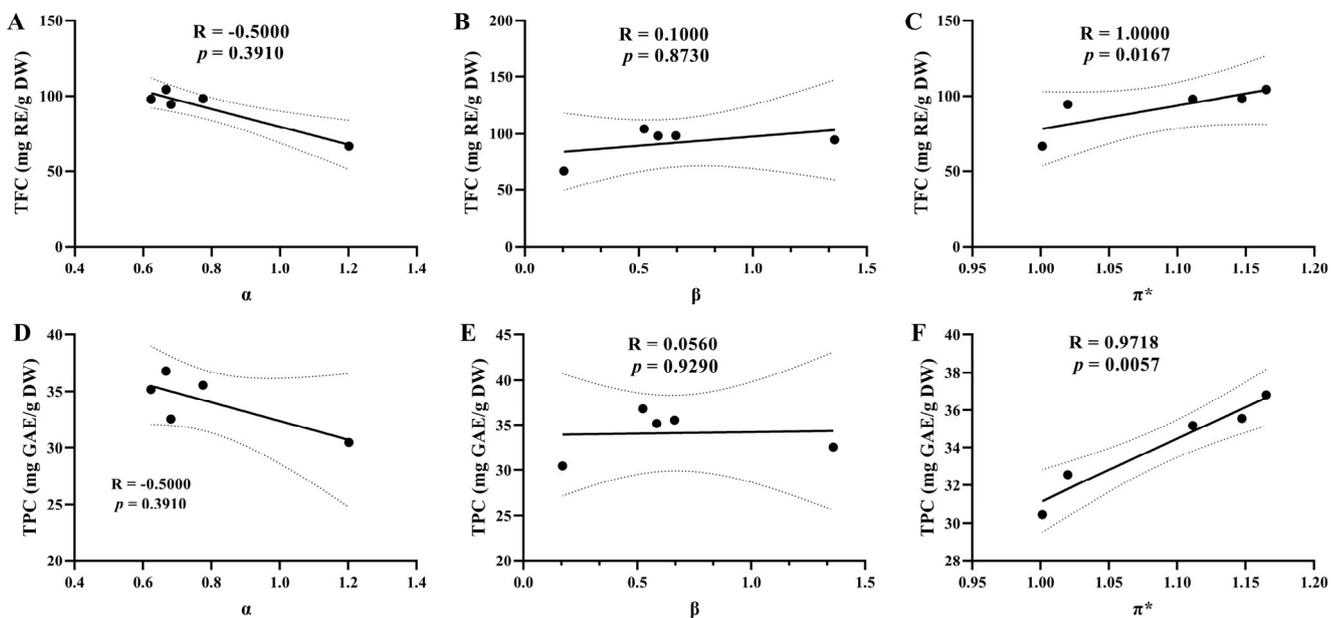
**Figure S1.** The external morphological characteristics of LjF (A). The microscopic characteristics of LjF. (B): non-glandular hair; (C): calcium oxalate clusters; (D): pollen grains; (E): glandular hair.



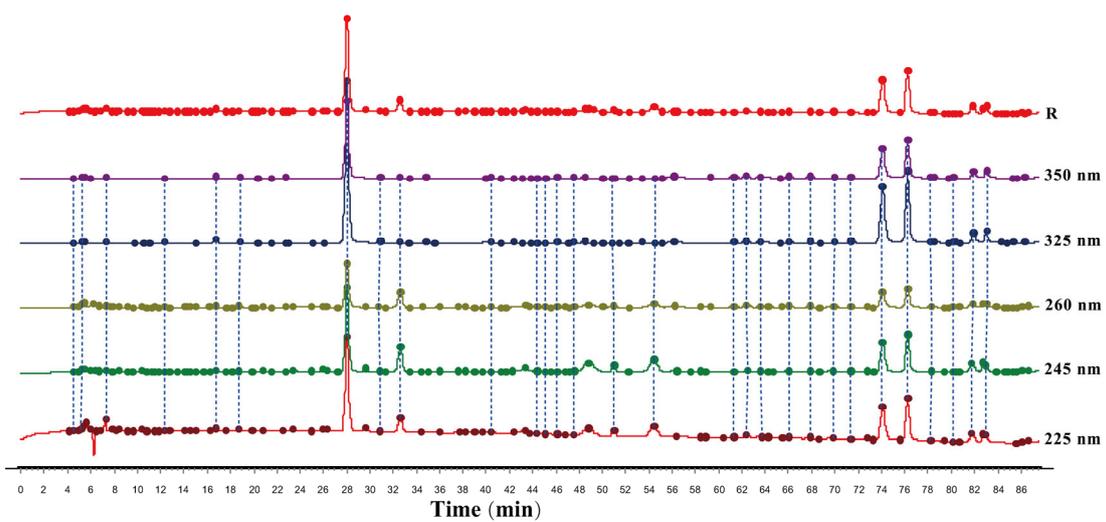
**Figure S2.** The FT-IR spectrum of TMAC-EG-1 DES.



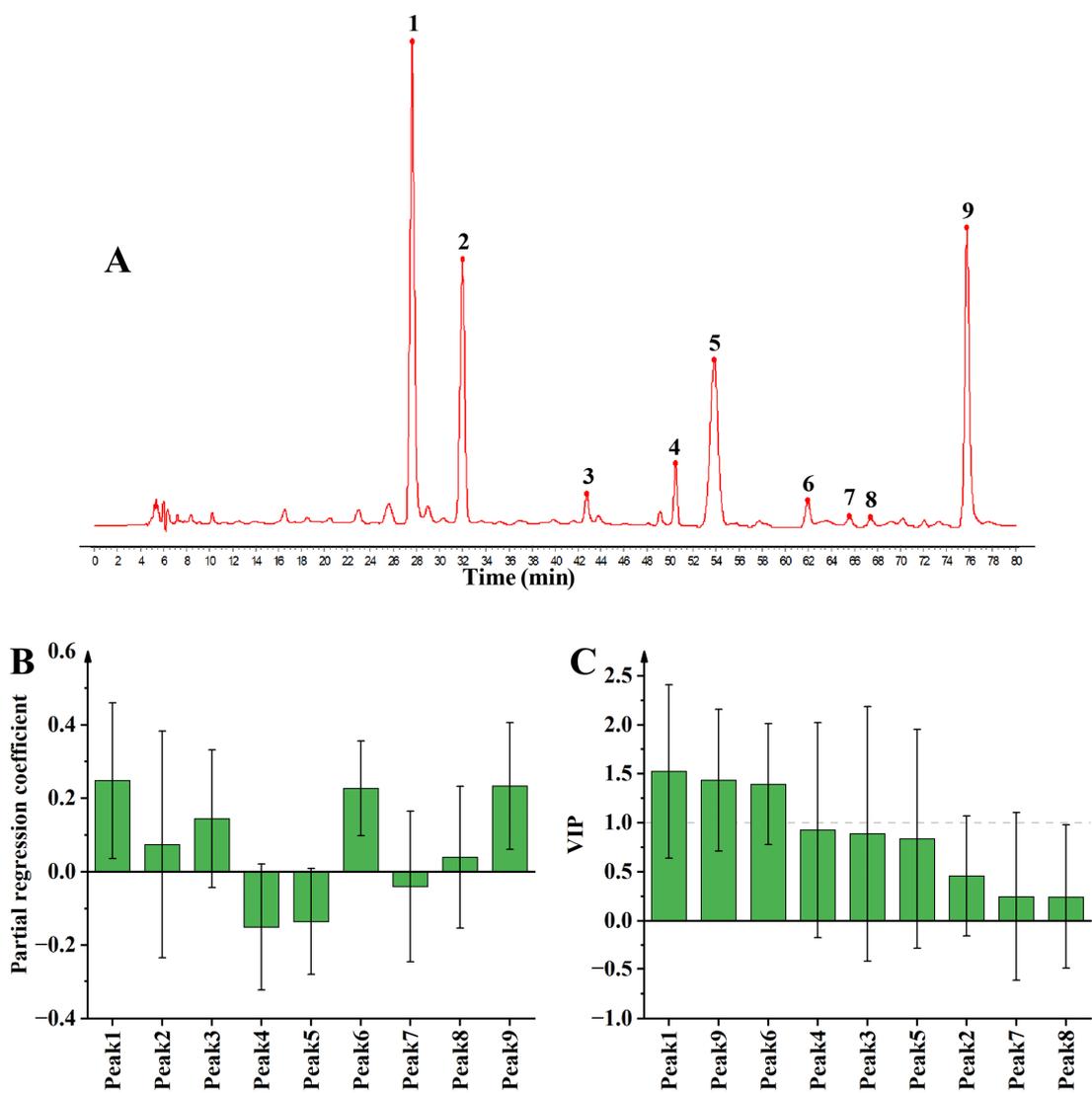
**Figure S3.** The antioxidant ability standard curve of Trolox on the DPPH• scavenging.



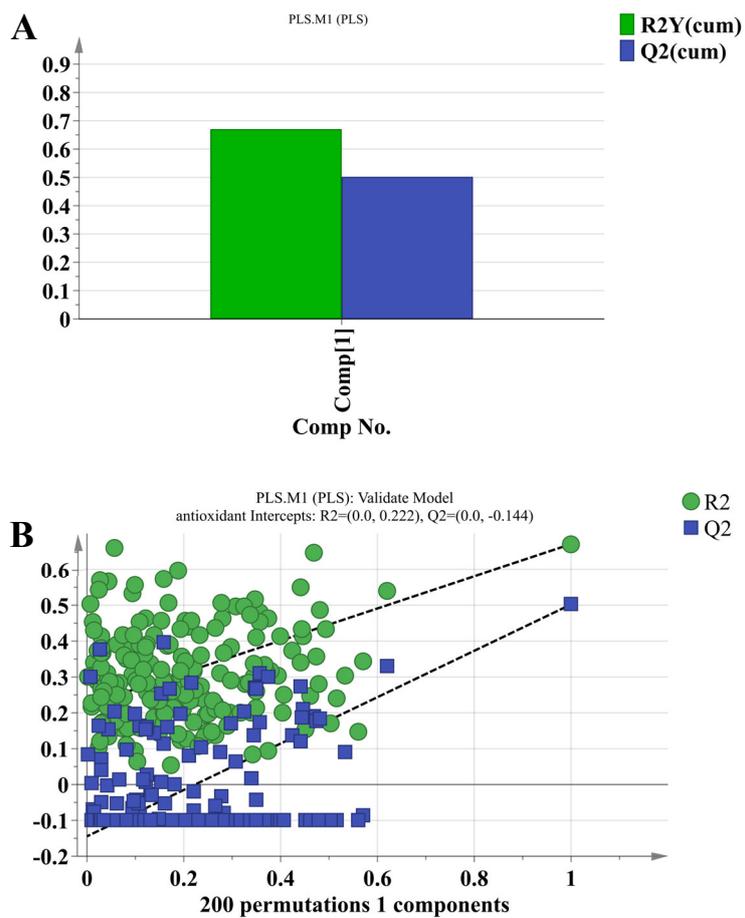
**Figure S4.** The correlation between TFC content of LJF and the  $\alpha$  (A),  $\beta$  (B) and  $\pi^*$  (C) subtraction value of DESs; The correlation between TPC content of LJF and the  $\alpha$  (D),  $\beta$  (E) and  $\pi^*$  (F) subtraction value of DESs.



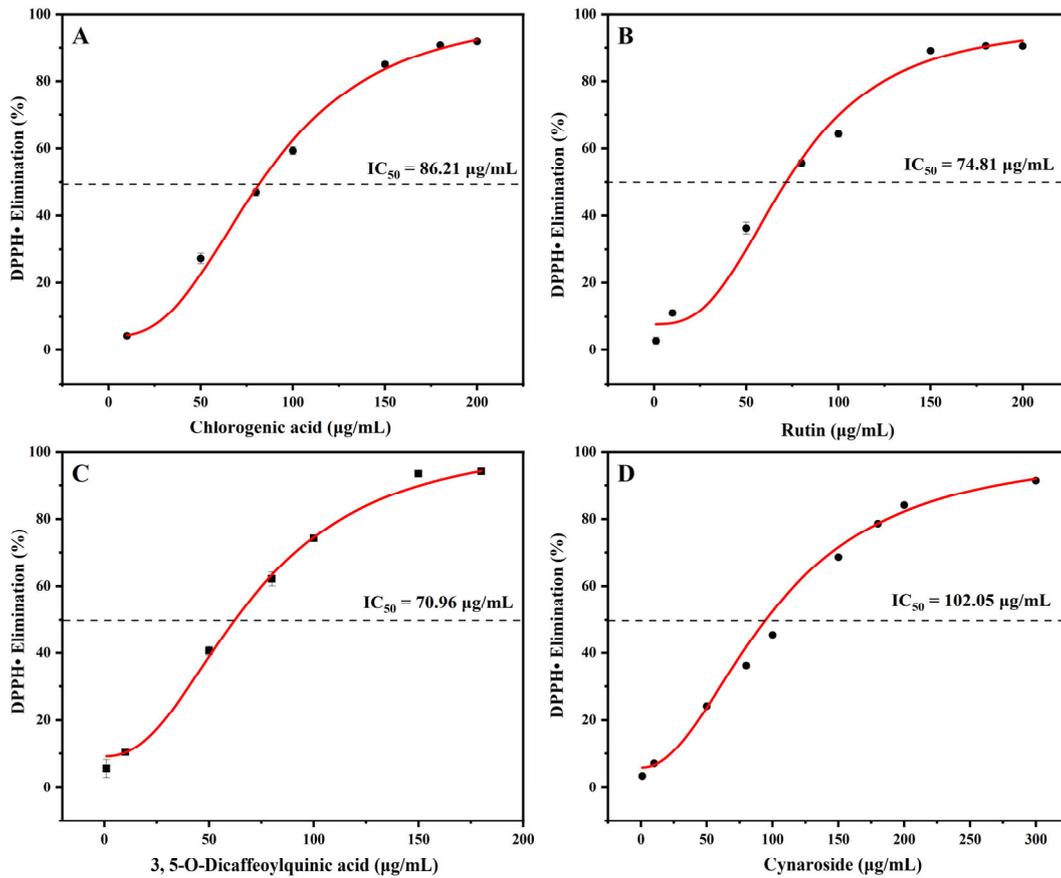
**Figure S5.** The HPLC-fingerprint of LJJ extracts at different UV detection wavelengths. The dots in the graph indicate the presence of chromatographic peaks.



**Figure S6.** Characteristic chromatogram of LJF (A). The partial regression coefficient of PLS model (B) and variable importance plot of PLS model (C).



**Figure S7.** The permutation test results (A) and replacement test results (B) of PLS model based on tailored DES extract from different LJF.



**Figure S8.** The elimination of DPPH• with different concentrations of chlorogenic acid (A), rutin (B), 3, 5-O-dicaffeoylquinic acid (C), and cymaroside (D).

## 4. Supplementary Tables

**Table S1.** The information of collected LJF and LF samples and their water content.

Sample No.	Species	Sources	Sample code	Collected time*	Water content (m/m, %)
1	LJF	Linyi, Shandong	LJF-SD1	August 2022	9.40
2	LJF	Pingyi, Shandong	LJF-SD2	June 2022	9.40
3	LJF	Pingyi, Shandong	LJF-SD3	May 2022	9.47
4	LJF	Pingyi, Shandong	LJF-SD4	June 2022	9.37
5	LJF	Pingyi, Shandong	LJF-SD5	May 2023	9.39
6	LJF	Julu, Hebei	LJF-HB1	May 2022	9.21
7	LJF	Julu, Hebei	LJF-HB2	July 2022	9.67
8	LJF	Julu, Hebei	LJF-HB3	June 2023	9.15
9	LJF	Julu, Hebei	LJF-HB4	July 2023	8.93
10	LJF	Julu, Hebei	LJF-HB5	August 2022	8.90
11	LJF	Fengqiu, Henan	LJF-HN1	June 2022	9.62
12	LJF	Fengqiu, Henan	LJF-HN2	July 2022	8.90
13	LJF	Fengqiu, Henan	LJF-HN3	May 2022	9.50
14	LJF	Fengqiu, Henan	LJF-HN4	May 2023	9.48
15	LJF	Fengqiu, Henan	LJF-HN5	July 2022	9.77
16	LF	Longhui, Hunan	LF1	July 2022	9.32
17	LF	Longhui, Hunan	LF2	May 2023	9.59
18	LF	Longhui, Hunan	LF3	July 2022	10.00
19	LF	Longhui, Hunan	LF4	August 2022	9.39
20	LF	Yulin, Guangxi	LF5	May 2022	9.43
21	LF	Yulin, Guangxi	LF6	June 2022	9.83
22	LF	Yulin, Guangxi	LF7	May 2023	9.02
23	LF	Yulin, Guangxi	LF8	May 2022	9.14

\* The collection times for LJF and LF were obtained by asking the pharmacy staff.

**Table S2.** Experimental design and results of Box-Behnken Design (BBD).

<b>Run</b>	<b>A: DES content (v/v, %)</b>	<b>B: Extraction temperature (°C)</b>	<b>C: Liquid-solid ratio (mL/g)</b>	<b>Response: Antioxidant ability (<math>\mu\text{mol TE/g DW}</math>)</b>
1	75.00	60.00	40.00	234.59
2	75.00	60.00	20.00	227.01
3	60.00	60.00	30.00	237.38
4	75.00	70.00	30.00	247.84
5	75.00	80.00	20.00	210.37
6	75.00	70.00	30.00	242.12
7	90.00	60.00	30.00	215.22
8	75.00	70.00	30.00	242.79
9	90.00	70.00	40.00	219.01
10	75.00	70.00	30.00	242.15
11	75.00	70.00	30.00	243.52
12	90.00	70.00	20.00	210.22
13	60.00	70.00	20.00	216.36
14	90.00	80.00	30.00	220.00
15	60.00	80.00	30.00	226.54
16	60.00	70.00	40.00	238.49
17	75.00	80.00	40.00	242.39

**Table S3.** ANOVA of the established BBD model.

A: DES content; B: extraction temperature; C: liquid-solid ratio.

Source	Sum of squares	Degree of freedom	Mean square	F value	p value	Prob>F
Model	2646.06	9	294.01	59.63	< 0.0001	significant
<i>A</i>	368.69	1	368.69	74.78	< 0.0001	
<i>B</i>	27.79	1	27.79	5.64	0.0493	
<i>C</i>	621.65	1	621.65	126.08	< 0.0001	
<i>AB</i>	60.93	1	60.93	12.36	0.0098	
<i>AC</i>	44.52	1	44.52	9.03	0.0198	
<i>BC</i>	149.23	1	149.23	30.27	0.0009	
<i>A</i> <sup>2</sup>	737.28	1	737.28	149.53	< 0.0001	
<i>B</i> <sup>2</sup>	135.14	1	135.14	27.41	0.0012	
<i>C</i> <sup>2</sup>	374.36	1	374.36	75.93	< 0.0001	
Residual	34.51	7	4.93			
Lack of fit	11.61	3	3.87	0.68	0.6107	not significant
Pure error	22.90	4	5.73			
Cor total	2680.58	16				

$R^2 = 0.9871$  Adjusted  $R^2 = 0.9706$  Predicted  $R^2 = 0.9174$

**Table S4.** K-T parameters of prepared DESs and organic solvents.

Solvents	K-T Parameters		
	$\alpha$	$\beta$	$\pi^*$
Water	0.300	0.209	1.270
Methanol	0.697	0.631	0.712
Ethanol	0.640	0.780	0.672
ChCl-EG-1	0.668	0.525	1.165
ChCl-Pro-1	0.623	0.585	1.111
ChCl-But-1	0.682	1.360	1.020
ChCl-Gly	0.776	0.664	1.147
ChCl-Aa-1	1.203	0.172	1.001

**Table S5.** The results of total polyphenols (TPC), total flavonoids (TFC), total reducing sugars (TRS) content and antioxidant ability of LJF extracts from different origins.

<b>Sample Code</b>	<b>TPC (mg GAE/g DW)</b>	<b>TFC (mg RE/g DW)</b>	<b>TRS (mg GE/g DW)</b>	<b>Antioxidant ability (μmol TE/g DW)</b>
LJF-SD1	34.61	122.58	25.07	207.85
LJF-SD2	38.05	105.51	28.70	218.03
LJF-SD3	34.04	116.99	46.62	180.67
LJF-SD4	35.26	105.96	28.91	201.80
LJF-SD5	33.90	123.16	23.95	172.03
LJF-HB1	31.64	108.40	18.91	207.55
LJF-HB2	32.12	101.06	43.21	220.90
LJF-HB3	28.60	105.44	24.80	210.12
LJF-HB4	31.43	111.01	21.25	220.97
LJF-HB5	30.94	113.75	24.68	197.80
LJF-HN1	34.23	120.77	23.95	240.19
LJF-HN2	30.87	104.03	81.05	220.47
LJF-HN3	33.58	125.44	26.10	236.02
LJF-HN4	34.96	119.80	27.65	183.67
LJF-HN5	35.73	129.82	30.04	224.51

**Table S6.** The results of grey relation analysis between total polyphenols (TPC), total flavonoids (TFC), total reducing sugars (TRS) content and antioxidant capacity of LJF extracts.

<b>Evaluation item</b>	<b>Degree of association</b>	<b>Range</b>
TPC	0.884	1
TFC	0.882	2
TRS	0.749	3

**Table S7.** The precision, repeatability and stability evaluation of HPLC fingerprint method of LJF extracts.

Common peaks	Precision (RSD%, n = 6)		Repeatability (RSD%, n = 6)		Stability (RSD%, n = 6)	
	Peak area	Retention time	Peak area	Retention time	Peak area	Retention time
1	0.00	0.00	0.00	0.00	0.00	0.00
2	3.89	0.14	2.48	0.12	3.57	0.13
3	2.93	0.33	0.50	0.17	2.01	0.20
4	2.93	0.43	3.15	0.21	3.08	0.27
5	0.80	0.42	0.55	0.21	0.62	0.28
6	1.49	0.48	3.13	0.21	1.72	0.32
7	4.50	0.48	0.96	0.22	2.93	0.33
8	2.42	0.49	1.07	0.22	2.47	0.34
9	0.59	0.51	0.55	0.24	0.70	0.36

**Table S8.** The results of similarity analysis of HPLC fingerprint of LJF different origins.

Sample code	Similarity	Sample code	Similarity	Sample code	Similarity
LJF-SD1	1	LJF-HB1	0.972	LJF-HN1	1
LJF-SD2	0.999	LJF-HB2	0.962	LJF-HN2	0.934
LJF-SD3	0.992	LJF-HB3	0.995	LJF-HN3	0.998
LJF-SD4	0.998	LJF-HB4	0.999	LJF-HN4	0.998
LJF-SD5	0.971	LJF-HB5	0.999	LJF-HN5	0.993

**Table S9.** MS data for characteristic peaks of compounds of LJF by HPLC-MS.

Peak	t <sub>R</sub> /min	[M-H] <sup>-</sup> /[M+H] <sup>+</sup>	MSn Fragment Ions	Formula	Compound identification	Reference
1	27.59	355.10040[M+H] <sup>+</sup>	372.12701; 355.10040; 163.03819	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	Chlorogenic acid	[54]
2	31.96	373.11005[M-H] <sup>-</sup>	179.05389; 159.05836; 123.04288; 119.03267	C <sub>16</sub> H <sub>22</sub> O <sub>10</sub>	Secologanic acid	[55]
3	42.77	359.13187[M+H] <sup>+</sup>	197.18006; 127.03860	C <sub>16</sub> H <sub>22</sub> O <sub>9</sub>	Sweroside	[54]
4	50.47	-	-	-	unknown	-
5	53.82	195.05200[M+H] <sup>+</sup>	89.05987; 117.03350	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	Ferulic acid	[56]
6	61.92	611.15808[M+H] <sup>+</sup>	303.04874; 85.02875	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	Rutin	[57]
7	65.96	465.10098[M+H] <sup>+</sup>	303.04895	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	Isoquercitrin	[55]
8	67.78	447.08966[M-H] <sup>-</sup>	285.03781	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	Cynaroside	[38]
9	75.57	515.11420[M-H] <sup>-</sup>	353.08459; 191.05368; 179.03256; 173.04309; 135.04289	C <sub>25</sub> H <sub>24</sub> O <sub>12</sub>	3, 5-O-Dicaffeoylquinic acid	[54]

**Table S10.** The result of grey relation analysis between the area of common peaks and antioxidant capacity of LJF extracts.

<b>Evaluation item</b>	<b>Degree of association</b>	<b>Rank</b>
Peak1	0.894	1
Peak9	0.864	2
Peak6	0.837	3
Peak2	0.831	4
Peak3	0.826	5
Peak8	0.749	6
Peak4	0.728	7
Peak7	0.703	8
Peak5	0.698	9

**Table S11.** The standard curves of chlorogenic acid, rutin and 3,5-O-Dicaffeoylquinic acid.

<b>Analyte</b>	<b>Linear equation (n = 3)</b>	<b>Linear range (µg/mL)</b>	<b>R<sup>2</sup></b>
Chlorogenic acid	$y = 62076 x - 64907$	20-200	0.9994
Rutin	$y = 44867 x + 24107$	0.25-80	0.9994
3,5-O-Dicaffeoylquinic acid	$y = 89339 x - 31059$	1-200	0.9997

**Table S12.** The content of chlorogenic acid, rutin and 3,5-O-Dicaffeoylquinic acid in LJF and LF from different origins.

Sample code	Content of three Q-makers (mg/g)		
	Chlorogenic acid	Rutin	3,5-O-Dicaffeoylquinic acid
LJF-HN1	34.89	2.51	15.34
LJF-HN2	28.14	2.09	12.63
LJF-HN3	29.88	2.92	14.97
LJF-HN4	28.68	2.14	13.87
LJF-HN5	33.44	3.43	16.94
LJF-HB1	24.18	0.97	0.53
LJF-HB2	30.07	1.37	1.44
LJF-HB3	32.11	1.58	1.41
LJF-HB4	34.33	2.33	1.47
LJF-HB5	34.98	2.51	1.48
LJF-SD1	36.93	16.11	22.75
LJF-SD2	32.89	21.42	21.50
LJF-SD3	34.87	15.56	21.43
LJF-SD4	29.50	19.25	20.38
LJF-SD5	27.25	16.06	17.07
LF1	50.49	0.00	24.91
LF2	52.13	0.00	22.17
LF3	55.51	0.00	24.36
LF4	53.28	0.00	22.65
LF5	51.87	0.00	17.87
LF6	48.01	0.00	19.47
LF7	49.98	0.00	21.40
LF8	48.06	0.00	19.99

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